Does Tryptophan Intercalate in DNA? A Comparative Study of Peptide Binding to Alternating and Nonalternating A·T Sequences†

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ABSTRACT: The interactions of a tetrapeptide, lysyltryptophylglycyllysine *tert*-butyl ester (KWGK), with synthetic double-stranded polynucleotides [poly(dA)·poly(dT), poly[d(A-T)], poly(rA)·poly(dT), and poly(rA)·poly(rU)], *Escherichia coli* DNA, and single-stranded polynucleotides [poly(rA), poly(rU), poly(dA), and poly(dT)] were studied in a low-salt buffer by absorption and fluorescence spectroscopy. From fluorescence quenching data, we determined the two binding constants K_1 and K_2 of the two-step mechanism previously proposed for lysyltryptophyllysine binding to polynucleotides [Brun, F., Toulmé, J. J., & Hélène, C. (1975) *Biochemistry 14*, 558–563]. The first complex $(PN)_1$ is purely due to electrostatic interactions between the lysyl residues and the phosphate groups. The second complex $(PN)_2$ involves an additional stacking of the indole moiety of the tryptophyl residue with the bases (or base pairs) of the polynucleotide and is in equilibrium with $(PN)_1$. K_2 measures the ratio of the concentrations of stacked and unstacked complexes. The fluorescence decay of the tryptophyl residue in KWGK was not significantly different in the presence and in the absence of double-stranded polynucleotides in agreement with the previous model, which assumes total quenching of tryptophan fluorescence in complex $(PN)_2$ and identical fluorescence characteristics for free KWGK and complex $(PN)_1$. The stacking of the tryptophyl residue with A·T base pairs in alternating poly[d(A-T)] was found to be 10 times more efficient than that with nonalternating poly(dA)·poly(dT). Among A·T-containing double-stranded polynucleotides, poly(rA)·poly(dT) was found to be the most favorable for tryptophan stacking. A similar behavior was previously demonstrated for several intercalating agents such as ethidium bromide, propidium iodide, and daunomycin. We demonstrated that quinacrine also behaved similarly; its binding decreased in the order poly(rA)·poly(dT) > poly[d(A-T)] ≫ poly(dA)·poly(dT). With single-stranded polynucleotides, tryptophan stacking decreased in the order poly(dT) > poly(rU) > poly(rA) > poly(dA). These results are discussed with respect to intercalation of the tryptophyl residue between A·T base pairs and to the differences in conformation between alternating and nonalternating A·T-containing polymers. The tryptophyl residue behaves as an intercalating agent with strong preference for alternating as compared to nonalternating A·T base pairs in the deoxyribo polymers. Stacking of tryptophyl residues of DNA-binding proteins might be involved in base sequence discrimination.

The fundamental processes of gene regulation rest upon the recognition of nucleic acid base sequences and/or nucleic acid conformations. The control of gene expression is usually achieved by regulatory proteins, but small RNAs might also play an important regulatory function (Green et al., 1986). The molecular mechanisms for the recognition of nucleic acid base sequences are not fully understood yet [see Hélène and Lancelot (1982) for a review]. A study of small oligopeptides may shed light on these processes by providing simple systems where molecular interactions can be characterized in detail (Hélène & Maurizot, 1981). It was previously shown that short oligopeptides containing basic and aromatic residues could differentiate between single-stranded and double-stranded structures by inserting their aromatic residue between successive bases. Such a stacking interaction is favored in single strands as compared to a double helix [see Hélène and Maurizot (1981) for a review]. This appears to be an important factor in the recognition of single-stranded nucleic acids by single-strand binding proteins (Coleman & Oakley, 1980). The insertion of an aromatic amino acid side chain into an apurinic site might form the basis for the selective recognition of such sites in apurinic DNA (Behmoaras et al., 1981a,b).

In double-stranded DNA it might be expected that the extent of stacking between the indole ring of a tryptophyl residue and the bases is dependent upon the base sequence. Whether the indole ring is fully inserted between base pairs or partially inserted is still unknown. On the basis of physicochemical and viscosimetric studies, it was suggested that the stacking of tryptophan with bases could induce a bend in the DNA structure as a result of a partial insertion between successive base pairs (Gabbay et al., 1976; Hélène & Maurizot, 1981).

