Interactions of Aromatic Residues of Proteins with Nucleic Acids. Fluorescence Studies of the Binding of Oligopeptides Containing Tryptophan and Tyrosine Residues to Polynucleotides†

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ABSTRACT: The binding of oligopeptides of general structure Lys-X-Lys (where X is an aromatic residue) to several polynucleotides has been studied by fluorescence spectroscopy. Two types of complexes are formed, both involving electrostatic interactions between lysyl residues and phosphate groups as shown by the ionic strength and pH dependence of binding. The fluorescence quantum yield of the first complex is identical with that of the free peptide. The other complex involves a stacking of the nucleic acid bases with the aromatic amino acid whose fluorescence is quenched. Fluorescence data have been quantitatively analyzed according to a model involving these two types of complexes. Association constants and the size of binding sites have been determined. Stacking interactions are favored in single-stranded polynucleotides as compared to double-stranded ones. A short oligopeptide such as Lys-X-Lys is thus able to distinguish between single-stranded and double-stranded nucleic acids. Fluorescence results are compared to those obtained by proton magnetic resonance and circular dichroism.

Specific interactions between amino acid side chains and nucleic acid bases are involved in the selective recognition of base sequences by proteins. Two types of such interactions have been described at the monomer level. These are (i) stacking of aromatic amino acids with purine and pyrimidine bases (Montenay-Garestier and Hélène, 1968, 1971; Dimicoli and Hélène, 1971, 1973; Wagner and Lawaczeck, 1972; (ii) hydrogen bonding of some amino acid side chains (tyrosine, carboxylic acids) with adenine and uracil (Sellini *et al.*, 1973). Interactions of nucleotides with immobilized amino acids have also been described (Saxinger *et al.*, 1971).

The first type of interactions has been demonstrated to take place in complexes involving nucleic acids and either aromatic amines (Hélène *et al.*, 1971a,b; Hélène, 1971a,b) or oligopeptides (Brown, 1970; Hélène and Dimicoli, 1972; Gabbay *et al.* 1972, 1973; Novak and Donhal, 1973; Dimicoli and Hélène, 1974a,b). The interactions of several oligopeptides with nucleic acids have also been described (Backer *et al.*, 1970; Fritzsche, 1972). In recent quantitative proton magnetic resonance studies we have shown that the behavior of aromatic residues of oligopeptides should depend both on the sequence of the oligopeptides and on the structure of the nucleic acid (Dimicoli and Hélène, 1974a,b). We present here the results of a fluorescence investigation of the binding of oligopeptides containing aromatic amino acids to several single-stranded and double-stranded polynucleotides. A quantitative analysis of fluorescence data has allowed us to propose a general mechanism for the binding of these oligopeptides to nucleic acids involving both electrostatic and stacking interactions.

Material and Methods

Oligopeptides Lys-Trp-Lys and Lys-Tyr-Lys were purchased from Schwarz/Mann. Lys-TyrOMe-Lys, Lys-TrpOMe, Lys-Phe-Lys, and Lys-Ala-Lys were synthesized in our laboratory by Dr. J. Rossi. Polynucleotides were obtained from Miles.

Solutions were made in a buffer containing 1 mM NaCl, 1 mM sodium cacodylate, and 2×10^{-4} M EDTA at pH 7. Polynucleotide solutions were dialyzed against this buffer before use. Ionic strength was changed by the addition of sodium chloride.

Fluorescence measurements were performed with a Jobin-Yvon spectrofluorimeter. Part of the incident irradiation beam was focused on a rhodamine B quantum counter (Parker, 1968) whose fluorescence was measured with a 6097 EMI photomultiplier. The sample fluorescence was measured with a 9558 EMI photomultiplier after dispersion through a prism monochromator. The ratio of the sample and reference intensities was recorded.

