INTERACTIONS OF OLIGOPEPTIDES WITH NUCLEIC ACIDS

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I. INTRODUCTION

Binding of oligopeptides to nucleic acids has been the subject of numerous studies during the past few years. These studies were usually undertaken to try to understand the nature and specificity of the interactions involved in protein-nucleic acid associations. The binding to nucleic acids of biologically active peptides whose target is — or is believed to be — DNA has also been investigated in some details. Some of these studies bring information or ideas on the types of interactions which might be responsible for the recognition of nucleic acid structures or base sequences by proteins. This review will not cover studies related to amino acid-base interactions nor those devoted to the association of polypeptides with mononucleotides or nucleic acids. It will be limited to those reports dealing with small oligopeptides interacting with ribo- or deoxyribopolynucleotides and nucleic acids.

Different types of interactions are responsible for the binding of oligopeptides to nucleic acids. They can be classified as

1. Electrostatic interactions between positively charged (basic) amino acids such as Lys, Arg or protonated histidine and negatively charged phosphates of the nucleic acid
2. Stacking interactions involving aromatic amino acids and nucleic acid bases
3. Hydrogen bonding interactions between several amino acid side chains or the peptidic amide group and nucleic acid bases, phosphates, ribose or d-ribose
4. Hydrophobic interactions between aliphatic amino acid side chains and the bases of nucleic acids

It should be pointed out that this classification might appear arbitrary. Electrostatic binding of an arginine side chain to a phosphate group involves hydrogen bonding interactions between $-NH$ groups of the guanidinium cation and oxygens of the phosphate group, as seen, for example, in the crystal structure of guanidinium phosphate.[1] The electrostatic contribution to hydrogen bond formation might be very important. In all types of interactions the same intermolecular and interatomic forces are involved with different weights depending upon the chemical nature, charge distribution and polarizabilities of the interacting species (Coulombic interactions between charges and/or dipoles; polarization, dispersion and repulsive forces, etc.). It is only for sake of clarity that the classification presented above has been used in the present review. Experimental data providing evidence for the different types of interactions involved in

oligopeptide-nucleic acid associations will first be reviewed. Then the specificity afforded by these interactions will be discussed, including different models which have been proposed to explain the selectivity of protein associations with nucleic acids.

II. ELECTROSTATIC INTERACTIONS

Electrostatic interactions between positively charged amino acids and phosphate groups of nucleic acids are involved in the great majority of associations of nucleic acids with proteins. Associations between basic polypeptides and polynucletides or nucleic acids have frequently been studied as model systems. Less studies have been devoted to the interactions between basic oligopeptides and nucleic acids. Very often basic oligopeptides have been investigated as reference compounds for the study of other types of interactions such as, for example, stacking interactions (see below). It is thus often difficult to make a clear comparison between the various results since they are scattered in several articles and are less systematic than for other types of interactions. Furthermore less methods are available to investigate the binding of basic peptides as compared, for example, to aromatic peptides.

Interactions of nucleic acids with oligopeptides which do not contain aromatic residues can be followed by nuclear magnetic resonance (NMR) spectroscopy. The resonance signals of bound molecules are expected to broaden due to a decrease in mobility. Differential broadening of different lines in the NMR spectra should reveal some structural features of the complexes. For example, Backer et al.[2] reported that binding of the dipeptide Gly-Gly to tRNA was characterized by a broadening of the α -CH_2 resonance of the N-terminal glycyl residue while that of the C-terminal residue was not broadened. This result was interpreted as revealing the electrostatic interaction of the terminal NH_3^+ group of the peptide with a phosphate group on the RNA. The extent to which the α-CH_2 resonance of glycyl residues was broadened also depended on the sequence and increased in the order Gly-Leu < Gly-Gly < Gly-Ser at a given tRNA concentration. However changing the sequence of the peptide may alter either the association constant of the peptide or the mobility of the α-CH_2 group in the *bound* peptide (or both). A detailed study of the association as a function of the concentrations of both partners or information from other techniques are required to propose an unequivocal interpretation of NMR results.

The binding of lysine oligopeptides to an oligodesoxynucleotide (A_3 GCT_3) has been recently investigated by nuclear magnetic resonance.[3] Downfield shifts of proton resonances of $(Lys)_4$ were observed as well as a reduction in the exchange rates of NH protons. An upfield shift of 1 ppm was reported for the ^{31}P resonance of 3′-5′ phosphate diesters.

A. Effect of Electrostatic Interactions on the Stability of DNA

The binding of basic oligopeptides to DNA changes the melting temperature as reported by several authors. However due to the difference in the origin of DNA, ionic strength, and ratio of peptide to DNA concentrations, it is difficult to make a quantitative comparison between these studies. Nevertheless the following main points emerge:

1. All basic oligopeptides increase the melting temperature of DNA upon binding.
2. Increasing the ratio of peptide to DNA concentrations leads to an increase of the melting temperature. It was reported by Brown[4] that this increase could be fitted by the empirical relationship

$$\frac{1}{\Delta T} = \frac{1}{\Delta T_o} + \frac{1}{kc} \qquad (1)$$

where Δ T is the rise in T_m due to binding at concentration c, ΔT_o is the maximum rise in T_m (at infinite peptide concentration) and k is a constant. A similar relationship was used by Gourêvitch et al.[5] to analyze the binding of several basic peptides and aliphatic diamines to DNA.

The efficiency in stabilizing the double-stranded structure of DNA is highly dependent on the number of basic residues in the peptide. For example Gourêvitch et al.[5] have compared the effect of two basic peptides AcGly-Arg-Arg-Gly(OCH_3) and AcGly-Arg-Gly(OCH_3). With the first peptide, the T_m of the complex rapidly reaches a limit value (80% of the total variation occurs for a ratio of peptide-to-nucleotide concentration smaller than one), whereas with the second peptide one needs about ten times more peptide to obtain a similar effect. Gabbay et al.[6] made similar observation with peptides containing one and two lysyl residues.

When the length of the peptide increases and if a high ratio of peptide-to-DNA is used, a biphasic melting can be observed. In a study of the interaction of oligolysines of various sizes (Lys_4, Lys_8, Lys_{14-18} and polylysine) Ollins et al.[7] reported that DNA-octalysine complexes show the emergence of a biphasic melting profile at a charge-to-phosphate ratio higher than 0.5. Longer oligomers and the polymer showed a pronounced biphasic melting curve characteristic of a cooperative interaction. This phenomenon has been observed with many basic polypeptides.[8-17]

B. Conformational Changes Induced by Electrostatic Interactions

The binding of basic oligopeptides to nucleic acids induces small conformational changes of the nucleic acid which can be demonstrated by circular dichroism and absorption spectroscopy. In a study on the interaction of various derivatives of lysine, lysinemethylester, Lys_2 and $(Lys)_2$ methyl ester, Lacombe and Laigle[18] observed that the binding of these compounds induced a decrease in the positive band of the CD signal of DNA and only a very small change in the negative band. Since these peptides did not show any absorption in the same wavelength range, the effect was ascribed to a conformational change of DNA. We observed similar changes upon binding of the peptide Lys-Ala-Lys to DNA.[169] Conformational changes in DNA due to peptide binding were interpreted as corresponding to a transition from the B to the C structure.[18] Taking into account the more recent results of Chan et al[19] and Boose and Johnson,[20] one could interpret these results as corresponding to an increase of the winding angle of the DNA by about 0.5°.

The binding of basic oligopeptides to single stranded poly(A) was also investigated using CD and difference absorption spectroscopy. The binding of the peptide Lys-Ala-Lys led to a small decrease of the positive band of the CD spectrum of poly(A) and a small increase of the negative band.[21] Similar results were obtained with lysine methylester and with cadaverine (1-5 diaminopentane). This was interpreted as being due to the fact that binding involved the N-terminal lysyl residue of Lys-Ala-Lys, which binds to poly(A) through the α and ε NH_3^+ groups. This change of poly(A) conformation does not correspond to a change in the degree of stacking of the polymer which would be expected to yield similar modifications of the positive and negative bands of the CD spectrum. It may correspond to a change in the winding angle or to a tilt of the bases. A similar decrease of the CD amplitude at 264 nm of poly(A) upon $(Lys)_3$ binding was recently reported.[22]

These results have been confirmed by difference absorption measurements. The binding of Lys-Ala-Lys, Lys(OMe), and cadaverine induced difference spectra similar for the three compounds. These difference spectra were different from those obtained either upon heating or upon cooling poly(A); i.e., by changing the stacking of the bases in the polymer.

With poly(U), addition of pentalysine induced a conformational change from the single stranded random coil to a double stranded helix as demonstrated by the effect

on the CD spectrum.[23] This effect was also observed with Lys-Ala-Lys[169] and with the tripeptides $(Arg)_3$ and $(Lys)_3$.[22] With other polynucleotides, changes in the CD spectrum upon interaction with $(Arg)_3$ or $(Lys)_3$ were reported to be small.[22]

The binding of $(Arg)_3$ and $(Lys)_3$ to poly(A) presents several phases depending on the ratio of peptide-to-poly(A) concentrations.[22] For example the circular dichroism amplitude of poly(A) at 264 nm decreased much faster than the absorbance at 260 nm upon addition of $(Lys)_3$. Complex titration curves were also obtained when the UV absorbance and CD amplitude of poly(A) were plotted vs. $(Arg)_3$ concentration. High oligopeptide concentrations caused opalescence and then coacervation.

A biphasic binding of oligopeptides to double-stranded nucleic acids was also reported by Ramstein[24] using proflavine as a probe to follow the association. At low peptide concentration the binding of tripeptides such as Lys-Ala-Lys or Lys-Tyr-Lys was accompanied by a conformational change in DNA resulting in a slightly different absorption spectrum for the intercalated dye. At higher concentrations the dye was released from its intercalating sites. Up to a ratio [peptide]/[DNA] of 0.05 isosbestic points were obtained in the absorption spectra indicating that a single type of complex was formed. For a ratio higher than 0.1, a second type of complex was obtained which was characterized by different isosbestic points in the absorption spectra.

C. Thermodynamic Studies

In a calorimetric study of the binding of tetralysine, polylysine, and spermine to DNA, Ross and Shapiro[25] showed that the enthalpy change during this interaction was very close to zero. ΔH values ranging from 0 to 0.8 μ J mol^{-1} were found for tetralysine and polylysine gave a ΔH of -1.2 kJ mol^{-1}. For spermine no enthalpy change was observed. More recently Giancotti et al.[26] investigated the binding of trilysine and polylysine by the same method and found a positive reaction enthalpy of $+1.2$ kJ mol^{-1} for trilysine and about 4 kJ mol^{-1} for polylysine. The discrepancy between the results of these two groups might be attributed to difference in the types of complex formed, since the ionic strength and the peptide-to-DNA ratios were different in the two studies.

Whatever is the exact value of ΔH corresponding to the binding of a lysyl residue to DNA, it is clear that this value is very small which suggests that this binding process is primarily entropy driven. Biltonen and co-workers[27] have measured the ΔH and ΔS values associated with the binding of CMP to ribonuclease and also reached the conclusion that the electrostatic component contributed to complex formation through a positive ΔS whereas the corresponding ΔH was nearly zero.

D. Effect of Ionic Strength

Interactions between negative phosphate groups and positive groups of basic amino acids are very sensitive to the ionic strength of the medium. They are associated with a concomitant release of low molecular weight ions that were previously associated with the charged groups. Because of ion release in this charge neutralization reaction, the equilibrium shifts to favor complex formation when the salt concentration is reduced.

Latt and Sober[28] in their study of complex formation between ε-DNP-L Lys $(Lys)_N$ and double-stranded poly(A)·poly(U), poly(I)·poly(C) and triple-stranded poly(A)·2 poly(U) have shown that the binding is highly dependent on the ionic strength. At a given Na^+ concentration in the range 0.15 to 0.5 *M*, the logarithm of the observed binding constant increased linearly with the chain length of the oligopeptides and the graphs for the various Na^+ concentrations extrapolated to a common intercept at N=O which was identified as the binding constant of the terminal lysyl residue labeled by a dinitrophenyl group. This result was also interpreted as indicating that only the ε-NH_3^+ groups of the oligomers interacted with the polymer. These data were reexamined by

Record et al,[29] introducing the concept of Na^+ condensation developed by Manning. Figure 1 shows the plots of the logarithm of the binding constant as a function of log Na^+ for various chainlengths N in the case of the complexes of oligolysine with poly(A)·poly(U). The slope of these lines increases in magnitude in direct proportion to N according to the following relationship

$$\frac{\log K_{obs}}{\log [Na^+]} = (0.9 \pm 0.05)\,N \qquad (2)$$

The theory developed by Record et al.[29] concludes that the slope of the linear plot $\log K_{obs} = f(\log Na^+)$ for the interaction between a charged ligand and a nucleic acid is $m'\psi$ where m' is the number of ion pairs formed in the interaction and ψ the fraction of counterions thermodynamically bound per phosphate. Since $\psi = 0.89$ for poly(A)·poly(U), it comes that the number of ion pairs formed is equal to the number of ε-NH_3^+ groups in the oligopeptide.

This theory may be applied to the binding of a molecule which does not involve only electrostatic interactions. In the case of the binding of the peptide Lys-Trp-Lys to single stranded poly(A) the formation of three ion pairs was deduced from the ionic strength dependence of binding.[29]

The release of ions which results from electrostatic binding of lysyl residues to phosphates can also be directly demonstrated by electric conductivity measurements using dielectric methods.[30] When the oligopeptide Lys-Tyr-Lys binds to DNA, at least two phosphate sites are electrostatically linked to the oligopeptide.

E. Role of Metal Cations in the Binding of Peptides to Nucleic Acids

Interactions between peptides and nucleic acids could be mediated through metal cations acting as bridges between the two partners. Very few studies have been devoted to this type of interaction which could play an important role in protein-nucleic acid associations. There have been several reports of ternary complex formation involving nucleotides, metal cations, and amino acids.[31,32] Using ^1H and ^{13}C NMR spectroscopies Weiner et al.[33] have shown that the carboxyl groups of glycine, β-alanine, γ-aminobutyric acid, and diglycine interact with Mn^{++} coordinated by tRNAs. The formation of ternary complexes between polynucleotides, Zn^{++} or Cu^{++} ions, and polypeptides containing tyrosine and glutamic acid residues has also been described.[34,35]

The structure of the complex formed by $(Gly)_2$, Cu^{++}, and cytidine has been solved by X-ray crystallography. The nucleoside is bonded to the Cu^{++} ion through the N(3) and O(2) atoms of the pyrimidine ring. The interaction involving O(2) is much weaker than that with N(3). The Cu^{++} ion is bonded to the peptide through the terminal carboxyl and amino groups and the NH of the amide peptidic group.[36]

There is no result available about the specificity of these interactions. However metal cations such as Zn^{++} which play an important role in the replication or transcription of DNA do exhibit some selectivity with respect to nucleotide binding which decreases in the order $C > A > G \gg U(T)$. On the other hand, coordination of these ions to the peptide is expected to occur mainly through carboxylic acid side chains (Glu and Asp) and the peptidic bond. Therefore metal cations might intervene in the correct positioning of a protein with respect to a nucleic acid. It has been shown[35] that formation of ternary complexes is accompanied by conformational changes which might also be important in establishing a selective interaction between a protein and a nucleic acid.