Recent investigations of intercalation between A·T base pairs in nonalternating poly(dA)·poly(dT)[1] and alternating poly[d(A-T)] showed that intercalation was strongly disfavored in the nonalternating polymer [Bresloff and Crothers (1981), Chaires (1983, 1986), Wilson et al. (1985), and Jones et al. (1986) and references cited therein]. Cooperative binding to poly(dA)·poly(dT) resulted from a conformational change in the polymer induced by intercalation. The number of base pairs per turn was determined to be lower in the nonalternating

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[1] Abbreviatons: KWGK, lysyltryptophylglycyllysine *tert*-butyl ester; EDTA, ethylenediaminetetraacetic acid; poly(dA)·poly(dT), double helix formed by the two homopolymers poly(dA) and poly(dT); poly[d(A-T)], double helix formed by two chains of alternating A's and T's.

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poly(dA)·poly(dT) (10.1) as compared to alternating poly[d-(A·T)] (10.6) (Strauss et al., 1981). A wedged structure was proposed earlier for poly(dA)·poly(dT) (Prunell et al., 1984; Trifonov, 1985). If tryptophyl residues partially inserted their indole ring between base pairs, a wedged polymer might be a better substrate by providing the appropriate structure for stacking interactions. Recent experiments have shown that poly(dA)·poly(dT) sequences adopt a nonusual conformation and induce a bending of the DNA double helix (Arnott et al., 1983; Koo et al., 1986; Hagerman, 1986).

Here we report a study of the interaction of the tetrapeptide lysyltryptophylglycyllysine *tert*-butyl ester with poly(dA)·poly(dT) and poly[d(A-T)]. A comparison is also made with other polynucleotides, either single stranded [poly(dA), poly(dT), poly(rA), poly(rU)] or double stranded [poly(rA)·poly(dT), poly(rA)·poly(rU)]. The *tert*-butyl ester of the peptide was used because removal of the negative charge borne by the terminal carboxylic group led to a more efficient electrostatic binding to nucleic acids without marked effects on stacking. This made it easier to quantitatively analyze stacking interactions of the tryptophyl residue with nucleic acid bases. Fluorescence spectroscopy was used to investigate peptide binding as it was previously shown that stacking of tryptophan with nucleic acid bases was accompanied by a complete quenching of fluorescence (Montenay-Garestier & Hélène, 1968, 1971; Montenay-Garestier et al., 1982). Fluorescence decay measurements were carried out to obtain further information on the environment of the tryptophyl residue in peptide–polynucleotide complexes. It is shown that the tryptophyl residue of the peptide KWGK behaves as an intercalating agent with a strong preference for alternating as compared to nonalternating A·T base pairs.

Materials and Methods

All polynucleotides were purchased from P-L Biochemicals except for poly(rA) (Boehringer, Mannheim). *Escherichia coli* DNA was obtained from Sigma Chemical Co., the peptide Lys-Trp-Gly-Lys-OtBu (KWGK) from Bachem, and quinacrine from Aldrich. All other chemicals used for preparing buffers were of analytical grade.

Unless otherwise specified, the polynucleotide solutions were made in a pH 7 buffer containing 1 mM NaCl, 0.2 mM EDTA, and 1 mM sodium cacodylate (referred to as SGH). The absorption experiments with quinacrine were performed in 100 mM NaCl and 10 mM sodium cacodylate. The pH of the solutions was always maintained at 7.0. The concentrations of polynucleotides were determined spectroscopically with the following molar extinction coefficients at 20 °C: poly(rA), ϵ_{260} = 9400 M^{-1}; poly(dA), ϵ_{257} = 8600 M^{-1}; poly(rU), ϵ_{260} = 8900 M^{-1}; poly(dT), ϵ_{264} = 8520 M^{-1}; poly(rA)·poly(rU), ϵ_{257} = 7000 M^{-1}; poly(rA)·poly(dT), ϵ_{257} = 6900 M^{-1}; poly(dA)·poly(dT), ϵ_{259} = 6000 M^{-1}; poly[d(A-T)], ϵ_{262} = 6600 M^{-1}; *E. coli* DNA, ϵ_{260} = 6500 M^{-1}. The concentrations of the peptide KWGK and quinacrine were determined with ϵ_{280} = 5600 M^{-1} and ϵ_{425} = 9750 M^{-1}, respectively. Double-stranded polynucleotides were characterized by determining the T_m (melting temperature) at the appropriate salt concentration and checked with the literature values.