Difference spectrophotometry experiments have shown that the binding of peptides to polynucleotides and nucleic acids induces changes in the absorption spectra of both molecules (unpublished results). In fluorescence investigations using Lys-Trp-Lys, the excitation wavelength was chosen at the isosbestic point (292 nm). In the case of tyrosine-containing peptides, the excitation wavelength was chosen at 275 nm where the absorption changes due to binding do not exceed 3% (the isosbestic point occurs at 258 nm and the lamp emission at this wavelength was too small to allow accurate measurements). At the excitation wavelength, light absorption by the nucleic acid produces a screening effect which must be taken into account when determining fluorescence quantum yields. This could be done in two ways.

(i) A mixture of nucleotides having the same base composition and the same absorbance at the excitation wavelength as the nucleic acid can be used to produce the same screening effect as the nucleic acid. Since nucleosides inter-

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act only weakly with aromatic amino acids (Dimicoli and Hélène, 1971, 1973; Wagner and Lawaczeck, 1972) the nucleoside mixture [in the concentration range investigated ($<5 \times 10^{-4}$ M)] will decrease the fluorescence intensity by its screening effect only.

(ii) Increasing the ionic strength leads to complex dissociation and consequently to an increase in fluorescence intensity (see below). Salt-induced dissociation can also be followed by proton magnetic resonance (pmr) (Dimicoli and Hélène, 1974a,b) or by circular dichroism (Durand *et al.*, 1975). Fluorescence experiments were carried out by progressively adding small amounts of a concentrated peptide solution to the polynucleotide and to the buffer, respectively, and measuring the ratio (R) of the fluorescence intensities in the presence and the absence of the polynucleotide (see Figure 1). Then sodium chloride was added up to a concentration of 0.4 M. A constant value of R was obtained when NaCl concentration increased above 0.1 M. As expected (Figure 1), this value decreased when the polynucleotide concentration increased or when the solution was excited at shorter wavelengths. Since the peptide is not bound at high ionic strength, this experiment allowed us to take into account the screening effect of the polynucleotide and then to determine the overall fluorescence quantum yield (ϕ) of the peptide.

Methods i and ii gave identical results. The second method (NaCl-induced dissociation) was routinely used in our experiments since it required only one series of measurement with the buffer as control when a series of polynucleotide concentrations was investigated.

Results and Discussion

Fluorescence Quenching. The fluorescence quantum yield of oligopeptides containing lysyl and aromatic residues is decreased in the presence of polynucleotides and nucleic acids (Figure 1). No change in the fluorescence spectrum could be detected except in the case of Lys-TyrOMe-Lys and Lys-TyrOMe-LysOMe where a new fluorescence band appeared at long wavelengths (~410 nm) in the presence of poly(A). The extent of fluorescence quenching depends on the concentrations of both peptide and polynucleotide (Figure 1), on the pH (Figure 2), and on the ionic strength (Figures 1 and 6).

In order to determine whether excitation energy transfer from bases to tryptophan residues could have introduced errors in fluorescence quantum yield measurements, the fluorescence intensity of Lys-Trp-Lys in the presence of poly(A) was measured under excitation at different wavelengths both when the peptide was bound to poly(A) (1 mM NaCl) and when the complex was dissociated (0.4 M NaCl). The intensity ratio (which takes into account the screening effect of the polynucleotide) was found to be practically independent of the excitation wavelength indicating that energy transfer from bases to tryptophan was negligible under our experimental conditions.

The pH dependence of fluorescence quenching is shown in Figure 2. The N-terminal α-amino group of the peptides has a pK value close to 7.5. Neutralization of the positive charge of this group by deprotonation leads to an increase in the fluorescence quantum yield of the peptides, indicating an interaction between this NH_3^+ group and the aromatic ring. In the presence of poly(A) the pH–titration curve of the peptide fluorescence is shifted by 0.8–0.9 pH unit toward higher pH as already observed by pmr spectroscopy (Dimicoli and Hélène, 1974a,b). This reflects the electrostatic binding of the N-terminal NH_3^+ group to negatively charged phosphate groups on the polynucleotide. Fluorescence quenching in the presence of the polynucleotide is still observed at pH 9 (although it is less important than at pH 7). This indicates that binding still occurs when the N-terminal α-NH_2 group is uncharged and involves the ϵ-NH_3^+ groups of lysyl side chains. Since several factors might contribute to the pH dependence of fluorescence quenching (change in binding constants, in fluorescence quantum yields, and lifetimes of bound peptides) a complete quantitative analysis of fluorescence data would be required at every pH. Such an analysis has been carried out at pH 7 only. At this pH, investigated peptides do not have their N-terminal α-amino group fully protonated. However, this pH value was chosen because poly(A) and poly(C) easily undergo a transition to a double-stranded structure in the presence of peptides at lower pH values in the low ionic strength medium used. Since binding of peptides to polynucleotides shifts the pK value of the N-terminal α-amino group to higher values (Figure 2), all bound peptide molecules have this group protonated at pH 7.