III. STACKING INTERACTIONS

Fluorescence studies of aggregates formed in frozen aqueous solutions first provided evidence for the existence of stacking interactions between aromatic amino acids and

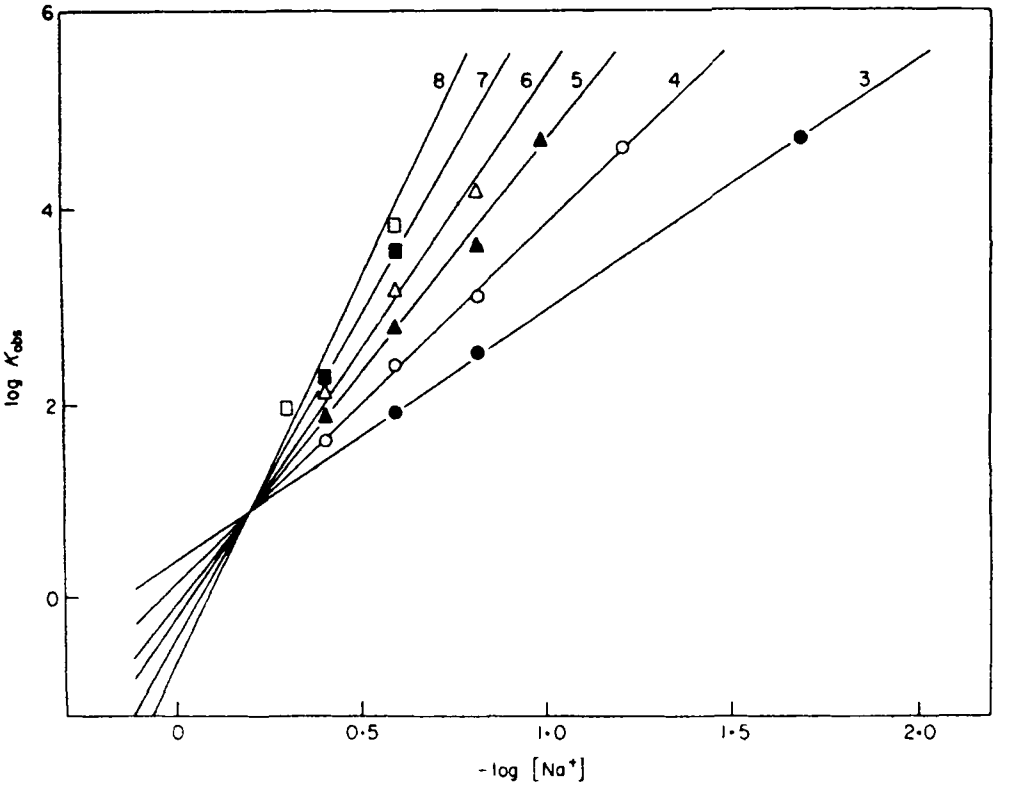


FIGURE 1. Variations of apparent association constants (K_{obs}) as a function of Na^+ concentrations. Data from Latt and Sober[18] for the binding of oligolysines to poly(A)·poly(U). Values of N are indicated on the figure. (With permission from Record, M. T., Jr., Lohman, T. M., and de Haseth, P., *J. Mol. Biol.*, 107, 145, 1976. Copyright by Academic Press, Inc. (London) Ltd.)

nucleic acid bases.[37, 38] The first observation which suggested that tryptophan residues of oligopeptides might stack with nucleic acid bases was made by Brown.[4] He investigated the binding of a series of basic dipeptide methyl esters to DNA by measurement of the melting temperatures of the complexes. The dipeptide Arg-Trp(OMe) showed an unexpectedly high differential stabilizing capacity when compared to other dipeptides Arg-X(OMe), including Arg-Arg(OMe). Brown speculated that tryptophan could act as an anchor ("bookmark" function) to prevent regulatory proteins moving from the site of repression. From NMR and fluorescence studies it was also concluded that the aromatic ring of amines such as tryptamine and serotonine was stacked with nucleic acid bases.[39, 40] Then different physico-chemical methods were used to study the binding of small oligopeptides containing aromatic amino acids to nucleic acids, either native or denatured. These include NMR, fluorescence, circular dichroism, viscosity, equilibrium dialysis, calorimetry, determination of melting temperatures, and flow dichroism. NMR and fluorescence are the methods of choice to investigate stacking interactions involving aromatic residues of oligopeptides.

A. NMR Studies

NMR resonance is a very powerful technique used to provide evidence for stacking interactions. Stacking should induce upfield shifts of proton resonances of a molecule due to the diamagnetic ring current effects of a neighboring aromatic ring. When an oligopeptide is bound to a macromolecule such as DNA, a broadening of the proton resonance lines is expected due to a restricted tumbling of the small molecule. However

this broadening will depend on the mobility of each particular group within the complex, and complexes with different stereochemical constraints should behave differently. When an oligopeptide containing an aromatic amino acid binds to denatured DNA or to single-stranded polynucleotides such as poly(A), upfield shifts of the aromatic and the CH_2 (β) proton resonances are usually observed together with a broadening of these resonance lines.[6,41-46] In complexes with double-stranded sonicated DNA, oligopeptides containing a tryptophyl or a phenylalanyl residue show upfield shifts of their aromatic resonances (Trp > Phe) whereas oligopeptides containing tyrosyl residues give rise to small — if any — upfield shifts.[6, 45, 46]

In recent experiments Mayer et al.[46] reported that no upfield shift could be observed for the complexes of native (sonicated) DNA with a series of oligopeptides containing tyrosine: Lys-Tyr-Lys, Lys-Tyr-Lys-NHEt, Ac Lys-Tyr-Lys NHEt, Lys-Ala-Tyr-Ala-Lys NHEt, and Ac Lys-Ala-Tyr-Ala-Lys NHEt, whereas complexes of the same peptides with poly(A) exhibited large upfield shifts. A similar result was reported by Novak and Donhal in a study of oligopeptides containing Gly and Tyr residues.[47]

Figure 2 shows the PMR spectra which have been obtained with three peptides (Lys-Trp-Lys, Lys-Tyr-Lys, and Lys-Phe-Lys) in the presence of sonicated double-stranded DNA and single-stranded poly(A). This allows a direct comparison of the upfield shifts which characterize the complexes formed by these three peptides: large upfield shifts in the case of poly(A) and smaller shifts with DNA decreasing in the order Trp > Phe > Tyr.

It must be kept in mind that a comparison of the behavior of different oligopeptides requires a quantitative analysis of complex formation since any upfield shift measured at a single peptide/DNA ratio depends on the relative concentration of bound and free peptide and on the chemical shift of the protons in the complex(es) (the shift that would be observed if all peptide molecules were bound to the DNA). Moreover if several complexes exist in equilibrium, the observed change in chemical shift will be the weighted average over the different complexes (which may exhibit different chemical shifts due to different stereochemical features). The observation of a single resonance for all peptide-nucleic acid mixture requires a fast chemical exchange between the different complexes and the free peptide. Since upfield shifts due to stacking interactions are usually smaller than 1 ppm, the rate contant for exchange between the different complexes should be higher than about $10^2 s^{-1}$. This always appears to be the case for small oligopeptides but might not be true for longer oligopeptides especially if they contain basic residues which give rise to strong electrostatic interactions with phosphate groups. In this last case the behavior of the PMR spectra is expected to depend on the ionic strength.

The pH dependence of the binding of oligopeptides to nucleic acids reveals the important role of the α-amino group.[44,48] In the absence of any protecting substituent this α-amino group participates in electrostatic interaction with a phosphate group. Consequently its pK is shifted to a higher value in the complex. Neutralization of the charge borne by this group results in a marked reduction of the binding strength. The presence of a free carboxylic group at the end of the peptide also decreases the binding efficiency as a result of electrostatic repulsion with the negatively charged phosphates. If one wants to extrapolate the conclusions drawn from a study of oligopeptide binding to proteins one must therefore use peptides which are protected at both the amino and carboxyl ends. When free, these groups may also participate in an intramolecular structuration of the peptide thereby interfering with the binding process.

The dependence of chemical shifts with peptide concentration is shown in Figure 3 in the case of Lys-Tyr-Lys and Lys-Tyr NH_2 binding to poly(A). Changes in chemical shifts are much higher with poly(A) than with DNA (Table 1). However, it should be noted that (1) adenine exhibits the highest ring current effects of the four DNA bases,[49]

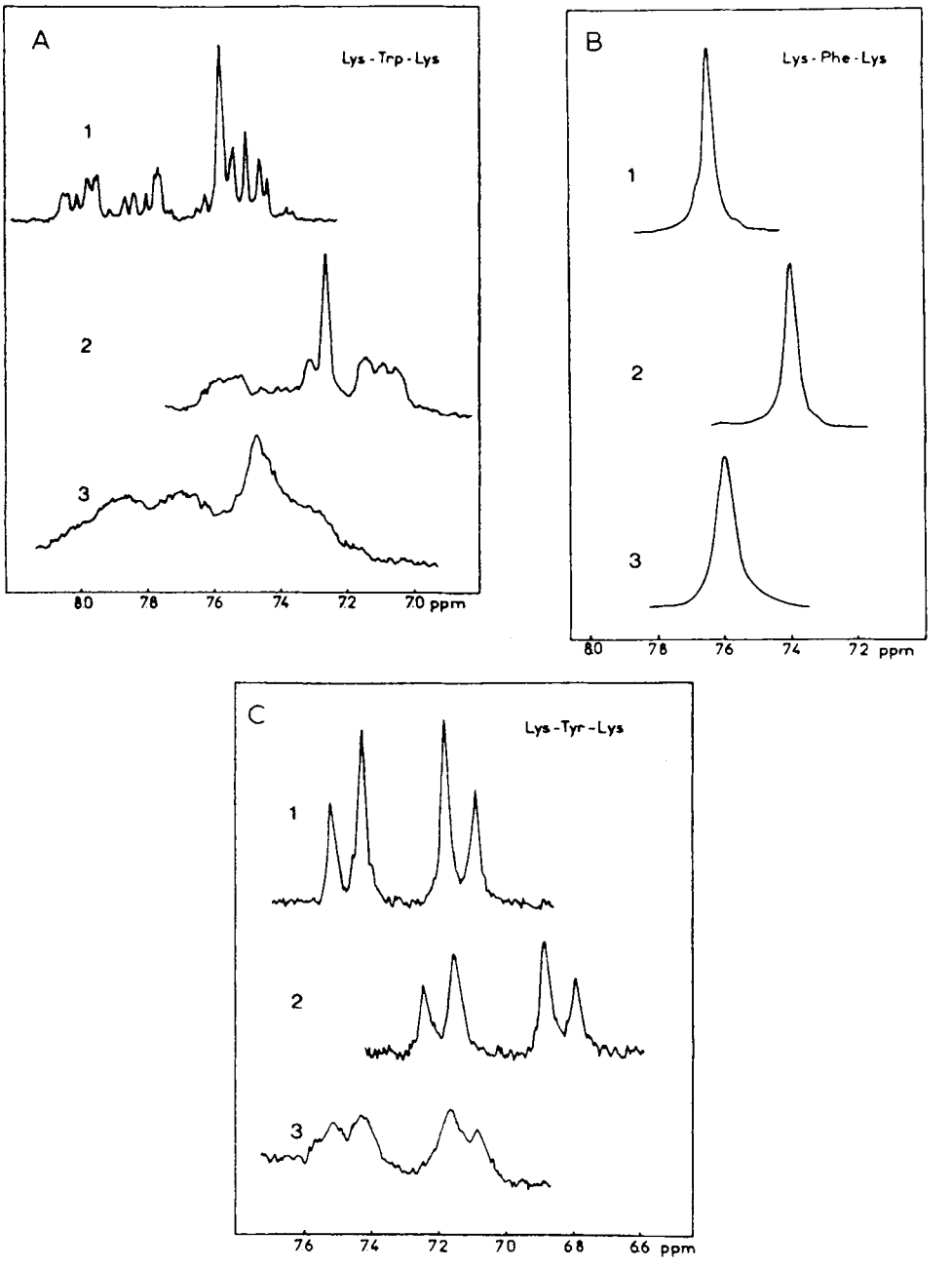


FIGURE 2. NMR spectra of aromatic protons of peptides ($2 \times 10^{-3} M$) in the absence (spectrum 1) and in the presence of poly(A) (spectrum 2) or DNA (spectrum 3). Concentration of poly(A) or DNA is $2 \times 10^{-2} M$ (phosphate/liter). A, Lys-Trp-Lys; B, Lys-Phe-Lys; C, Lys-Tyr-Lys

and (2) the structure of the stacked complexes (overlap of aromatic rings) is certainly different with DNA and poly(A). Therefore any quantitative comparison of the upfield shifts obtained with these two macromolecules must take these factors into account.

Stacking of an aromatic amino acid with a nucleic acid base should elicit upfield shifts of both the base and the amino acid proton resonances. Measurements cannot

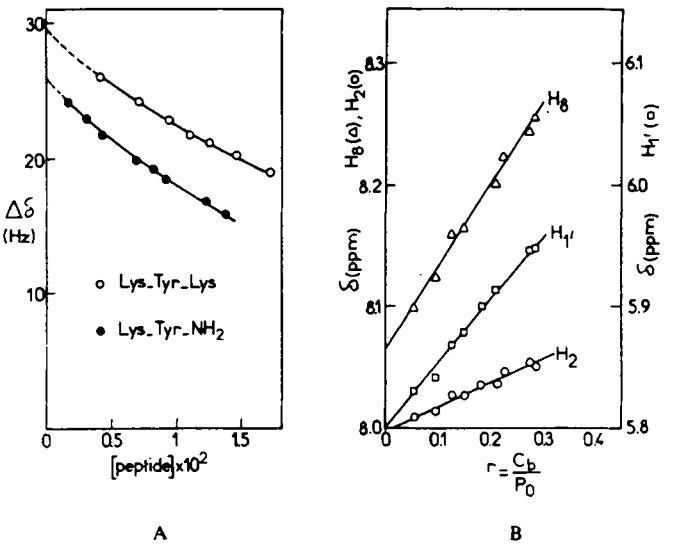


FIGURE 3(A). Concentration dependence of the upfield shifts of the H_ϵ tyrosine proton in the presence of poly(A) ($3.75 \times 10^{-2} M$ in the case of Lys-Tyr-Lys (O) and $2.9 \times 10^{-2} M$ in the case of Lys-Tyr-NH_2 (•). (B) Change in chemical shifts of poly(A) protons as a function of the ratio r of the concentrations of bound Lys-Tyr-NH_2 (C_b) and poly(A) (P_o).(Reprinted with permission from Dimicoli, J. L. and Hélène, C., *Biochemistry*, 13, 724, 1974. Copyright by the American Chemical Society.)