Absorption Measurements. All the absorption spectra, melting curves, and difference spectra were recorded on an Uvikon 820 spectrophotometer. The temperature was usually maintained at 5 °C except for poly(rA)·poly(rU), where the spectra were recorded at 3 °C. A total of 400 μL of polynucleotide and peptide was pipetted into the separate compartments of tandem cells. After the base line was recorded, the solutions of the sample cell were mixed thoroughly, and the difference spectrum was recorded. The base line was checked once again after the solutions were mixed in the reference cell.

The experiments with quinacrine were carried out in 100 mM NaCl and 10 mM sodium cacodylate at pH 7.0 and 20 °C. The absorption changes of quinacrine (5×10^{-6} M) were followed by adding the polynucleotide in small aliquots until the molar ratio [nucleotide]/[quinacrine] was about 50.

Fluorescence Measurements. The fluorescence spectra were recorded on an Aminco-SPF 500 spectrofluorometer at 5 °C in 5-mm path length cells. The excitation wavelength was chosen at 290 nm, the isosbestic point in the difference absorption spectra (see Figure 1). The concentrations of the polynucleotide generally ranged from 1×10^{-4} to 5×10^{-4} M, and the peptide concentration was from 1×10^{-6} to 7×10^{-6} M. The fluorescence spectra of the peptide had a maximum at 355 nm both in the presence and in the absence of polynucleotides. The fluorescence quantum yields were expressed relative to that of the free peptide (Q_F). The screening effect of the polynucleotide was taken care of by the addition of sodium chloride at high concentration (0.5 M) to dissociate the complexes (Brun et al., 1975; Hélène & Maurizot, 1981).

On the basis of circular dichroism, NMR, and fluorescence studies, we previously showed that the binding of a small peptide containing aromatic and basic residues to a nucleic acid follows a two-step mechanism involving two complexes: $(PN)_1$ involves only electrostatic interactions between the NH_3^+ groups of the peptide and phosphate groups of the polynucleotide while $(PN)_2$ involves an additional stacking interaction of the aromatic side chain with nucleic acid bases. P represents the peptide and N the polynucleotide (eq 1).

$$P + N \overset{K_1}{\rightleftharpoons} (PN)_1 \overset{K_2}{\rightleftharpoons} (PN)_2 \qquad (1)$$

The fluorescence of the peptide in complex $(PN)_1$ was assumed (and confirmed from the fluorescence decay studies and fluorescence polarization; Montenay-Garestier et al., 1982) to have the same quantum yield as that of the free peptide while in $(PN)_2$ it is completely quenched. So, the apparent fluorescence yield Q of a peptide–polynucleotide mixture would be

$$Q = Q_F \frac{[(PN)_1] + [P]}{[P_0]}$$

where [P] and [P_0] are the concentrations of free and total peptide, respectively. At low concentrations of the peptide, Q tends to a limiting value, Q_L. One can evaluate K_1 and K_2 with (Brun et al., 1975)

$$\frac{Q_F}{Q_F - Q_L} = \left(1 + \frac{1}{K_2}\right) + \frac{1}{K_1 K_2} \frac{1}{[N]} \qquad (2)$$

where [N] is the concentration of polynucleotide. The correction due to the screening effect of the polynucleotide was done as explained earlier (Brun et al., 1975). It should be noted that the average fluorescence quantum yield of the bound peptide Q_B is given by $Q_B = Q_F/(1 + K_2)$.

Fluorescence Decay Experiments. Fluorescence decay studies were carried out on an Edinburgh instrument with the single photon counting technique. The data were analyzed after deconvolution by a nonlinear curve-fitting technique. Two exponential components were usually sufficient to fit the decay curves.