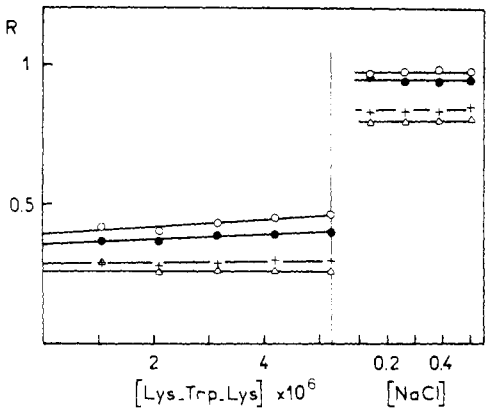

FIGURE 1: Concentration dependence of uncorrected fluorescence intensity of the peptide Lys-Trp-Lys in the presence of poly(A) at different concentrations: 5×10^{-4} M (Δ), 2.9×10^{-4} M (+), 1.4×10^{-4} M (●), and 8.6×10^{-5} M (○). R is the ratio of fluorescence intensities in the presence and the absence of poly(A). Sodium chloride was added as described in the text to dissociate the complexes and to determine the screening effect of poly(A). Excitation wavelength, 292 nm.

Quantitative Analysis of Fluorescence Data. The fluorescence quantum yield of the investigated peptides is decreased in the presence of the polynucleotides. Measurements of the fluorescence decay curves under flash excitation (as described by Wahl, 1969) do not reveal any marked difference (within experimental error) in the fluorescence lifetime of Lys-Trp-Lys and Lys-Tyr-Lys in the presence of polynucleotides even when the fluorescence quantum yield is decreased by as much as 50%. This result indicates that in the presence of the polynucleotide one must distinguish between two kinds of molecules: those which have the same fluorescence quantum yield and lifetime as the free peptide and those whose fluorescence quantum yield is zero (and therefore cannot be seen in the decay curve). In a previous communication (Hélène and Dimicoli, 1972) we analyzed fluorescence data assuming that only one type of complex was formed whose fluorescence quantum yield was zero. A linear plot was obtained in a Scatchard representation. However, when investigating different nucleic acid concentrations, different values of the association constant were obtained indicating that the above assumption was not correct. We were thus led to postulate the existence of at least two complexes according to one of the following mecha-

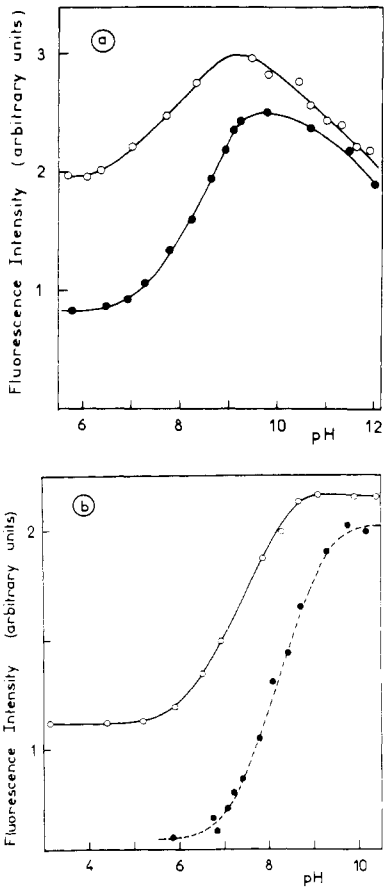

FIGURE 2: (a) pH dependence of the fluorescence of Lys-Trp-Lys (3.3×10^{-5} M) in the absence (○) and the presence (●) of 5×10^{-4} M poly(A). (b) pH dependence of the fluorescence of Lys-TrpOMe (5×10^{-5} M) in the absence (○) and the presence (●) of 5×10^{-4} M poly(A).