Table 1
UPFIELD SHIFTS (IN PPM) OF AROMATIC PROTONS OF OLIGOPEPTIDES UPON BINDING TO POLY(A) AND DNA EXTRAPOLATED TO ZERO PEPTIDE CONCENTRATION[44,45]

	poly(A)	DNA
Lys-Trp-Lys[a]	0.38	0.23
Trp-Lys[a]	0.31	0.14
Tryptamine[a]	0.30	0.62
5-methoxytryptamine[a]	0.40	0.75
Lys-Tyr-Lys[b]	0.31	0.025
Lys-Tyr-NH_2[b]	0.28	0.09
Tyramine[b]	0.25	0.06
p-methoxyphenethylamine[b]	0.38	0.21

Note: These values represent weighted averages of upfield shifts for the different types of complexes which could be formed in the presence of poly(A) and DNA (see text).

[a] H_ϵ proton of the indole ring.
[b] Average value for ortho and meta protons of tyrosine.

Table 2
CHANGE IN CHEMICAL SHIFT OF POLY(A) PROTONS (IN PPM) PER BOUND AMINE OR PEPTIDE MOLECULE AT 298 K[44,45]

	H_8	H_2	$H_{1'}$
Lys-Trp-Lys	−0.73[a]	−0.04	−0.62
Trp-Lys	−0.08	+0.19[b]	−0.09
Tryptamine	−0.22	+0.08	−0.15
5-methoxytryptamine	−0.28	+0.10	−0.19
Lys-Tyr-Lys	−0.67	−0.17	−0.53
Lys-Tyr NH_2	−0.70	−0.18	−0.52
Tyramine	−0.29	−0.06	−0.20
p-methoxyphenethylanine	−0.32	−0.07	−0.24

[a] (−) Indicates downfield shift.
[b] (+) Indicates upfield shift.

be made, however, with native DNA because even sonicated DNA does not show a high resolution PMR spectra due to a strong broadening of its resonance lines resulting from the long rotational correlation times. Only short pieces of double-stranded helices or highly folded structures (such as tRNAs) are expected to exhibit a high resolution PMR spectrum. Recent experiments, however, showed that long pieces of DNA double helix (several hundred base pairs) exhibit a NMR spectrum even though this spectrum might be difficult to exploit in the study of interactions with other molecules.[50] No NMR data are available yet for the binding of oligopeptides to such systems with the exception of the peptide part of actinomycin D.[51]

Single-stranded polynucleotides do show a high resolution PMR spectrum and are therefore amenable to a study of the effects of oligopeptides on their proton resonances. The results would be easy to interpret if bases were not stacked in the absence of oligopeptides. This is the case with poly(U) which shows upfield shifted resonances in the presence of oligopeptides containing tryptophan or tyrosine (Lys-Trp-Lys and Lys-Tyr-Lys), whereas Lys-Ala-Lys has no effect. The ring current effect of uracil is small,[52] (the smallest of all four bases) and therefore only very small upfield shifts of the aromatic amino acid resonances are observed.

When the polynucleotide possesses stacked bases, the stacking of an aromatic amino acid requires that the bases be first unstacked. This unstacking gives rise to a downfield shift which will be superimposed on the upfield shift due to base-amino acid stacking. The net result will depend on the chemical nature of both the base and the aromatic amino acid. Adenine and the indole ring of tryptophan have a rather similar ring current anisotropy. It is therefore expected that the effect of base unstacking in poly(A) will be more or less compensated by base-tryptophan stacking. Tyrosine has a much smaller ring current effect than tryptophan and downfield shifts are expected when, e.g., Lys-Tyr-Lys binds to poly(A). This is what has been experimentally observed[44,45] as shown in Figure 3B where the change in chemical shifts of adenine protons is plotted against the concentration of bound peptide (determined from data shown in Figure 3A). Changes in chemical shifts are small with tryptophan-containing peptides whereas important downfield shifts are observed with tyrosine-containing peptides (Table 2). It should be noted that electrostatic binding of the lysyl residues to phosphate groups induces a downfield shift of adenine proton resonances due to a change in poly(A) conformation as demonstrated by circular dichroism studies.[21] The resonances of the H_2 and H_8 protons of poly(A) are shifted downfield upon binding of Lys-Ala-Lys.

Stacking interactions of histidine with 5'-AMP have been reported by Mantsch and Neurohr.[53] They showed that proton resonances of histidine free or engaged in thyro-

tropin-releasing hormone TRH (a tripeptide of sequence pGlu-His-Pro-NH_2) were shifted upfield in the presence of 5'-AMP. Association constants of 10 M^{-1} (TRH) and 16 M^{-1} (histidine) were calculated from the NMR data.

Fritzche has investigated the binding to DNA of the dipeptide carnosine (β-alanyl-histidine) and the tripeptide Gly-His-Gly by NMR spectroscopy.[54] He observed a specific broadening of the H-2 and H-4 protons of the histidine residue without upfield shifts. These results are quite similar to those reported by Mayer et al. upon binding of tyrosine-containing peptides to DNA.[46] This can be interpreted as indicating a restricted mobility of the aromatic ring when the peptide is bound to DNA without stacking with bases. It is possible that tyrosine and histidine form hydrogen bonds with some groups on the nucleic acid. A comparison of oligopeptides containing Tyr and Tyr(OMe) residues, however, showed that it is unlikely that Tyr forms hydrogen bonds with the nucleic acid.[46] Histidine residues when protonated may bind to phosphate groups through electrostatic interactions. In the absence of any information on the pH at which experiments are carried out, it is difficult to conclude about the nature of the interactions involved in the binding of histidine-containing peptides to DNA.

Using amino acids immobilized on a polyvinyl resine through covalent binding of their carboxyl groups, Saxinger et al.[55] have determined selectivity coefficients defined as the ratio of the apparent association constants of nucleotides and phosphate. Nucleotides bind better to arginine than to lysine and guanosine-5'-monophosphate better than other nucleotides. These results are therefore comparable to those obtained in solution when nucleotides bind to poly-L-lysine or poly-L-arginine.[56] The highest selectivity coefficients were obtained with aromatic amino acids and evidence for stacking interactions were also obtained by the same authors from a nuclear magnetic resonance study of the binding of amino acid esters to polynucleotides.[57]

The results obtained by photochemically induced dynamic nuclear polarization (photo-CINDP) on the complexes formed by Lys-Tyr-Lys and poly(A) are also in agreement with a stacking of the tyrosyl ring with adenine bases.[58] The upfield shifts of the tyrosyl protons are retained in the emission signal observed for the 3,5 ring protons in the photo-CINDP experiments. The intensity of this emission signal is reduced by about 30% as compared to the free peptide, which might be explained by a fast exchange between bound and free peptide molecules if only the tyrosyl residue of the free peptide is polarizable.

B. Fluorescence Investigations

The effect of stacking interactions between aromatic amino acids and nucleic acid bases on the fluorescence of these aromatic molecules was first investigated in frozen aqueous solutions.[37,38,59] When dilute aqueous solutions are frozen down to 77 K, solute molecules do not remain isolated but form aggregates in cavities of the ice microcrystalline structure. This property was first used in the field of nucleic acids to produce thymine dimers in a very efficient way upon UV irradiation of frozen thymine solutions.[60] Thymine in fluid medium is very resistant to UV irradiations whereas stacked thymines in the aggregates formed in ice dimerize very readily. Although nucleic acid bases are only very weakly fluorescent in fluid medium ($\phi_F \sim 10^{-4} - 10^{-5}$), they exhibit much higher fluorescence quantum yields in the frozen state.[61] Aromatic amino acids emit fluorescence of high quantum yield both in fluid and in frozen aqueous solutions.[62] When an equimolar mixture of tryptophan and a nucleic acid base (or nucleoside) is frozen down to 77 K, the fluorescence of both molecules is completely quenched and a new fluorescence emission of much lower quantum yield appears at longer wavelengths. The wavelength of the new fluorescence maximum increases in the order Guo < Ado < Cyt, < Urd, Thd. These phenomena have been interpreted as resulting from the formation of charge-transfer stacked complexes between tryptophan

and nucleic acid bases. Even though charge-transfer contribution to the ground state stability of the complexes is certainly weak, as shown by reflectance studies of the frozen solutions,[38] its importance in the excited state is considerably increased. This behavior is characteristic of "exciplexes' although this term was coined to designate complexes which form in the excited state in the absence of any interaction in the ground state. In the aggregates formed in frozen aqueous solutions, the ground state interaction is responsible for the appearance of a new absorption on the long wavelength side of the absorption bands of the two interacting molecules,[38] although this effect is of much smaller amplitude as compared to the shift of the fluorescence spectrum.

The fluorescence of tyrosine is quenched by nucleic acid bases in the aggregates formed in frozen aqueous solutions. The fluorescence of pyrimidines is quenched by tyrosine whereas that of purines is not greatly affected.[59] The quenching in the former case is therefore very likely due to the formation of an electron donor-acceptor complex as described above for tryptophan. Quenching by purines is more likely due to energy transfer from tyrosine to the base (see below).

When oligopeptides containing an aromatic amino acid bind to a polynucleotide or a nucleic acid (RNA or DNA), a quenching of the oligopeptide fluorescence is observed.[41] It should be noted that the fluorescence quantum yield of the nucleic acids is very weak (three to four orders of magnitude smaller) as compared to that of aromatic amino acids.[61,62] Therefore the fluorescence emission from the nucleic acid can be neglected except if it contains a modified base of high fluorescence quantum yield (N^6-ethenoadenine, for example). Nevertheless, the nucleic acid interferes with fluorescence intensity measurements of the oligopeptide complex due to its absorption at the wavelength where this fluorescence is excited. Correction for this so-called "screening" or "inner-filter" effect can be made in different ways. An empirical correction curve can be constructed by recording the fluorescence intensity of a noninteracting indole derivative (such as *N*-acetyltryptophanamide) as a function of the nucleic acid concentration under the same experimental conditions as used to investigate oligopeptide-nucleic acid complexes.[48] A correction curve can also be calculated which depends on the absorbance of the nucleic acid and on geometrical parameters of the spectrofluorimeter used in these investigations.[63] In most of our own studies we found it more convenient to measure the screening effect after dissociation of the oligopeptide-nucleic acid complexes at high ionic strength. This was made possible by the fact that the main contribution to the binding of oligopeptides such as Lys-X-Lys is due to electrostatic interaction with the phosphate groups. Therefore most of the complexes are completely dissociated above 0.1 *M* NaCl. Under these conditions the fluorescence intensity is reduced as compared to that of the oligopeptide alone as a result of the screening effect of the nucleic acid which can thus be determined.[48] (See Figure 4.)

A fluorescence investigation of the binding of Trp-containing peptides to DNA was also made by Szekerke and Erchegyi.[64] The peptides were Ser-Trp(OMe), Lys-Trp(OMe), Orn-Trp(OMe), and Glu-Trp(OMe). However there is no indication in this report of correction for the screening effect of DNA ($2 \times 10^{-5} - 8 \times 10^{-4}$ *M*), which might explain the apparent high association constants found by these authors even at high ionic strength.

In order to analyze fluorescence quenching data in terms of association constants and binding sites, one has to know how many different types of complexes are formed as well as their fluorescence characteristics. The determination of fluorescence lifetimes can help to obtain some information on these questions. For example it was shown that the fluorescence decay upon flash excitation of Lys-Trp-Lys was not changed upon binding to poly(A) and DNA even under conditions where the fluorescence inten-

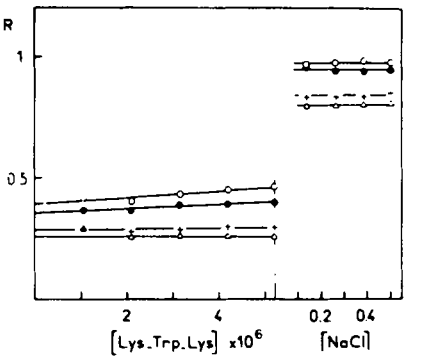
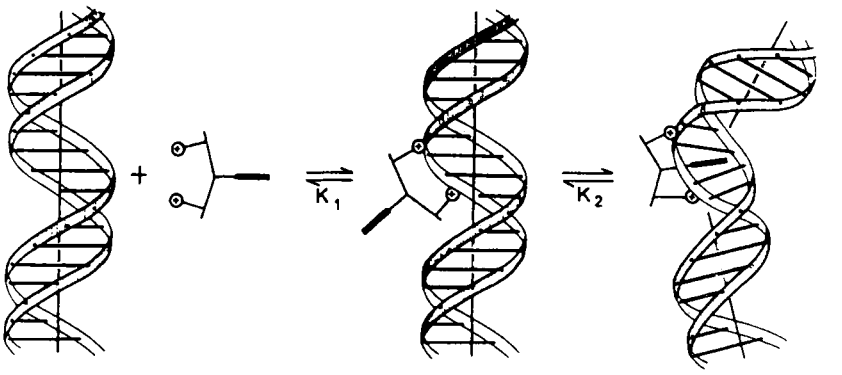


FIGURE 4. Concentration dependence of uncorrected fluorescence intensity of the peptide Lys-Trp-Lys in the presence of poly(A) at different concentrations: $5 \times 10^{-4} M$ (Δ), $2.9 \times 10^{-4} M$ (+), $1.4 \times 10^{-4} M$ (•), and $8.6 \times 10^{-5} M$ (O). R is the ratio of fluorescence intensities in the presence and the absence of poly(A). Sodium chloride was added to dissociate the complex and determine the screening effect of poly(A). (Reprinted with permission from Brun, F., Toulmé, J. J., and Hélène, C., *Biochemistry*, 14, 558, 1975. Copyright by the American Chemical Society.)



SCHEME 1. Schematic drawing of the binding of a peptide bearing one tryptophyl and two lysyl residues (Lys-Trp-Lys) to DNA, showing the two types of complexes with a bending of DNA induced by stacking.

sity was reduced by more than 50% in the presence of poly(A) or denaturated DNA.[48] This result indicated that two *classes* of complexes *at most* could exist: those which have a fluorescence quantum yield and a lifetime identical to those of the free peptide and those whose fluorescence quantum yield is reduced to zero (and which do not appear, of course, in the fluorescence decay). The simplest scheme which could account for both the fluorescence intensity and lifetime measurements is therefore the following

$$\text{peptide} + \text{nucleic acid} \rightleftharpoons \text{complex} \qquad (3)$$

in which the fluorescence quantum yield of the complex is reduced to zero. However this scheme was not able to fit either the experimental fluorescence quenching data nor the circular dichroism results (see below). This could be achieved only by assuming the formation of two types of complexes (I and II) with respective fluorescence quantum yields $\phi_I = \phi_F$ and $\phi_{II} = 0$ where ϕ_F is the fluorescence quantum yield of the free peptide (see Scheme 1).