nisms (where P represents the polynucleotide):

$$\begin{aligned} &P + \text{free peptide} \overset{K_I}{\rightleftharpoons} \text{complex A} \\ &P + \text{free peptide} \underset{K_{II}}{\rightleftharpoons} \text{complex B} \end{aligned} \qquad (1, \alpha)$$

$$P + \text{free peptide} \overset{K_1}{\rightleftharpoons} \text{complex A} \overset{K_2}{\rightleftharpoons} \text{complex B} \qquad (1, \beta)$$

In both schemes, complex A would have the same fluorescence characteristics as the free peptide and complex B would have its fluorescence completely quenched. If excitation is performed at the isosbestic wavelength or if absorbance changes are small (see Materials and Methods), the overall fluorescence quantum yield (ϕ) of the peptide can be written as eq. 2, where ϕ_F is the fluorescence quantum

$$\phi = \phi_F \frac{C_F + C_A}{C_t} \qquad (2)$$

yield of the free peptide and C_F, C_A, and C_B are the concentrations of free and bound (A and B) peptides with $C_t = C_F + C_A + C_B$.

We are dealing with the binding to one-dimensional homogeneous lattices of ligands which cover more than one lattice residue. For an infinitely long polymer the concentration of potential binding sites for a naked polynucleotide is P_0 where P_0 is the phosphate concentration.[1] At a very low degree of saturation one can therefore write:

$$C_A = K_I P_0 C_F, \qquad C_B = K_{II} P_0 C_F \qquad (3, \alpha)$$

$$C_A = K_1 P_0 C_F, \qquad C_B = K_1 K_2 P_0 C_F \qquad (3, \beta)$$

where K_I, K_{II}, and K_1 are association constants for the binding of the peptide to a free binding site and K_2 is an isomerization constant. The two schemes α and β are therefore equivalent from the thermodynamical point of view with $K_{II} = K_1 K_2$. Formation of complex B results from an isomerization of complex A in scheme β, whereas complexes A and B exchange between each other in scheme α. All the quantitative results will be given according to scheme β (see the discussion below related to the structure of the complexes).

As can be seen in Figure 1, fluorescence intensity varied linearly with total peptide concentration (C_t) at low concentrations. Extrapolation to zero peptide concentration allowed us to determine a limit value ϕ_L of the overall fluorescence quantum yield. The following equation can be deduced from eq 2 and 3:

$$\frac{\phi_F}{\phi_F - \phi_L} = 1 + \frac{1}{K_2} + \frac{1}{K_1 K_2} \frac{1}{P_0} \qquad (4)$$

A plot of $\phi_F/(\phi_F - \phi_L)$ *vs.* $1/P_0$ should give a straight line as experimentally observed (Figure 3). The values of K_1 and K_2 which can be calculated from these plots are given in Tables I and II.

For any degree of saturation of the polynucleotide, the concentration of bound molecules ($C_A + C_B$) can be calculated after K_2 has been determined as described above:

$$\frac{C_A + C_B}{C_t} = \frac{\phi_F - \phi}{\phi_F - \phi^*} \qquad (5)$$

where

$$\phi^* = \phi_F/(1 + K_2).$$

Then a Scatchard representation can be used where r/C_f is plotted against r ($r = (C_A + C_B)/P_0$). These curves are nonlinear as expected for the binding of a ligand which covers and makes inaccessible more than one polynucleotide residue (Daune, 1972; McGhee and Von Hippel, 1974, and references therein). The number n of phosphate groups made inaccessible can be estimated from an extrapolation of plots of $1/r$ *vs.* $1/C_F$ (Daune, 1972) or from the linear part of the plots shown in Figure 4 which intercepts the x axis at $r = 1/(2n - 1)$ (McGhee and Von Hippel, 1974). Values of n between 3.5 and 4 were obtained. Since there are two electrostatic interactions involved in complex formation (see below), this result would mean that about two other phosphate groups are masked by the bound peptide molecule which is in agreement with the peptide size and model building.

It should be noted that two other factors at least contribute to the nonlinearity of the Scatchard plots. The electrostatic potential of the polynucleotide is expected to decrease as binding proceeds. The association constant for electrostatic binding will therefore decrease at high values of r. Also, electrostatic repulsion between bound peptides will contribute to the anticooperativity of binding.

Structure of the Complexes. There are several ways by which the fluorescence of Trp and Tyr residues could be quenched upon binding of the peptides to polynucleotides.

[1] Since the lattice is polar (3′→5′ polynucleotide), bound peptides may have two orientations with respect to the lattice and two binding constants should be introduced. If it is assumed that these two constants are equal then a factor of 2 should be inserted in eq 3 and the values of K_1 in Tables I and II should be divided by two. It might also be hypothesized that complexes A and B correspond to the two orientations of the bound peptide with respect to the lattice.

Table I: Association Constants for the Binding of Lys-Trp-Lys to Different Polynucleotides Obtained from Plots According to Eq 4.[a]

	K_2	$10^{-4}K_1$ M^{-1}	$10^{-4}K_1 \cdot (1 + K_2)$ (M^{-1})
Poly(A), 2°	2.2	1.9	6.1
26°	2.4	1.0	3.5
42°	2.7	0.37	1.4
Poly(U), 2°	3.8	0.65	3.1
26°	3.45	0.41	1.8
Poly(C), 2°	1.05	0.51	1.05
Poly(A)·poly(U), 2°	0.56	4.15	6.5
Poly(G)·poly(C), 2°	0.48	1.65	2.45

[a] Fluorescence measurements were made at pH 7 in 1 mM sodium cacodylate, 1 mM NaCl, and EDTA (2×10^{-4} M). The values of K_2 and K_1 were obtained by a least-squares procedure applied to plots of $\phi_F/(\phi_F - \phi_L)$ *vs.* $1/P_0$.

Table II: Association Constants for the Binding of Peptides to Poly(A) at 10° at pH 7 (Sodium Cacodylate (1 mM)–NaCl (1 mM)).

	K_2	$10^{-3}K_1$ (M^{-1})	$10^{-4}K_1 \cdot (1 + K_2)$ (M^{-1})
Lys-Trp-Lys	2.2	17	5.5
Lys-Tyr-Lys	3.85	3.1	1.5
Lys-TyrOMe-Lys	6.7	2.2	1.7

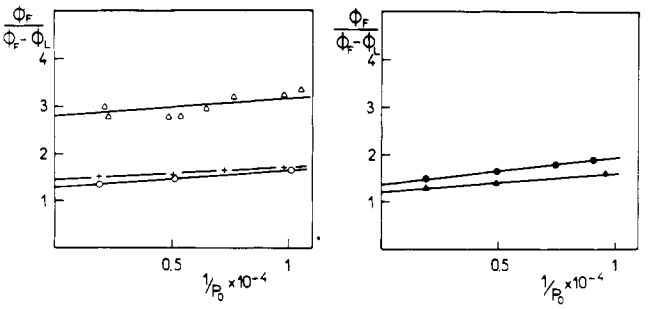

FIGURE 3: Plots of $\phi_F/(\phi_F - \phi_L)$ *vs.* $1/P_0$ according to eq 4: (left) Lys-Trp-Lys binding to poly(A) (+), poly(U) (○), and poly(A)·poly(U) (△); (right) binding of Lys-Tyr-Lys (●) and Lys-TyrOMe-Lys (▲) to poly(A).