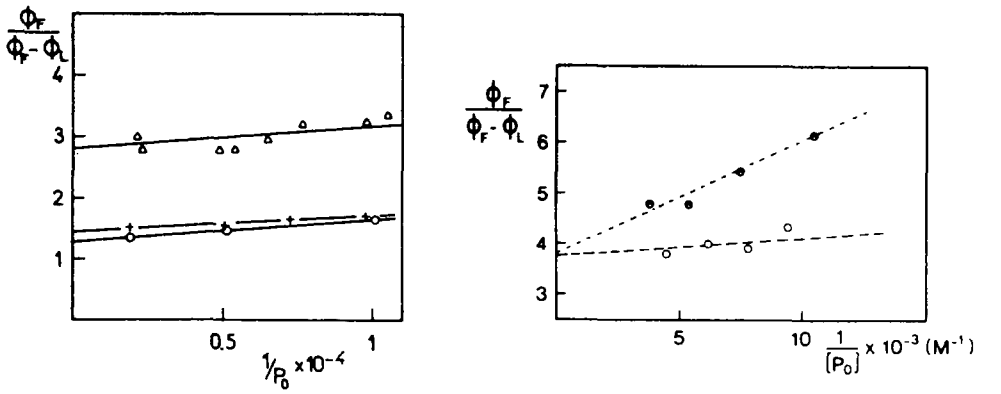


FIGURE 5. Analysis of fluorescence data for the binding of Lys-Trp-Lys to different nucleic acids according to Equation 5. (A) poly(A) (+), poly(U) (O) and the complex poly(A)·poly(U) (Δ). (Reprinted with permission from Brun, F., Toulmé, J. J., and Hélène, C., *Biochemistry*, 14, 558, 1975. Copyright by the American Chemical Society.) (B) *E. coli* DNA at two NaCl concentrations, 1 m*M* (O) and 9 m*M* (Θ). (From Toulmé, J. J. and Hélène, C., *J. Biol. Chem.*, 252, 244, 1977. With permission.)

Table 3
VALUES OF K_1 AND K_2 (EQUATION 4) DETERMINED FROM ANALYSIS OF FLUORESCENCE DATA ACCORDING TO EQUATION 5 FOR THE BINDING OF LYS-TRP-LYS TO POLYNUCLEOTIDES AND DIFFERENT DNAs AT 277 K (1 m*M* NaCl, 1 m*M* NA-CACODYLATE, 0.2 m*M* EDTA)[48,65]

	polyA	polyU	polyA·polyU	polyC
$K_1 \times 10^{-4}$ (M^{-1})	1.9	0.65	4.15	0.51
K_2	2.2	3.8	0.56	1.05

	polyG·polyC	Calf thymus DNA	Denatured calf thymus DNA
$K_1 \times 10^{-4}$ (M^{-1})	1.65	1.75	1.8
K_2	0.48	0.43	5.15

$$\text{peptide} + \text{nucleic acid} \underset{K_1}{\rightleftharpoons} \text{complex I} \underset{K_2}{\rightleftharpoons} \text{complex II} \qquad (4)$$

The equilibrium constants K_1 and K_2 could be determined from the following equation

$$\frac{\phi_F}{\phi_F - \phi_L} = 1 + \frac{1}{K_2} + \frac{1}{K_1 K_2} \frac{1}{P_o} \qquad (5)$$

where ϕ_L is the apparent fluorescence quantum yield obtained at a low level of saturation of the nucleic acid whose concentration is P_o. ϕ_L was determined from an extrapolation to zero peptide concentration of the curve obtained by plotting fluorescence intensity vs. peptide concentration. Figure 5 reports some results obtained with native and denatured DNA and different polynucleotides. Table 3 gives some examples of K_1

and K_2 values.

Several arguments can be put forward to support the two-step model presented above (Equation 4). First, the overall association constant K_1 $(1 + K_2)$ determined from fluorescence data is in very good agreement with that determined from circular dichroism measurements.[48, 21] No agreement was found if only one complex was assumed to exist with a fluorescence quantum yield reduced to zero (Equation 3). The fluorescence data could be accounted for by a one-step model if the fluorescence quantum yield of the complex was not zero. For example, in the case of poly(A) complex with Lys-Trp-Lys this quantum yield (and therefore the corresponding fluorescence lifetime) should be 0.3 that of the free peptide. Under the experimental conditions used in fluorescence decay measurements this should have been easily detected. Moreover, measurements of the electric dichroism of Lys-Trp-Lys-poly(A) complexes suggest that the tryptophyl ring is stacked parallel to adenine bases.[171] If the fluorescence quantum yield of these stacked tryptophans was 0.3 that of the free peptide, one should expect a high polarization of their fluorescence. This is not experimentally observed. The residual fluorescence is only slightly polarized (0.05).[65] These results are more in agreement with the two-step model in which the fluorescence originates from complex I which does not involve stacking interactions. The quantum yield of this complex is the same as that of the free peptide (hence the identical fluorescence decay) and the tryptophyl residue has sufficient mobility to explain the rather low degree of polarization.

Second, the two-step model predicts that K_1 which measures the electrostatic contribution should decrease when the ionic strength increases, whereas K_2 should be independent of ionic strength. This is what has been experimentally observed. The value of K_1 for native DNA or poly(A) can be changed by one or two orders of magnitude without any change in the value of K_2.[48,65] When temperature increases, the value of K_2 slightly increases with both poly(A) and DNA (before melting starts).[48,65] In the case of DNA this behavior is probably related to premelting which enhances the probability of tryptophan stacking with bases. In the case of poly(A) complexes one could have expected that unstacking of the bases upon increasing the temperature should have led to an increase of K_2. However, one should keep in mind that Lys-Trp-Lys binding to poly(A) locally induces a conformational change as indicated by circular dichroism and difference absorption spectroscopy.[21,66] A conformational change is induced by purely electrostatic interactions as observed with Lys-Ala-Lys. Therefore one can imagine that what determines the stacking efficiency of the tryptophyl residue is the local conformation of the complex and not the overall structure of the polynucleotide.

Third, there is a very efficient energy transfer process at the triplet level between adenine bases and tryptophan in stacked complexes. This was first demonstrated in mixed aggregate formed in frozen aqueous solutions.[37] Subsequently the same phenomenon was shown to occur in poly(A)-Lys-Trp-Lys complexes.[67] The energy transfer process between stacked adenine bases in poly(A) is very efficient at the triplet level. Around 100 bases are involved in this process.[67] A tryptophan residue inserted between these bases is expected to act as an energy trap because its triplet level is lower in energy as compared to poly(A). This is what was experimentally observed with Lys-Trp-Lys-poly(A) complexes, one Lys-Trp-Lys molecule being able to trap the excitation energy of a collection of about 60 adenine bases. Triplet energy transfer occurs by an electron exchange mechanism and requires a good orbital overlap of the electron clouds of donor and acceptor molecules. This is clearly favored in a stacked complex between adenine and tryptophan and would not occur if the two aromatic molecules were lying side by side.

The phosphorescence spectrum of tryptophan inserted between bases is red-shifted as compared to free tryptophan. The effect of stacking on the triplet state properties

of the indole ring is also reflected in a modification of its electron spin resonance spectrum.[67, 68]

Finally, a recent spectroscopic method has been developed to provide evidence for stacking interactions in oligopeptide-nucleic acid complexes. It makes use of the well-known external heavy atom effect on the spectroscopic properties of a neighboring aromatic molecule.[69] Using 5-mercuripyrimidines it was possible to demonstrate the heavy atom effect of mercury on a stacked indole ring either in mixed aggregates in frozen aqueous solutions[70] or in Lys-Trp-Lys complexes with poly 5-mercuriuridylic acid.[71] This effect results from the perturbation of spin orbit coupling. It is characterized by a complete quenching of tryptophan fluorescence, an enhancement of its phosphorescence, and a drastic shortening of the phosphorescence lifetime (three orders of magnitude).

It is possible to introduce mercuripyrimidines in both DNA or RNA using DNA or RNA polymerases.[72,73] In order to extrapolate this spectroscopic probe to protein-nucleic acid complexes, one must avoid the reaction of mercury atoms with the −SH groups of the protein. This can be achieved by chemical modification of cysteinyl residues or by reaction of the mercuripyrimidines with mercaptans.

Because the heavy atom effect requires a van der Waals contact between the heavy atom and the perturbed molecule, this "spin-orbit probe" should give information on the distance between a tryptophan residue and a mercury-substituted base. Taken together with other methods (NMR or fluorescence quenching in the absence of heavy atom) it should provide a strong basis for conclusions regarding the existence of stacking interactions involving tryptophan in protein-nucleic acid complexes.

C. Conformational Stability

The value of K_2 for denatured DNA ($\sim$5) is much higher than that for native DNA (0.3) (see Equation 4 and Table 3). However, the overall association constant for peptide binding is K_1 $(1 + K_2)$. The value of K_1 which reflects the electrostatic contribution is expected to be higher for double-stranded DNA whose electrostatic potential is higher than that of a single strand. The value of K_2 for native DNA increases when temperature increases even before melting starts to be detected by absorption spectroscopy. This probably reflects the premelting of DNA[74] which enhances the probability for an aromatic amino acid to stack with the nucleic acid bases. Of course in the temperature range where melting takes place, the value of K_2 strongly increases. Nevertheless this increase of K_2 for denatured DNA is not sufficient to overcome the lower value of K_1 as compared to the double helix. Consequently, the melting temperature of DNA increases in the presence of Lys-Trp-Lys and Lys-Tyr-Lys as a result of a higher overall association constant with the double helix. However this T_m increase is slightly smaller than for Lys-Ala-Lys which exhibits only electrostatic interactions.[169] An increase of the melting temperature of DNA upon binding of oligopeptides containing aromatic amino acids has been reported by several authors.[4,6,75-77] In the series Tyr-$(Gly)_{1-4}$-Tyr, maximal thermal stabilization of calf thymus DNA was observed with the peptide Tyr-$(Gly)_2$-Tyr.[77] The alternating peptides X-Gly-X-Gly-X where X = Trp,Tyr,His were found to be equally effective (although less than Tyr-$(Gly)_2$-Tyr) in stabilizing DNA. Surprisingly the alternating peptide with X = Phe afforded only little stabilization.

Gabbay et al.[6] have also shown that oligopeptides containing aromatic residues increased the Tm of the helix-coil transition of salmon sperm DNA. Dipeptide amides with the aromatic residue on the C-terminal end stabilized the DNA double helix to a greater extent than did the corresponding N-terminal isomers. The stabilization afforded by tyrosine-containing peptides was higher than that due to phenylalanine-containing peptides of similar structures.

In many cases measurements have been made with only one ratio of peptide-to-DNA concentrations. When comparing different oligopeptides it might be difficult to draw conclusions from these studies because the effect on DNA helix-coil transition depends not only on the relative binding of the oligopeptide to the helix and the coil forms in the temperature range where melting takes place, but also on the amount of peptide effectively bound to DNA. The intrinsic affinities may vary from peptide to peptide, especially for peptides which contain a different number of basic residues. The results reported by Brown[4] showed for example that the binding of Arg-Trp to DNA was weaker than that of Arg-Arg but the maximum rise in melting temperature ΔTm was higher. Szekerke et al.[75] reported that the dipeptide Lys-L Tyr(OMe) stabilized DNA more than the corresponding diastereoisomer Lys-D Tyr(OMe). Lysine-containing peptides had a higher stabilizing effect than ornithine-containing peptides.

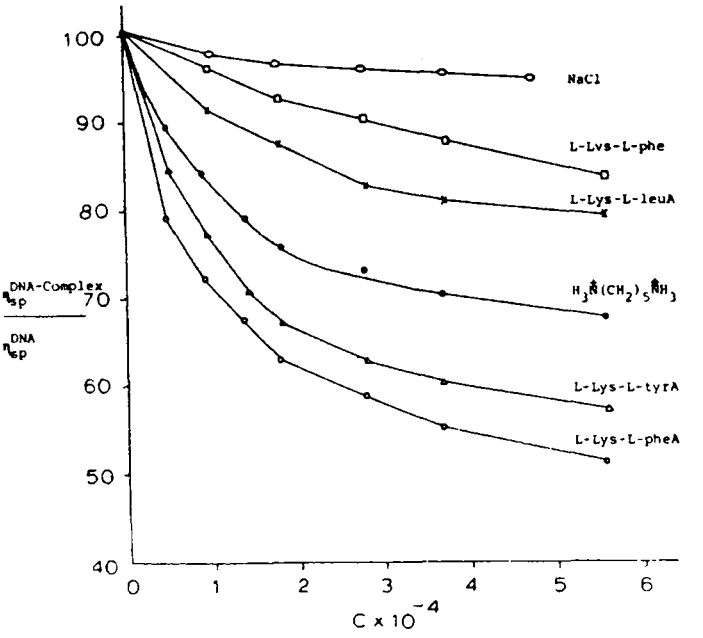
The binding to DNA of sequential and random lysine copolymers containing aromatic residues has also been studied.[17] The presence of aromatic residues reduces the Tm of the complexes as compared to poly-lysine The thermal stability of DNA decreases in the order $(Lys)_n > (Lys, Tyr)_n > (Lys, Phe)_n > (Lys, Tyr(OMe))_n$. The results obtained with copolymers are however difficult to rationalize in view of the possible nonrandom distribution of amino acid residues. The stoichiometry data show that DNA phosphates are completely neutralized by the lysyl side chains independently of the composition and sequence of the copolypeptides. The effect of aromatic residues has been ascribed to the preferential stacking within single-stranded structures.

D. Conformational Changes Induced in Nucleic Acids

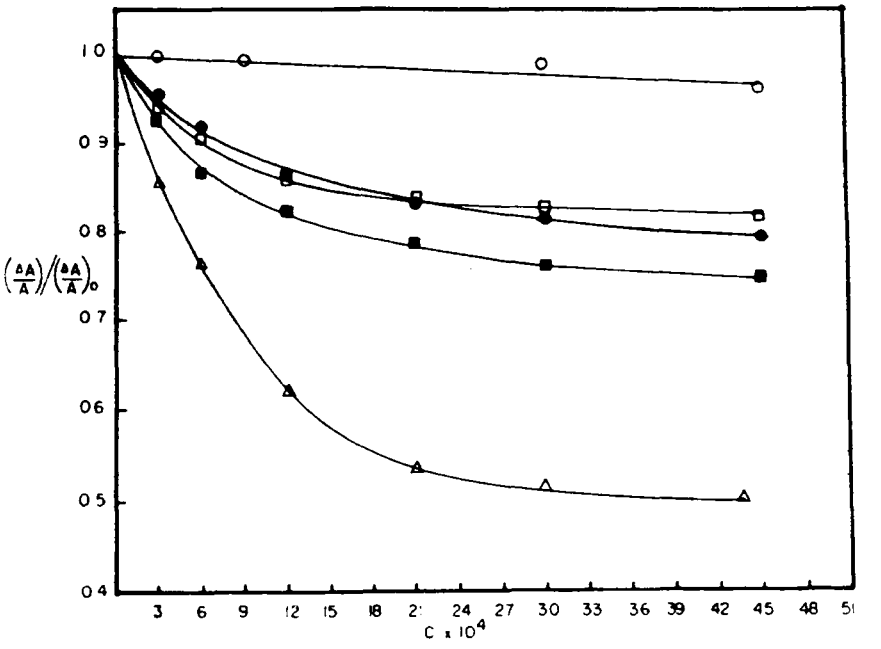
Jacobsen and Wang[78] have reported that they could not observe any unwinding of DNA upon binding of tryptamine, tyramine phenethylamine, and histamine. These aromatic amines did not significantly change the sedimentation coefficient of covalently closed λ DNA containing approximately 15 negative superhelical turns. No change was observed either in the sedimentation behavior of λ DNA which had been covalently closed by ligase in the presence of tryptamine. However, using sucrose gradients might weaken the binding of amines in the first type of experiments while the ionic conditions used in the second type of studies (10 m*M* Tris-HCl, 2 m*M* $MgCl_2$, 1 m*M* Na_3EDTA) probably dissociate any tryptamine-DNA complexes. There is no report of the effect of oligopeptides on the sedimentation behavior of circular DNA. Using peptides of sequence Lys-X-Lys would be of interest although ionic interactions due to the lysyl residues would probably play an important role.

The viscosity of DNA solutions decreases upon binding of oligopeptides containing aromatic amino acids (Figure 6). This behavior is in contrast to that observed when planar aromatic dyes such as proflavine or ethidium bromide intercalate between base pairs of DNA. This led Hélène, Gabbay, and co-workers to propose that aromatic amino acids were only partially inserted leading to a binding of the DNA double helix.[40,42,48,79,80] Such a conclusion can also be reached from a consideration of the respective sizes of a nucleic acid base and an aromatic amino acid.[40]

The reduced linear dichroism ΔA/A of DNA, measured by flow dichroism experiments, decreases upon binding of oligopeptides containing aromatic amino acids, lending some support in favor of the partial insertion model (Figure 6).[79,80] That oligopeptides containing aromatic residues induce conformational changes in DNA and other nucleic acids is clearly demonstrated by circular dichroism studies. In the wavelength range 250 to 300 nm, peptides containing aromatic residues exhibit only a very weak CD signal which can be neglected when compared to that of the nucleic acid. Therefore this wavelength range can be used to obtain information on the conformation of the nucleic acid during the binding process. At wavelengths shorter than 250 nm both the



A



B

FIGURE 6(A). The effect of increasing concentrations of peptide, diamine, or NaCl on the specific viscosity, η_{sp}, of DNA ($6.0 \times 10^{-4} M$). (Reprinted with permission from Gabbay, E. J., Stanford, K., and Baxter, C. S., *Biochemistry*, 11, 3429, 1972. Copyright by the American Chemical Society.) (B) The effect of increasing concentrations of NaCl (○), 1,5-diaminopentane 2 HCl (□), L-Lys-L-Leu NH_2 (○), L-Lys-D-Phe-NH_2 (□), and L-Lys-L-Phe-NH_2 (△) on the relative reduced dichroism of DNA at 260 nm (△ A/A) where △ A = A // − A ⊥. Reprinted with permission from Gabbay, E. J., Adawadkar, P. D., and Wilson, W. D., *Biochemistry*, 15, 1·6, 1976. Copyright by the American Chemical Society.)

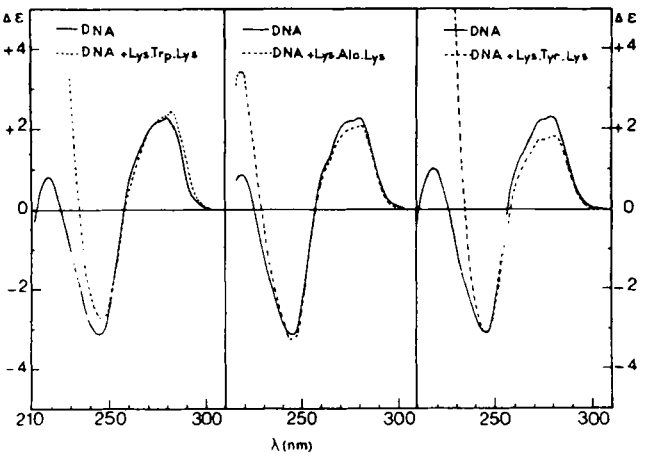


FIGURE 7. Circular dichroism spectra of calf thymus DNA ($1.3 \times 10^{-3} M$) alone (—-) and with an equimolar concentration of peptide (as indicated on the figure).

peptide and the nucleic acid contribute to the CD signal which makes it more difficult to extract useful data.

Figure 7 shows the CD spectrum of DNA and its equimolar mixture with Lys-Trp-Lys, Lys-Ala-Lys, and Lys-Tyr-Lys. All these peptides lead to conformational changes of DNA. As mentioned in a previous section, the peptide Lys-Ala-Lys leads to a conformational change similar to that observed with Lys(OMe) or $NH_3^+(CH_2)NH_3^+$. This has been attributed to the binding of the α- and ε-amino groups of the N-terminal lysyl residue with two adjacent phosphates of the DNA molecule. To this conformational change is superimposed a second one due to the presence of the aromatic group. It corresponds to an increase of the intensity of the positive band of the CD spectrum for Lys-Trp-Lys and to a decrease of this band for Lys-Tyr-Lys. Similar results were obtained in a study of the binding of simpler derivatives of aromatic amino acids: tyramine and tryptamine. Tryptamine binding to DNA induced an increase of the CD signal of DNA larger than that produced by Lys-Trp-Lys, probably as a result of the "negative" contribution of the N-terminal lysyl residue to the conformational change of DNA. These results, as well as those of NMR experiments, clearly demonstrate that the binding of the peptide containing tryptophan is different from that of the peptide containing tyrosine since different conformational changes are induced in the nucleic acid.[46]

The CD spectrum of poly(A) is well understood[81, 82] and this makes it easier to study its interactions with the oligopeptides from both a qualitative and a quantitative point of view. It was shown by Durand et al.[21] that the binding of the peptides Lys-Trp-Lys, Lys-Phe-Lys, and Lys-Tyr-Lys led to a large decrease of the intensities of the positive and negative bands of the CD spectrum of poly(A) without alteration of its overall shape. As previously mentioned, the peptide Lys-Ala-Lys induced a decrease of the intensity of the positive band one order of magnitude smaller than that due to peptides containing an aromatic residue. A very small increase of the negative band was observed. The change of CD spectrum induced by the binding of aromatic peptides was attributed to an unstacking of the polynucleotide bases similar to that observed during the melting process of poly(A) alone. Thus CD experiments are in agreement with

NMR results[44,45] in demonstrating that the stacking of the aromatic amino acid with adenines in poly(A) requires that the bases are first unstacked.

The variation of the CD signal as a function of peptide concentration at various concentrations of poly(A) was used to analyze quantitatively the binding process.[21,83] The binding constants obtained by this method were in agreement with those obtained by fluorescence measurements only if a two-step model was assumed as described above (Equation 4).

The quantitative analysis of the CD results showed that the relative decrease of the CD signal was linearly related to the degree of saturation of poly(A), r, by the following relationship

$$\frac{\Delta\epsilon}{\Delta\epsilon_o} = 1 - \alpha r \qquad (6)$$

The value of the constant α can be thought of as representing the average number of adenine bases whose contribution to the CD spectrum of poly(A) has been eliminated per bound peptide. It was found that the value of α depended markedly on the nature of the aromatic amino acid, as shown below.

Peptide	α
Lys-Trp-Lys	1.47
Lys-Tyr-Lys	1.0
Lys-Phe-Lys	0.73

With another single-stranded polynucleotide, poly(C), almost no change was observed in the CD spectrum in the presence of aromatic oligopeptides. This could be due to an absence of binding to this polynucleotide or to a binding without major effect on the conformation. In fact, fluorescence experiments[48] demonstrated that the peptide Lys-Trp-Lys did bind to poly(C), even though the value of K_2 (Equation 4) was much smaller than that of poly(U) or poly(A). Using a series of random copolynucleotides of adenine and cytosine it was demonstrated that the value of this constant drastically decreased when the content of cytosine increased in the copolymer (see Figure 10).[84] Thus in the case of poly(C), the stacking interaction between the aromatic amino acid and the base does not seem to require a drastic change of the polynucleotide conformation. The geometry which has been proposed for single stranded poly(A)[85] is quite different from that of poly(C).[86] This may be the main reason explaining the difference of behavior of these polynucleotides with respect to the aromatic peptides.

E. Thermodynamic and Kinetic Studies

There are very few quantitative determinations of thermodynamic parameters for the association of oligopeptides containing aromatic amino acids with nucleic acids. From the temperature dependence of the association constant for the binding of Lys-Trp-Lys to poly(A), Brun et al.[48] estimated a ΔH of ~ -29 kJ mol^{-1}. Calorimetric studies of the binding of 5-methoxytryptamine to DNA and poly(A) led to the thermodynamic parameters reported in Table 4.[87]

Calorimetric data for the binding of Lys-X-Lys to DNA have been published.[88] The ΔH values are all close to zero. This is not unexpected since the most important interactions are electrostatic in nature. There is no reason *a priori* to expect a ΔH value comparable to that obtained with intercalating dyes. The tryptophyl ring has the same size as a purine, and tyrosine that of a pyrimidine. Therefore it is very unlikely that

Table 4
THERMODYNAMIC PARAMETERS FOR THE BINDING OF 5-METHOXYTRYPTAMIN TO DNA AND POLY(A)[87]

	T(°C)	$K \times 10^{-4}$ (M^{-1})	ΔH(kJ mol^{-1})	ΔS(J mol^{-1} K^{-1})
DNA	25	2	−10.25	−46
	40	1.6	−10.45	−46
poly(A)	25	0.5	−8.35	−92

stacking of these aromatic residues with bases requires an unstacking of two base pairs together with an unwinding of the double helix. It is more likely that stacking interactions involve only one strand of the double helix with a possible bending of DNA at the site of stacking (the so-called "partial insertion" model).[79, 80] In the two-step model presented above (Equation 4), the association constants for the stacked and unstacked complexes are K_1K_2 and K_1, respectively. For native DNA ($K_2 \sim 0.3$), the gain in free energy which can be attributed to stacking is therefore of the order of −0.7 kJ mol^{-1}. In the case of denatured DNA ($K_2 \sim 5$), the gain in free energy which can be attributed to the involvement of stacking is about −4.5 kJ mol^{-1}.

In all cases it should be kept in mind that stacking of an aromatic amino acid with nucleic acid bases requires that the two bases involved in the process first unstack. Therefore the energy gained from amino acid-base stacking could be partially compensated by the energy lost as a result of base-base unstacking. As a matter of fact the stacking efficiency (K_2 value) of Lys-Trp-Lys decreases in the order poly(U) > poly(A) > poly(C) which follows the order of increasing stacking energy of the bases.

Field-jump techniques have been used to obtain information on the kinetics of oligopeptide binding to polynucleotides.[22] If the association involves an electrostatic contribution, then electric field pulses will induce a dissociation of the complexes. When the electric field is switched off, reassociation occurs and the process can be analyzed to obtain association and dissociation rate constants (at zero field strength). Some typical values are given in Table 5 for the binding of different oligopeptides to oligonucleotides and polynucleotides. It should be noted that binding to oligonucleotides can usually be analyzed as a one-step process even when the oligopeptide contains an aromatic residue.[89] A two-step model has to be postulated for a few complexes. For example, in the case of $(A)_6 + (Lys)_3$, the bimolecular reaction between the two species is followed by a conformational change in the complex (attributed to some stacking rearrangement in the oligoadenylate). A one-step mechanism can account for the kinetic data when *basic* oligopeptides such as $(Lys)_3$ or $(Arg)_3$ bind to *poly*nucleotides at low concentration of ligands. A second step is observed at higher concentrations. When Lys-Trp-Lys binds to poly(A), a two-step model (analogous to Equation 4) has to be postulated to account for the relaxation process which cannot be fitted by a single exponential. The main difference in the binding of simple basic peptides and, e.g., Lys-Trp-Lys is the fact that the second step observed for Lys-Trp-Lys binding is slower than that expected for a "simple" stacking rearrangement, whereas the second step observed in the case of simple basic peptides is relatively fast and consistent with a "simple" stacking rearrangement. There is still some discrepancy about the K_2 value obtained from relaxation measurements[22, 170] and from fluorescence quenching.[48] Although there is no explanation for this discrepancy yet, it should be noted that the relaxation data are in agreement with a two-step model and not with two independent equilibria leading to the two different complexes I and II.

Table 5
ASSOCIATION AND RATE CONSTANTS FOR THE BINDING OF OLIGOPEPTIDES TO OLIGO- AND POLYNUCLEOTIDES. DATA FROM PÖRSCHKE[22,89] USING THE FIELD-JUMP TECHNIQUE

		$K(M^{-1})$	$k_a(M^{-1}s^{-1})$	$k_d(s^{-1})$
Arg_3	$(I)_6$	4.9×10^4	2.5×10^{10}	5.4×10^5
	polyI	1.0×10^7	4.1×10^{11}	
	$(U)_6$	1.5×10^4	2.0×10^{10}	1.5×10^6
	polyU	6.5×10^5	2.8×10^{11}	
	$(A)_6$	3.8×10^4	1.3×10^{10}	3.5×10^5
	polyA	5.4×10^6	4.4×10^{11}	
Lys_3	$(I)_6$	1.2×10^4	1.5×10^{10}	1.4×10^6
	polyI	6.4×10^5	3.4×10^{11}	
	$(U)_6$	8.9×10^3	2.0×10^{10}	2.3×10^6
	polyU	6.4×10^5	2.6×10^{11}	
	$(A)_6$	1.7×10^4	1.1×10^{10}	7.5×10^5
	polyA	4.1×10^6	2.6×10^{11}	
Lys-Tyr-Lys	$(A)_6$	2.9×10^3	5.6×10^9	1.9×10^6
	$(U)_6$	2.6×10^3	7.8×10^9	3.0×10^6
Lys-Phe-Lys	$(A)_6$	2.4×10^3	7.0×10^9	1.9×10^6
	$(U)_6$	1.8×10^3	6.2×10^9	4.0×10^6
Lys-Gly-Lys	$(A)_6$	1.8×10^3	4.6×10^9	2.3×10^6
	$(U)_6$	1.1×10^3	—	—

Note: K was obtained from an analysis of the field-jump amplitudes; k_a and k_d were determined from the relaxation process; $K = k_a/k_d$. Measurements were carried out at 20°C in the following buffers: for oligonucleotides, 1 m*M* sodium cacodylate, pH 5.9, 50 μM EDTA; for polynucleotides, 1 m*M* Tris, pH 8.0, 50 μM EDTA. In the case of $(A)_6$ + $(Lys)_3$ or $(Arg)_3$ a simple bimolecular reaction between the oligonucleotide and the oligopeptide does not fit the relaxation data. A second step (conformational change in the complex) has to be postulated. In the case of polynucleotides the rate constants reported in this table have been calculated according to a sphere model (see Reference 22).