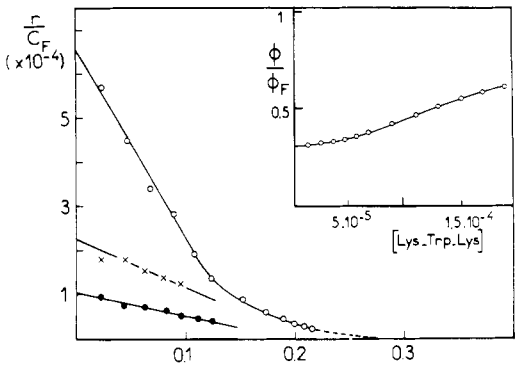

FIGURE 4: Scatchard plots for the binding to 5×10^{-4} M poly(A) of (○) Lys-Trp-Lys. (×) Lys-Tyr-Lys, and (●) Lys-Trp-Lys in the presence of 10^{-4} M Lys-Ala-Lys. (Inset) Concentration dependence of the ratio of fluorescence intensities of Lys-Trp-Lys in the presence and in the absence of 5×10^{-4} M poly(A). The screening effect of the polynucleotide has been taken into account.

When fluorescence results are compared to pmr data (Dimicoli and Hélène, 1974a,b), it is clear that the main interaction of the aromatic amino acid with bases involves a stacking of both aromatic rings. Low-temperature fluorescence measurements have also shown that stacking of aromatic amino acids with nucleic acid bases leads to a quenching of the amino acid fluorescence (Montenay-Garestier and Hélène, 1971; Hélène *et al.*, 1971a). Also energy transfer experiments have demonstrated that Trp residues in Lys-Trp-Lys or Lys-TrpOMe are stacked with adenine bases of poly(A) and act as energy traps for the triplet excitation energy which is delocalized in single-stranded poly(A) (Hélène, 1973). All these experiments lead to the conclusion that the aromatic amino acid is stacked with bases in complex B and that this stacking is responsible for fluorescence quenching.

The fluorescence of tyrosine could also be quenched as a result of excited-state proton transfer in a hydrogen-bonded complex (Feitelson, 1964). We investigated the behavior of the peptide Lys-TyrOMe-Lys where the hydroxyl group of tyrosine has been methylated and can no longer act as a hydrogen donor. The fluorescence of TyrOMe is also quenched in the presence of polynucleotides. The K_2 value is higher than that obtained with Lys-Tyr-Lys whereas K_1 is of the same order of magnitude (Table II). The differences in K_2 values between Tyr and TyrOMe might reflect differences in stacking energy with adenine bases.

Energy transfer from Tyr to bases which could take place at the singlet level (Montenay-Garestier, 1974) might also be involved in tyrosine fluorescence quenching. If such a transfer occurs, this will lead to a K_2 value which is too high. Notice that energy transfer from Trp to bases is unexpected (Montenay-Garestier, 1974) and that energy transfer from bases to Trp has been ruled out by our experiments (see above).

The structure of complex A should be such that the aromatic amino acid ring does not interact with the polynucleotide bases. The fluorescence quantum yield of the peptide in this complex would not be expected to be markedly affected as compared to the free peptide. As shown in Figure 4, nonaromatic peptides such as Lys-Ala-Lys compete efficiently with Lys-Trp-Lys for binding to polynucleotides. Binding of peptides to polynucleotides is very likely accompanied by a change in peptide conformation. The energy required for this conformational change as well as the initial peptide conformation might depend on the particular peptide investigated. This could explain the differences in association constants calculated for different peptides (Table II).

The results reported in Table I show that the values of K_1 depend on the nature of the base in the polynucleotide and decrease in the order poly(A) > poly(U) $\gtrsim$ poly(C). Since K_1 reflects mainly the electrostatic interaction between the peptide and the polynucleotide, this result would mean that the electrostatic potential of the polynucleotides decreases in the same order or that the relative position of phosphates groups is less favorable to electrostatic binding of peptide amino groups in poly(C) or poly(U) as compared to poly(A). It should be noted that a similar order was found for the binding of Mg^{2+} ions (Sander and Ts'o, 1971).

The value of K_2 is much smaller for double-stranded

than for single-stranded polynucleotides (Table I). This constant represents the concentration ratio of stacked and unstacked complexes. As expected, stacking is favored in single-stranded structures.