IV. HYDROGEN BONDING

Hydrogen bonding between functional groups of nucleic acids and proteins is certainly involved in a large number of biological complexes. Hydrogen bonding of the nucleic acid may involve the bases, the carbohydrates (ribose or deoxyribose), and the phosphate groups with nucleic acid bases certainly representing the most specific sites by which specificity may be achieved. Each nucleic acid base possesses several chemical groups which may act as donor or as acceptor sites for hydrogen bonding. When the nucleic acid is double stranded, some of these groups are involved in maintaining the bihelical structure but others are still available (see Figure 8).[90] On the other hand, a large number of amino acid side chains possess chemical groups which may form hydrogen bonds: the hydroxyl group of serine, threonine and tyrosine; the carboxylic and carboxylate group of glutamic and aspartic acids; the amino group of lysine; the guanidinium group of arginine, the imidazole ring of histidine, sulfur-containing groups of cysteine, cystine, and methionine; and the amide group of asparagine and glutamine. To this list it is necessary to add the peptide bond which may act either as donor or as acceptor of the hydrogen bond.

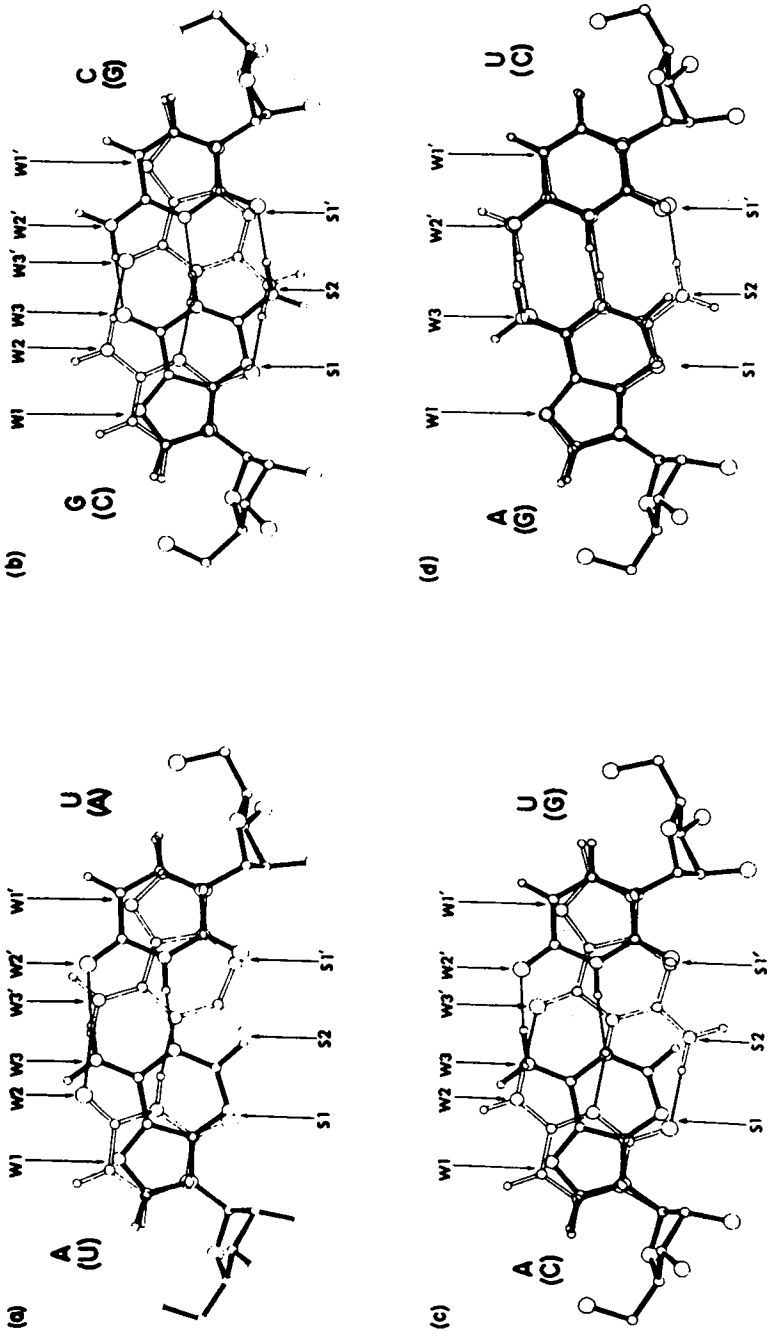
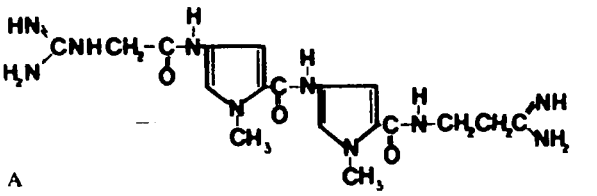


FIGURE 8. Diagram showing the stereochemistry of double helical A-U and G-C base pairs. The base pairs are superimposed upon each other with one base pair drawn with solid bonds and the other with outlined bonds, (a) through (d) representing all of the possible base pair comparisons. W refers to a potential recognition site in the major groove, while S refers to a potential site in the minor groove of the double helix. (From Seeman, N. C., Rosenberg, J. M., and Rich, A., *Proc. Natl. Acad. Sci. U.S.A.*, 73, 804, 1976. With permission.)



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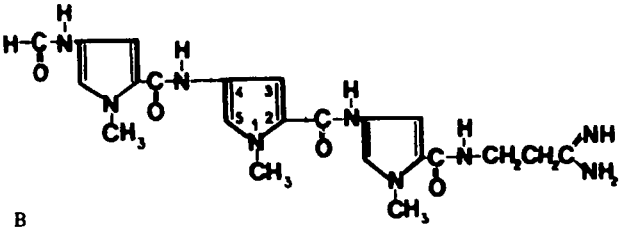

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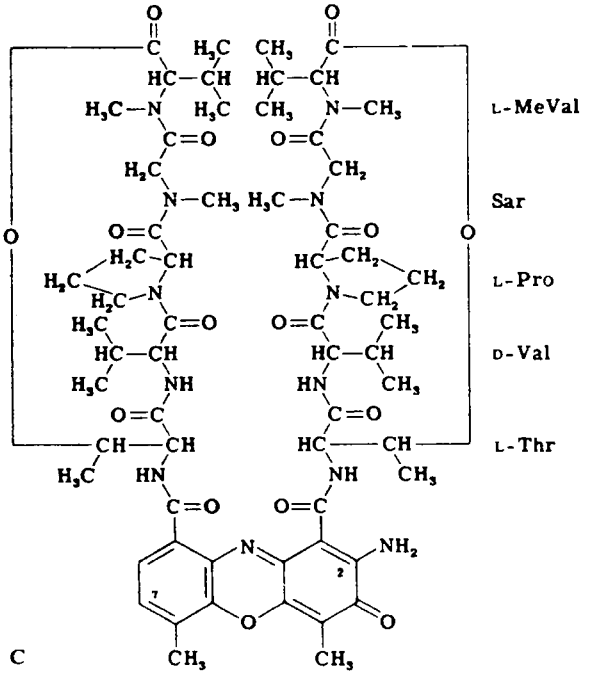

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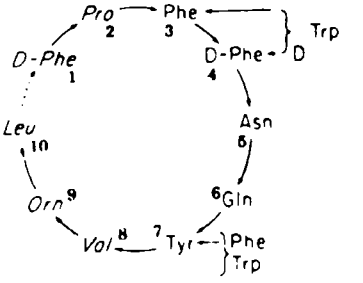

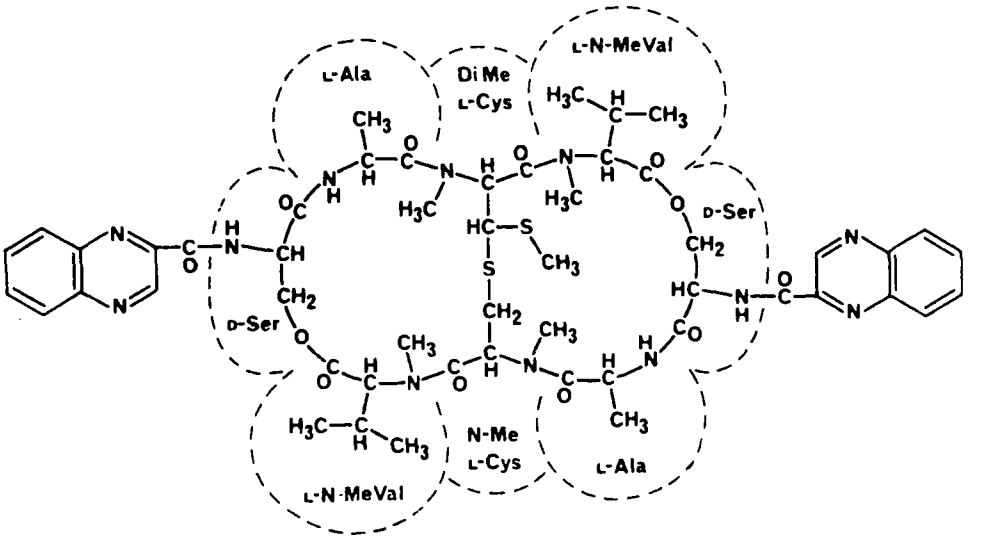

ECHINOMYCIN

FIGURE 9D

FIGURE 9. Chemical structure of (A) netropsin, (B) distamycin A, (C) actinomycin, (D) tyrocidine, and (E) echinomycin.

peptides (Figure 9). Its binding to DNA has been investigated by several methods. It shows a strong specificity for G-containing nucleic acids.

Based on the geometry of an actinomycin-deoxyguanosine complex determined by X-ray crystallography, Sobell et al.[92,94] have proposed a model for the binding of actinomycin to DNA. The main features of this model are as follows:

1. The phenoxazone ring is intercalated between the base pairs of DNA.
2. The binding of actinomycin induces a conformational change of the DNA, including an unwinding of the helix, a tilt of some bases, and a change in the puckering of the deoxyribose from 2′ endo-3′ exo to 3′ endo-2′ exo.
3. The peptide portion of the antibiotic lies in the minor groove of DNA where several hydrogen bonds are formed with guanine residues.
4. In addition, several van der Waals contacts exist between hydrophobic groups of the peptidic part of the molecule and of DNA, providing additional stabilization to the interaction.

The two cyclic peptide chains are related to one another by a twofold symmetry. This model predicts a base sequence binding preference of the type dG-dC/dC-dG which has been verified in solution using model dinucleoside phosphates and synthetic polymers.[111,112]

C. Tyrocidine

Tyrocidines belong to a family of cyclic decapeptides which inhibit transcription both in vivo and in vitro. It has been suggested that tyrocidine acts in vivo by inhibiting the initiation process[113] and it has been proposed that in the sporulating bacterium *Bacillus brevis*, where it is found, it may act as a nonspecific repressor turning off transcription during sporulation.

VI. SPECIFICITY OF PEPTIDE INTERACTIONS WITH NUCLEIC ACIDS

Evidence has been provided in the preceding sections for different types of interactions between functional groups of peptides and nucleic acids. It is of general interest to determine whether these interactions present some specificity with respect to the structure or the base sequence of the nucleic acid. This problem is crucial to the understanding of the properties of natural peptides whose target is a nucleic acid inside the cell. The specificity of peptide interactions with nucleic acids might also improve our knowledge of the origin(s) of the selectivity of recognition which characterizes many associations of proteins with nucleic acids in vivo.

A. Electrostatic Interactions

Do electrostatic interactions between basic residues of proteins and phosphate groups of nucleic acids participate in the selectivity of recognition between these polymers? An *a priori* answer would be "no" since a purely electrostatic interaction between a positive and a negative charge should not depend on the nature of the base. In fact the problem is more complicated, especially with peptides containing more than one basic residue, since geometrical factors may be involved at the level of both the peptide and the nucleic acid. Electrostatic interactions between phosphate and the positively charged side chain of arginine involve the formation of two hydrogen bonds and are expected to be very directional. Lysine and protonated histidine also form hydrogen bonds with phosphate groups. The base composition of the nucleic acid and the sequence of the peptide might therefore influence the binding.

No specificity has been reported for the binding of basic peptides to DNA of various base compositions. Using a dialysis method, Shapiro et al.[125] performed direct competition experiments to study the binding of tetralysine to DNAs which differed by 34% in AT contents. No measurable preference for one of the DNAs was found. This result is in contrast to what has been demonstrated by several authors at the polypeptide level. Leng et al.[126] have shown that under certain conditions polylysine preferentially and stochiometrically precipitates AT-rich DNAs. Polyarginine under the same conditions shows a marked preference for GC-rich DNAs. More recently, similar conclusions were obtained by Wehling et al.[127] by an affinity chromatography technique using poly-lysine and poly-arginine bound to agarose. Two DNAs with different base compositions but equal molecular weight were loaded and detached by a NaCl gradient. The difference in NaCl concentrations required for the elution of the two DNAs confirms the AT and GC specificity of poly-lysine and poly-arginine, respectively. Introduction of neutral residues in these polypeptides reduces the specificity.

Using ^{14}C labeled oligopeptides Standke and Brunnert[128] performed equilibrium dialysis experiments with several basic peptides. The binding constants were determined using the Scatchard representation. The peptides had sequences of the following types: Lys-X-Lys, Lys-X-X-Lys, and Lys-X-X-X-Lys, with X being an aliphatic or an aromatic residue. The dipeptides Lys-Lys and Arg-Arg were used for comparison. As far as X is an aliphatic residue there is very little, if any, change in the value of the binding constant. This implies that the distance between lysyl residues has no influence on the binding process. The nature of the aliphatic residue between two lysines slightly affects the binding strength which decreases in the order Lys-Gly-Lys > Lys-Ala-Lys > Lys-Leu-Lys.

In the same work it was found that the binding of the dipeptide $(Arg)_2$ was stronger than that of $(Lys)_2$ ($K = 8.9\ mM^{-1}$ and $2.9\ mM^{-1}$, respectively). As observed by several authors, increasing the chain length of the peptides containing only basic residues drastically increased the binding.[28, 5]

In a study of the binding of oligolyines labeled with a dinitrophenyl group on one ε-NH_2, Latt and Sober[28] observed that binding to poly(I)·poly(C) was stronger than to poly(A)·poly(U). Porschke[22] investigated the binding of the basic peptides $(Lys)_2$, $(Arg)_2$, $(Lys)_3$, and $(Arg)_3$ to single-stranded polynucleotides poly(A), poly(U), poly(I), and poly(C). Using the field relaxation technique he found that the binding constant of $(Arg)_3$ for these various polynucleotides was only slightly higher than that of $(Lys)_3$, except in the case of poly(I) where the ratio $K(Arg_3)/K(Lys_3)$ was about five. Also $(Arg)_3$ did not induce helix association of poly(I) whereas $(Lys)_3$ strongly induced the formation of the poly(I) tetramer helix. These results were ascribed to hydrogen bonding of the guanidino group of arginine with inosine. This type of hydrogen bonding scheme had previously been postulated to explain a specific recognition of guanine by arginine side chains.[90, 129, 130]

In a model proposed by Woo et al.[122] on the basis of the X-ray structure of putrescine diphosphate, the lysyl α-amino group could form hydrogen bonds with O(2) and N(3) atoms of two successive A-T bases and with the O(1′) atom of a deoxyribose on the same strand. This model could contribute to explain the selectivity of polylysine binding to A-T-rich DNA.

B. Stacking Interactions

We have shown above that stacking interactions are strongly favored in single-stranded nucleic acids. The value of K_2 (see Equation 4) which is the ratio of the concentrations of stacked and unstacked Lys-Trp-Lys complexes increases from about 0.3 for native DNA to about 5 for denatured DNA. Table 3 gives some examples of K_2 values for different polynucleotides as determined from fluorescence quenching data.

The selectivity of stacking interactions for single-stranded structures is even more striking in the case of tyrosine-containing peptides. Mayer et al.[46] have shown that a series of oligopeptides including Lys-Tyr-Lys and Lys-Ala-Tyr-Ala-Lys do not exhibit any stacking when bound to a double helix whereas they readily form stacked complexes with single-stranded polynucleotides. Tyrosine-containing peptides have not been subjected to the same quantitative analysis as tryptophan-containing peptides due to the fact that tyrosine fluorescence can be quenched by energy transfer to the bases even in the absence of stacking. This makes it more difficult to evaluate the contribution of stacking interactions to the quenching as compared to tryptophan. The values reported for K_2[42] are thus certainly overestimated.

Novak and Donhal have reported that oligopeptides containing glycyl and tyrosyl, tryptophyl, or histidyl residues facilitate the renaturation of double helices and that this "snapback" property is more specific of GC-rich sequences.[76, 77] A linear correlation was found between the thermal stabilization afforded to DNA by the investigated peptides and their ability to induce DNA strand renaturation, with Tyr-Gly-Gly-Tyr being the most efficient.[76] These interesting observations have not been confirmed or extended further since their publication in 1974.

Since stacking interactions of aromatic amino acids are strongly favored in single strands, it was of interest to determine whether this specificity was also dependent upon the base sequence in single-stranded polynucleotides. Maurizot et al. have investigated the binding of Lys-Trp-Lys to random copolymers of U and A[131] and of C and A.[84] The results have been interpreted as showing a decreased affinity of tryptophan for the sequences UU > UA, AA > AC,CC. No data is available for guanine-containing polynucleotides. Figure 10 shows the analysis of fluorescence data according to the two-step model described above (Equation 4). The experimental values of K_2 are plotted against the fraction of cytosine bases in copolynucleotides of C and A. Theoretical curves were calculated according to different assumptions (see legend of Figure 10). Assuming that random copolymers behave as a collection of dinucleotides, the

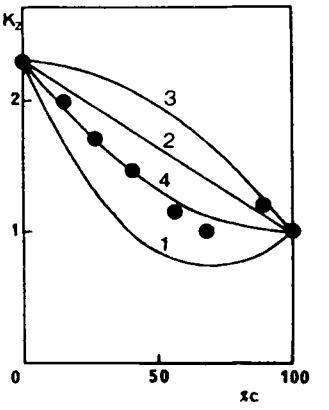

FIGURE 10. Variation of constant K_2 (see Equations 4 and 5) for copolymers of A and C calculated according to different assumptions: (1) $K_{2AC} = 0$; (2) $K_{2AC} = (K_{2AA} + K_{2CC})/2$; (3) $K_{2AC} = K_{2AA}$; (4) $K_{2AC} = K_{2CC}$; (•••), experimental points. (Reprinted with permission from Maurizot, J. C., Boubault, G., and Hélène, C., *Biochemistry*, 17, 2096, 1978. Copyright by the American Chemical Society.)

experimental data fit the theoretical curve quite well if the sequences AC and CA (which are not distinguished in this model) have the same K_2 value as the sequence CC as far as insertion (stacking) of tryptophan is concerned. Sequences AA have a higher value of K_2 (~2.4) as compared to cytosine-containing sequences (~1).

The nature of the carbohydrate residue also affects the binding process of peptides containing aromatic residues. It was shown that with single stranded poly(dA) the interaction of the peptide Lys-Trp-Lys could also be accounted for by a two-step mechanism, but that the values of constants K_1 and K_2 were about half that obtained under similar conditions for the ribopolymer as shown below:

	poly(rA)	poly(dA)
K_1	$1.3 \times 10^4 M^{-1}$	$0.7 \times 10^4 M^{-1}$
K_2	2.4	1.15

Template chromatography has been used to investigate the binding of amino acids and peptides to oligodeoxythymidylic acids $(pT)_n$ and oligodeoxyadenylic acids $(pA)_n$.[132, 133] The oligodeoxynucleotide was covalently linked to polyvinylalcohol and then mixed with DEAE-cellulose. This gives a stable stationary phase under elution conditions. This column chromatography technique allowed Schott et al.[132] to determine the degree of interaction of different molecules in the mobile phase with the oligonucleotide from their retardation values. It was shown that complementary and noncomplementary oligonucleotides could be separated on such a column. Also peptides with different sequences were shown to be retarded differently. Oligopeptides containing successive Tyr or Trp residues exhibited a greater retardation when the number of residues increased. Insertion of nonaromatic residues decreased the effect.

On $(dT)_n$ columns, $(Trp)_2$ had a behavior similar to $d(pA)_3$ whereas $d(pA)_4$ interacted less strongly than $(Trp)_3$. However, this method does not give any quantitative information either on the binding strength, or on the nature of the interactions involved in the retardation process. It is difficult to determine the respective roles of the cellulose matrix, the polyvinylalcohol, and the oligonucleotide.

C. Recognition of Chemically-Modified DNA by Oligopeptides Containin Aromatic Amino Acids

Since it was shown that stacking interactions were strongly favored in single-stranded nucleic acids, it was of interest to investigate the binding of oligopeptides containing aromatic amino acids to DNA which has been chemically modified by different agents. Different types of DNA modifications have been investigated:

1. Pyrimidine dimers induced by UV light[134]
2. Modifications of guanines by N-acetoxy-N-2-acetylaminofluorene (AAAF), a carcinogen which reacts with the CH(8) and NH_2(2) groups of guanine (80 and 20% of the adducts, respectively)[135]
3. Methylation of guanine N(7) and adenine N(3) followed by depurination of DNA with or without strand cleavage at the apurinic sites[136]

In all cases it has been suggested that chemically modified DNAs have a locally altered structure. The results reported so far show that stacking interactions of the tripeptide Lys-Trp-Lys increase in the order methylated DNA < UV irradiated DNA < nicked apurinic DNA < DNA-AAF < apurinic DNA.

The most interesting results were obtained with apurinic DNA which exhibits a very strong affinity for Lys-Trp-Lys. Analysis according to the two-step model already mentioned (Equation 2) led to a K_2 value of $\sim$200 for apurinic sites as compared to $\sim$0.3 for native sites.[136] Introduction of a strand break at the apurinic sites strongly reduced the value of K_2. Removal of a purine in a double helix leaves a "hole" which has the same size as the indole ring of tryptophan. This creates a very favorable situation for inserting the tryptophyl residue of the tripeptide Lys-Trp-Lys. No energy has to be provided to unstack bases prior to insertion of the indole ring. If a chain break is introduced at the apurinic site, then this favorable situation is lost, probably as a result of an important structural change in the vicinity of the break.

The preferential binding of Lys-Trp-Lys at DNA sites where guanine has reacted with AAAF has been interpreted as resulting from a local opening of the double-helical structure. This local opening of several base pairs had already been revealed by other methods. These results showing that aromatic residues are capable of "recognizing" distorted regions in a modified DNA might be of importance in explaining how specific proteins recognize damaged DNA in repair and/or protection processes.

D. Hydrogen Bonding Interactions

Formation of hydrogen bonds between amino acid side chains (or the peptidic group) and nucleic acid bases (or ribose, d-ribose, and phosphate groups) may be responsible for specific interactions between oligopeptides and nucleic acids. Examination of different hydrogen bonding possibilities led several authors to postulate that formation of pairs of hydrogen bonds should lead to some specificity.[90, 129, 130] Recognition of base pairs can be achieved from the small or large groove of the nucleic acid. Amide groups such as Gln or Asn could form two hydrogen bonds with A in A-T base pairs in the large groove and with G in G-C base pairs in the small groove. The same expectation holds for carboxylic acids in their *unionized* state (it must be pointed

out that the pK of an ionizable side chain might be considerably shifted in a protein — or peptide — nucleic acid complex).

The guanidinium group of arginine should also be able to form a pair of hydrogen bonds with guanine in G-C base pairs (in the large groove of DNA). However, this group is positively charged and has a strong tendency to interact with negatively charged phosphate groups on the nucleic acid. A model was proposed by Hélène[130] to overcome this objection. The side chain of arginine could be "neutralized" inside a peptide or a protein by a neighboring ionized carboxylic acid of glutamic or aspartic acid. Formation of an ion pair between these two residues would still leave the possibility of forming two hydrogen bonds between the arginine side chain and a G-C base pair. From model building studies it was proposed that the simplest oligopeptide which could form such an ion pair was the dipeptide Arg-Glu. This hypothesis was fully confirmed by a PMR investigation of this dipeptide.[137] Ion pair formation was also observed in other oligopeptides such as Glu-Arg, Arg-Glu-Lys, and Arg-Ala-Gly-Glu.[138] There is no report yet as to the specificity of interaction between these oligopeptides and double-stranded nucleic acids. Only guanine was shown to interact strongly with the dipeptide Arg-Glu but this was due to the strong interaction of guanine (N[1] and NH_2[2]) with carboxylic anions[137] which disrupts the Arg...Glu ion pair. When the guanine groups involved in this interaction (N[l] and NH_2 [2]) are blocked by methylation, guanine does interact with Arg in the Arg...Glu ion pair. Cytosine is also able to form two hydrogen bonds with Arg without disrupting the Arg...Glu ion pair. However, the functional groups of cytosine involved in this interaction (N[1] and O[2]) are not available when this base is engaged in a G-C base pair.

The only very specific hydrogen bonding interaction thus far reported is that of carboxylate anions (of ionized Glu and Asp side chains).[104] Two hydrogen bonds are formed and the G...carboxylate "pair" thus obtained is more stable than the G...C base pair itself. The original study was made in an organic solvent (DMSO) but a similar conclusion was recently reached in aqueous solutions.[139] Although water molecules do compete with hydrogen bonding interactions, the *relative* strength of the associations between guanine and carboxylate or cytosine remains the same. No result is available yet concerning the specific interaction of Glu side chains of oligopeptides with guanine bases in oligonucleotides or nucleic acids.

Bruskov,[129] Hélène,[130] and Rein et al.[140] have suggested that several oligopeptide sequences should be specific for nucleic acid bases, base pairs, or base sequences. Bruskov has tried to utilize all hydrogen bonding possibilities of nucleic acid bases to determine the oligopeptide sequence which should be best adapted to any of the four bases. For example, model building studies suggest that three hydrogen bonds could be formed between cytosine and the dipeptide Asn-Ser or between thymine and the dipeptides Asn-Asn and Asn-Ser.... Four hydrogen bonds could allow the specific recognition of adenine by the tripeptide Glu-X-Glu where X is any amino acid. Guanine makes five hydrogen bonds with the tripeptide Asp-Ser-Arg.

Hélène has proposed a model in which Arg-Glu sequences should be able to recognize selectively G-C base pairs.[130] Rein et al.[140] have suggested that a right handed twisted β-ribbon carrying in every other position (1-3-5...) either Gln(or Asn) to recognize A-T, or Arg to recognize G-C, should provide a basis for the stereospecific recognition of a base pair sequence. Glu or Arg residues should alternate with any residue compatible with a β structure. To our knowledge none of these models has been tested experimentally with the exception of the Arg-Glu sequence and other sequences containing Arg and Glu residues which have been shown to form a selective and strong complex with guanine (see above).

Carter and Kraut[141] have constructed a model in which a pair of antiparallel β segments fits into the minor groove of an RNA double helix. Hydrogen bonds are formed

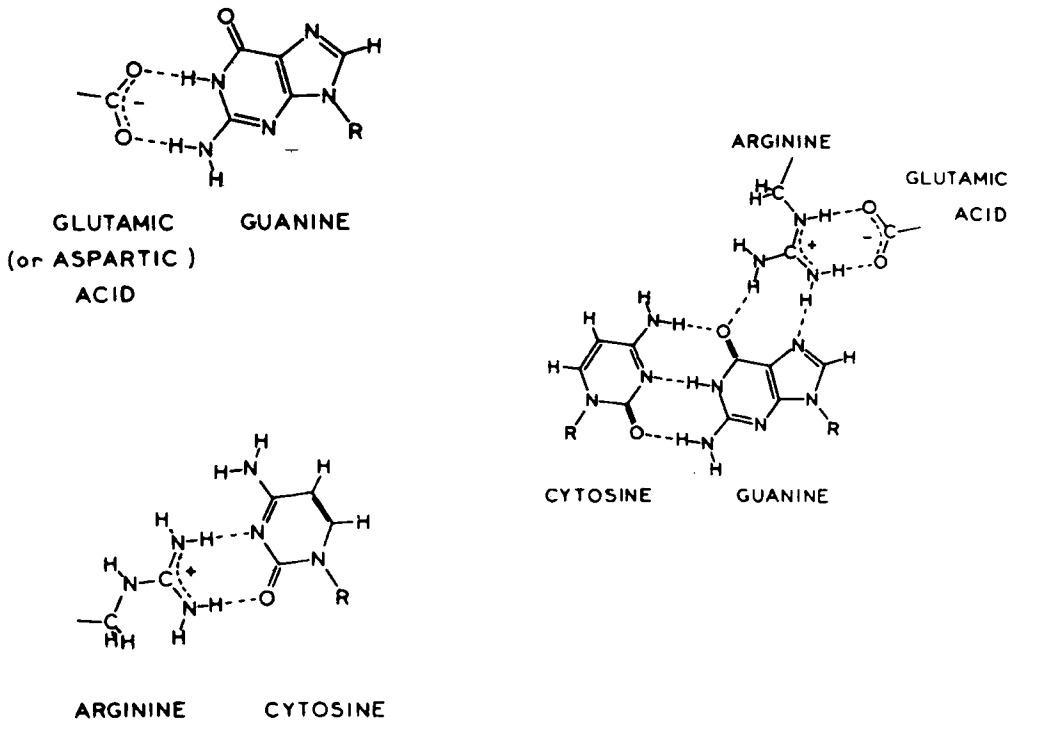

FIGURE 11. Hydrogen bonding of carboxylate anion and guanidinum cation to G and C and model for the interaction of the sequence Arg-Glu with a G-C base pair.

between the ribose 2′-hydroxyl groups and polypeptide carbonyl oxygens. The antiparallel β chains are twisted in a right-handed sense as observed in proteins,[142] and adjacent polypeptide and polynucleotide chains run parallel in the complex. A water molecule could bridge alternate peptide backbone NH groups (not involved in β-sheet formation) with the 2′-hydroxyl groups and furanose ring oxygen atoms.