Ionic Strength and Temperature Dependence. To obtain quantitative information on the electrostatic contribution to the binding of peptides to polynucleotides, we have investigated the binding process at different ionic strengths. The results shown in Figure 5 demonstrate that K_2 is not markedly affected but that K_1 decreases when the ionic strength increases. It has been shown by Daune (1972) that the number of electrostatic bonds involved in a protein–nucleic acid complex could be determined from an analysis of the ionic strength dependence of the binding constant. Plotting log K_1 *vs.* log [NaCl] gives a straight line whose slope is equal to the number of electrostatic bonds (Figure 5). This number is about 2 for the binding of Lys-Trp-Lys to poly(A). Since we have already shown (from the pH dependence of binding) that the terminal NH_3^+ group of the peptide is involved in the interaction, the above result means that one of the lysyl side-chain amino groups is also involved. A comparison of circular dichroism and absorption changes induced in poly(A) by the binding of different oligopeptides and diamines led us to the conclusion that the N-terminal lysyl side chain was involved in the binding process (Durand *et al.*, 1975).

We have investigated the temperature dependence of the binding of Lys-Trp-Lys to poly(A). Whereas the value of K_2 slightly increased when the temperature was raised, the value of K_1 decreased (Table I). A value of $\Delta H \simeq -7$ kcal mol^{-1} was calculated from the temperature dependence of K_1. The structure of poly(A) changes with temperature so that the above values reflect changes in both destacking of poly(A) and binding of Lys-Trp-Lys. Since the number of binding sites does not appear to change markedly with temperature and since the value of K_2 does not vary significantly between 2 and 42° it can be concluded that Lys-Trp-Lys binds both to stacked and unstacked regions of poly(A).

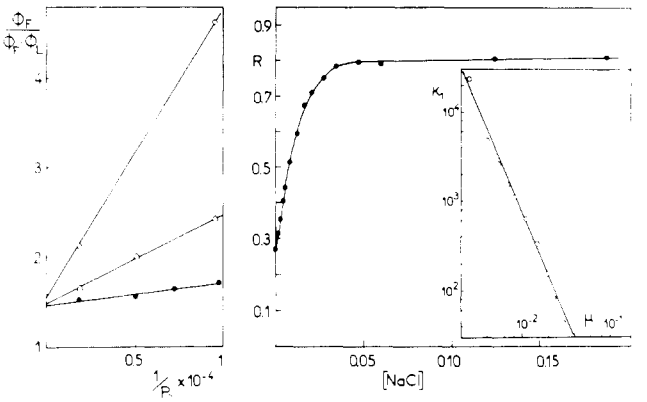

FIGURE 5: Ionic strength dependence of the binding of Lys-Trp-Lys to poly(A). (Left) Plots of $\phi_F/(\phi_F - \phi_L)$ *vs.* $1/P_0$ at different ionic strengths: 10^{-3} (●), 5.3×10^{-3} (△), and 9.6×10^{-3} (○). (Right) NaCl-induced dissociation of the complex Lys-Trp-Lys–poly(A) followed by fluorescence. The fluorescence intensity ratio was measured as described under Materials and Methods (see also Figure 1). Lys-Trp-Lys and poly(A) concentrations were 3.5×10^{-6} and 5.3×10^{-4} M, respectively. (Inset) Logarithmic plot of the association constant K_1 *vs.* ionic strength (μ). The slope (2) gives the number of electrostatic interactions involved in complex formation (see Daune, 1972).

Conclusion

Aromatic amino acids have been proposed to play an important role in protein–nucleic acid interactions (Brown, 1970; Hélène, 1971a,b; Gabbay *et al.*, 1972, 1973; Hélène and Dimicoli, 1972; Novak and Donhal, 1973; Dimicoli and Hélène, 1974a,b). Investigation of the binding of oligopeptides containing aromatic residues to nucleic acids of different structures and base compositions provides some insight into the origin and the nature of interactions that can take place between aromatic amino acids and nucleic acid bases. The results presented above show that oligopeptides of the general formula Lys-X-Lys (with X aromatic) form at least two different types of complexes when binding to single-stranded and double-stranded polynucleotides. One of them involves only electrostatic interactions between lysyl residues and phosphate groups without direct interactions of the aromatic amino acid with bases. In the second one, the aromatic residue X interacts with bases. This interaction involves a stacking of the aromatic ring with purine and pyrimidine bases which is expected to lead to a quenching of the aromatic amino acid fluorescence (Montenay-Garestier and Hélène, 1971; Hélène, *et al.*, 1971b). Tyr residues could also form hydrogen bonds especially in the case of double-stranded polynucleotides and DNA. Pmr and infrared data have already provided evidence for hydrogen bonding of Tyr to nucleic acid bases (Sellini *et al.*, 1973). Proton transfer in the excited state of these H-bonded complexes could lead to fluorescence quenching (Feitelson, 1964). One must also keep in mind that Tyr could transfer its excitation energy to nucleic acid bases and this could contribute to fluorescence quenching (Montenay-Garestier, 1974).