Kim et al.[143] have proposed that an antiparallel β-ribbon could fit into the narrow groove of DNA with hydrogen bonds formed between the peptidic NH groups not involved in the β structure and the 3′ oxygens of the polynucleotide backbone. The polarity of each DNA strand should be antiparallel to the peptide backbone with which hydrogen bonds are formed.

In both models amino acid side chains of the β-ribbon are pointing alternatively inward (toward the nucleic acid) and outward. In the DNA model any basic residue attached to the α-carbons on the *outside* of the β-structure will be able to engage electrostatic interactions with phosphate groups. Amino acid side chains on the *inside* of the β-ribbon have the possibility of "recognizing" the narrow groove side of the base pairs. In the RNA model there seems to be more restriction as to the size of the amino acid side chains which can be accommodated on the inside of the β-sheet. The N-2 amino group of guanine would prohibit the presence of at least one inward-pointing β-carbon atom.

Bruskov and Poltev,[144] Ivanov,[145] and Seeman et al.[90] have noted that the small groove of RNA and DNA provides analogous positions for hydrogen bond acceptor groups: O(2) of C and T, N(3) of A and G. The only discrimination between A-T and G-C base pairs in this groove comes from the presence of the NH_2 group of guanine. This observation led Ivanov to propose that recognition of base pairs in the small groove (for example, by serine or cysteine residues of a β-ribbon) could be based on

the impossibility of these residues to form a hydrogen bond with N(3) when guanine is present. The model proposed by Carter and Kraut also prohibits the presence of even alanine as an inward-pointing residue when the nearest base is guanine. Using these arguments Ivanov has proposed a binary "G- non G" code which he applied to the recognition of tRNAs by aminoacyl-tRNA synthetases. In this model the amino acids of the synthetase involved in the recognition of the CCA or DHU stems are assumed to be inward-pointing residues of a β-ribbon associated with the small groove of the stems and are located in such positions that they do not encounter Gs in the cognate tRNA. The presence of Gs at these positions in another tRNA would preclude the association. The model also predicts that an artificial tRNA with inosines instead of guanines will fit any synthetase. To our knowledge this prediction has not been tested experimentally.

The model proposed by Gursky et al.[146, 147] also implies the stereospecific recognition of the small groove of DNA by an antiparallel β-sheet. In this model half of the hydrogen bonds existing normally in β-structures are broken upon complex formation with DNA. New hydrogen bonds are formed between amide NH groups and O(2) or N(3) atoms of pyrimidines and purines, respectively, and between amide C=O groups and NH_2 of guanines. The structure of the β-sheet as well as that of the DNA double helix are slightly distorted as compared to the "classical" structures. One chain of the β-sheet is assumed to form all contacts with guanines (the g-chain). This assumption rests upon the observation that in regulatory regions of DNA which are specifically recognized by proteins, guanines are very often on the same strand. The other chain of the β-sheet (t-chain) forms hydrogen bonds with all bases. Six amino acid residues will prevent hydrogen bonding of C=O with guanines when present on the t-chain. These residues are Ser, Thr, Asn, His, Cys and Glu. They will form hydrogen bonds with the C=O group thus weakening or breaking its interaction with G. They can be considered as "AT-coding" residues. This forms the basis of a code controlling specific binding of regulatory proteins to DNA which has been applied to predict the regions of proteins (lac and λ repressors, cro protein from λ phage, ribosomal protein S8) which could be involved in the specific recognition of nucleic acids base pair sequences (operators, ribosomal 16 S RNA).

All the models discussed above imply the binding of a β-sheet polypeptidic structure to the small groove of DNA or RNA. There is no experimental evidence as yet to support these models. Gursky et al. have undertaken a study of oligopeptides which are expected to form β-structures such as $(Val)_n$ or $(Thr)_n$. These oligopeptides bind to nucleic acids and exhibit some preference for A-T ($[Thr]_n$) or G-C ($[Val]_n$) base pairs although the origin of this preference is not yet clear. Gursky et al.[147] have also reported an interesting attempt to link oligopeptides forming β-structures to distamycin. This antibiotic has been shown to bind in the small groove of DNA and to be specific for A-T clusters (see below). Dimers can be formed through association of the oligopeptides tails containing 5 to 10 valine residues. A greater binding specificity as compared to the free antibiotic has been reported.[147]

One of the restrictive aspects in all the above models predicting an association through β-sheet polypeptidic structures remains the exclusive utilization of the narrow groove. There is ample evidence suggesting that specific contacts between regulatory proteins such as the *lac* and λ repressors are made in the *large* groove of DNA.[149, 153] In the case of the *lac* repressor the main difference between the nonspecific and the specific complexes rests mostly upon interactions which involve the large groove in the latter case.[152, 154] The λ repressor does not protect the small groove of the λ operators against methylation by dimethylsulfate. Also the results now available demonstrate that regulatory proteins make contacts with base pairs on one side of the DNA double

helix.[152] This rules out the involvement of long stretches of polypeptidic β-structures wrapped around the DNA helix.

E. Stereospecific Binding of Diastereoisomeric Peptides

In a study of the thermal stabilization of several double helices, Gabbay et al. concluded that LL-lysyl dipeptides stabilize the nucleic acid double helices to a greater degree than the corresponding DD-lysyl dipeptides.[155] A model was presented to account for this result.[156] The N-terminal lysine interacts with two neighboring phosphate groups on the same polynucleotide chain and the most stable configuration is obtained with the L-enantiomer. The greater stabilization effected by LL-lysyl dipeptides is expected to be the same irrespective of the nature of the bases and the multiplicity of the helix.

More recently it has been shown by the same group[79, 80] that the two diastereoisomeric dipeptide amides L Lys-L Phe (NH_2) and L Lys-D Phe(NH_2) exhibit quite different behaviors with respect to DNA binding. They have different effects on the DNA structure. The relative specific viscosity of DNA and its relative reduced flow dichroism decrease more rapidly upon binding of L Lys-L Phe(NH_2) than L Lys-D Phe(NH_2). The proton magnetic resonance spectra of these two dipeptides in the presence of DNA are also quite different. Whereas L Lys-D Phe(NH_2) exhibits only slight broadening and upfield shift of the aromatic protons, L Lys-L Phe(NH_2) shows two broad resonance lines which are shifted upfield as compared to the single line of the free peptide. Also the spin-lattice relaxation times are different. These results have been interpreted by assuming that the aromatic ring of L Lys-L Phe(NH_2) points toward the helix leading to partial insertion between the bases whereas the aromatic ring of L Lys-D Phe(NH_2) points outward toward the solvent. It has been concluded that the N-terminal L-Lysyl residue of the two diasereoisomeric peptides binds stereospecifically to DNA through its α- and ε-amino groups and dictates the positioning of the aromatic ring of the C-terminal phenylalanyl residue.

These studies have been extended to longer oligopeptides.[43] The above model forms the basis for the proposal that oligopeptides might adopt a slightly modified single-stranded β-sheet structure which would be wrapped around the DNA helix in a manner similar to that described previously.[157] with oligopeptides composed of L-amino acids, the side chains would alternatively point "into" and "out" of the helix. Oligopeptides composed of alternating L- and D-amino acids can form two types of complexes with all side chains pointing either "into" or "out of" the DNA double helix. Gabbay and co-workers[43] have studied the PMR spectra of 16 different oligopeptide amides starting with the sequence L Lys-L Phe and composed of L- and D-amino acids. Most of the peptides (14 out of 16) conform to the prediction based on the model. For example, L Lys-L Phe-D Ala (NH_2) should exhibit a closer contact of the phenylalanyl ring than L Lys-L Phe-L Ala (NH_2). A similar result was expected for L Lys-L Phe-D Ala-L Val (NH_2) compared with L Lys-L Phe-L Ala-L Val (NH_2). In both cases a hydrophobic D-amino acid in position 3 should allow a closer contact of the aromatic ring of L-phenylalanine with DNA bases. This is experimentally observed except when D Leu is present in position 3. This last result could be due to a steric effect of the bulky side chain of the D Leu residue. However it must be emphasized that the analysis of Gabbay et al. rests upon PMR experiments at two DNA/peptide ratios and that no data is reported concerning the association constants for the different peptides. A higher upfield shift might reflect a higher association constant and not a closer contact of the phenylalanyl ring with the bases in the complexed peptide. It would be very interesting to know how the presence of D-amino acids in even or odd positions of the oligopeptide sequence affects the binding strength of the oligopeptide.

VII. PHOTOCHEMICAL BEHAVIOR OF PEPTIDE-NUCLEIC ACID COMPLEXES

The conformational changes induced in nucleic acids by the binding of oligopeptides might alter photochemical reactions in the nucleic acids. Moreover if the peptide contains an absorbing group it might contribute to the photochemical behavior of the complex by acting as a sensitizer or by introducing cross-links. All these phenomena should be involved in the photochemistry of protein-nucleic acid complexes.

Two types of photosensitized reactions have been described in oligopeptide-DNA complexes:

1. Tyrosyl residues can photosensitize the formation of thymine dimers in DNA.[158] This is also true of N′-formylkynurenin (FK), the photooxydation product of tryptophan, which has been shown to photosensitize dimer formation in complexes of DNA with Lys-FK even though FK is able to photosensitize dimer splitting in frozen aqueous solutions.[159]
2. Tryptophyl residues photosensitize the splitting of thymine dimers in DNA which has been submitted to UV radiations.[160] The mechanism of this reaction most probably involves an electron transfer reaction from the excited indole ring to the dimer.[161]

A study of the excited states of peptide-nucleic acid complexes has revealed aspects which might be of interest in understanding the photochemistry of protein-nucleic acid complexes. Energy transfer has been demonstrated at the singlet and triplet levels from tyrosine to nucleic acid bases.[162] On the contrary, tryptophan is able to accept the energy from excited bases at the triplet level.[67] Calculation of Forster distances for singlet energy transfer between aromatic residues of oligopeptides and nucleic acid bases reveals that tyrosine may transfer efficiently whereas tryptophan is not a good energy donor, except if the excited tryptophyl residue cannot be relaxed by solvent molecules, which might well be the situation in protein-nucleic acid complexes.[168]

The sulfhydryl group of cystein residues and the disulfide group of cystine might also be involved in the photochemical reactions of protein-nucleic acid complexes. There is no report available concerning this type of reaction for oligopeptides bound to nucleic acids. It has been shown, however, that the disulfide bridge of cystamine is an efficient trap for the triplet-state energy of polynucleotides such as poly(A).[163]

VIII. CONCLUSION

This review was not intended to cover all studies which have been devoted to interactions between amino acid side chains and nucleic acid constituents, but focused mainly on the binding of oligopeptides to nucleic acids. The main goal of these studies was to provide information and models for the associations of nucleic acids with proteins. Most — if not all — of these associations involve electrostatic interactions which are also observed in many of the investigated oligopeptide-nucleic acids complexes. Oligopeptides have been used to test the theory developed to determine the number of ion pairs in any particular nucleic acid-protein complex and to compare the respective affinities of lysyl and arginyl side chains, of single stranded vs. double stranded structures, etc.

Stacking interactions have been shown to introduce a specificity with respect to single-stranded structures as compared to double helices. They might play an important role in the discrimination between such structures or in the specific recognition of chemically damaged DNA.

Hydrogen bonding between amino acid side chains or peptidic groups and nucleic acid bases is certainly the basis for the recognition of base sequences by oligopeptides or proteins. However, experimental data dealing with this type of interaction are very limited — even though many models have been proposed — due to the experimental difficulty in providing evidence for hydrogen bonding interactions in aqueous solutions with small oligopeptides. Much effort has to be devoted to designing significant experiments in this field.

The role of hydrophobic interactions in the association of oligopeptides with nucleic acids has also proved to be difficult to demonstrate. Experiments showing that the replacement of thymine by uracil in *lac* operator sequences decreases the binding of the *lac* repressor might indicate that the methyl group of thymine in the large groove of DNA is a candidate for hydrophobic contacts (although replacing thymine by uracil might also induce a slight conformational change or an altered conformational stability).[148] Recent reports on the role of organic solvents (such as ethylene glycol) on lysine copolymer-DNA[164] have also emphasized the role that could be played by hydrophobic interactions in the association between proteins and nucleic acids and in the structural organization of the complexes.

The study of associations between nucleic acids and synthetic oligopeptides of defined sequence or protein fragments might provide the most interesting models to investigate the specific interactions which are responsible for the selective recognition of nucleic acid base sequences. Recently the synthesis of a 34-residue polypeptide that interacts with nucleic acids has been reported.[165] This peptide and its dimer were shown to interact strongly with cytidine monophosphates and single stranded DNA. The dimer exhibited a ribonuclease activity with a high preference for cleavage at the 3′-end of cytosine bases.

The 1-59 and 1-51 peptides obtained by trypsin cleavage of the N-terminal part of the *lac* repressor have been shown to bind specifically to the *lac* operator.[152] It is not yet possible to determine the nature of the interactions involved in the binding of these protein fragments. However the contacts formed with the base pairs of the specific DNA sequence are similar to those of the intact *lac* repressor (4 subunits of 360 amino acids each). Proteolytic cleavage of the λ repressor also yields an N-terminal fragment (92 amino acids) which binds specifically to the λ operators.[166, 167]

A combined approach using synthetic oligopeptides, protein fragments, and semisynthetic fragments should provide the fundamental elements of the "molecular code" which governs the selective recognition of nucleic acid base sequences by proteins. Although structural aspects of these interactions already have been the subject of many studies, there is much less data on the kinetic behavior of such complexes. Research in this area should be developed if one wants to understand how proteins recognize nucleic acids.

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