The results obtained by fluorescence spectroscopy must be compared to those obtained by pmr and circular dichroism (CD) (Dimicoli and Hélène, 1974a,b; Durand *et al.*, 1975). Pmr data provide clear evidence for a stacking interaction of the aromatic amino acid with bases. The upfield shifts of Trp and Tyr protons in the complexes Lys-X-Lys–poly(A) (with X = Trp or Tyr) are quite similar (Dimicoli and Hélène, 1974a,b). This suggests that in both cases stacking of the aromatic amino acid with adenine bases is the main interaction. The values of $K_1(1 + K_2)$ and n which can be obtained from CD measurements (Durand *et al.*, 1975) are in good agreement with those reported above.

It can be seen in Table I that the value of K_2 which measures the ratio of the concentrations of stacked and unstacked Lys-Trp-Lys complexes is much larger for single-stranded than for double-stranded polynucleotides. This means that stacking is favored, as expected, in single-stranded structures. Although the main force involved in complex formation is of electrostatic origin, the direct stacking interaction between the aromatic amino acid of Lys-X-Lys and the base contributes to the stability of the complexes. The observation that aromatic amino acids can "recognize" differently single-stranded and double-stranded structures is certainly important in protein–nucleic acid interactions. We have shown that a simple oligopeptide such as Lys-Trp-Lys is able to distinguish between denatured and native regions of DNA, for example in ultraviolet-irradiated DNA (Toulmé *et al.*, 1974). The conformational changes of the polynucleotides or DNAs which are induced by the binding of peptides could also be required for further activity of protein–nucleic acid complexes.

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Interaction of Aromatic Residues of Proteins with Nucleic Acids. Circular Dichroism Studies of the Binding of Oligopeptides to Poly(adenylic acid)†

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ABSTRACT: The binding of peptides containing lysyl and aromatic residues to poly(A) in its single-stranded form at pH 7 leads to a change of its circular dichroism (CD) spectrum, which is mainly due to the stacking of the aromatic amino acid with the bases of poly(A). Comparison is made between the binding of peptides having different primary structures which gives indications on the way the peptides bind to poly(A). A method is described which allows the calculation of the binding parameters from CD data. The magnitude of the association constant depends on the size of the aromatic ring and decreases in the order tryptophan > tyrosine > phenylalanine. The CD amplitude decreases linearly with the concentration of bound molecules. These results are discussed with respect to the role played by aromatic amino acids in complex formation between nucleic acids and proteins.

In previous papers we have shown that aromatic amines could interact with poly(A) to form stacked complexes with adenine bases (Hélène *et al.*, 1971a,b; Durand *et al.*, 1975). This leads to an unstacking of the adenine bases of poly(A) as shown by the change in the circular dichroism (CD) spectrum, as well as in the proton magnetic resonance (pmr) spectrum of poly(A) (Hélène *et al.*, 1971a,b; Razka and Mandel, 1971). This study has been extended to oligopeptides containing aromatic residues. Attention has been focused on peptides whose general formula is Lys-X-Lys, where X is tryptophan, tyrosine, phenylalanine, and, for comparison, alanine. At neutral pH, these peptides bear three positive and one negative charge, so that we can expect that the binding to poly(A) will be enhanced as compared to the aromatic amines as long as it depends on electrostatic forces. Furthermore, due to the increase of the binding constant, it has been possible to obtain a better

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