Fluorescence decay measurements of the peptide KWGK in the presence and absence of polynucleotides were all carried out in SGH buffer at 5 °C. The peptide concentration was

Table I: Fluorescence Decay Parameters of KWGK (3.9×10^{-5} M) in the Absence and in the Presence of Polynucleotides (9×10^{-4} M) in a pH 7.0 Buffer Containing 1 mM NaCl, 0.2 mM EDTA, and 1 mM Sodium Cacodylate at 5 °C (See Equations 3 and 4)[a]

	τ_1 (ns)	a_1	τ_2 (ns)	a_2	$\langle\tau\rangle$	χ^2	Q_B/Q_F
KWGK alone	2.0	0.70	3.9	0.30	2.6	1.1	1.00
poly(dA)·poly(dT)	2.0	0.64	4.1	0.36	2.8	1.2	0.88
poly[d(A-T)]	1.9	0.64	4.0	0.36	2.7	1.6	0.44
poly(rA)·poly(dT)	1.4	0.52	3.3	0.48	2.3	2.0	0.20

[a] The relative fluorescence quantum yields of the bound (Q_B) relative to the free peptide (Q_F) are given in the last column.

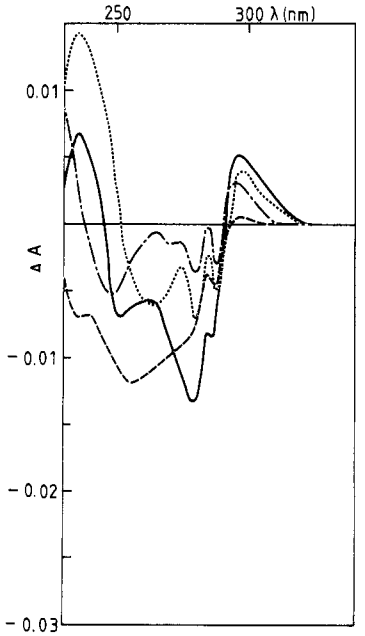

FIGURE 1: Difference absorption spectra of KWGK in the presence of poly(rA)·poly(dT) (—), poly[d(A-T)] (···) poly(dA)·poly(dT) (– –), and *E. coli* DNA (–·–) in 1 mM NaCl, 0.2 mM EDTA, and 1 mM sodium cacodylate, pH 7.0, at 5 °C. The concentrations of KWGK and polynucleotide were 2.4×10^{-5} and 1.55×10^{-4} M, respectively.

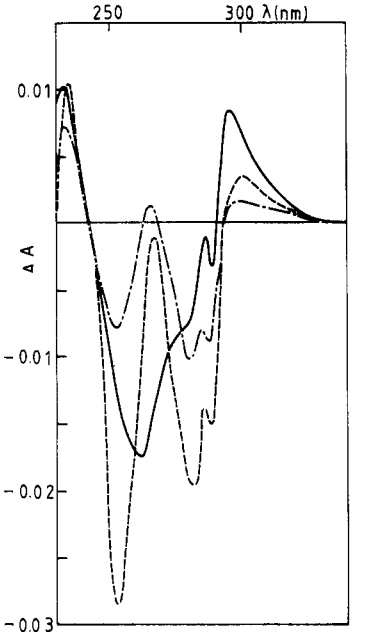

FIGURE 2: Difference spectra of KWGK (2.4×10^{-5} M) in the presence of single-stranded polynucleotides (1.6×10^{-4} M): poly(rA) (– –), poly(dA) (–·–), and poly(dT) (—). Experimental conditions are identical with those of Figure 1.

3.9×10^{-5} M, and the polynucleotide concentration was 9×10^{-4} M. At these concentrations, all the peptide molecules were bound to the polynucleotide. The excitation and emission wavelengths were chosen at 290 and 360 nm, respectively, after a wide range of wavelengths was scanned to obtain maximum emission intensity.

Results

Absorption Spectroscopy. Figure 1 shows the difference absorption spectra obtained after mixing KWKG with different polynucleotides: poly(dA)·poly(dT), poly[d(A-T)], poly(rA)·poly(dT), or *E. coli* DNA. The common feature that could be noted for all four systems was the presence of a positive peak around 295 nm and two negative peaks centered around 250–260 and 280 nm with a shoulder at 288 nm. The crossover point for all the difference spectra was around 290 nm. The ΔA observed at 295 nm was maximum for poly(rA)·poly(dT) and decreased in the order poly(rA)·poly(dT) > poly[d(A-T)] > *E. coli* DNA > poly(dA)·poly(dT). The ΔA value of the negative band at 280 nm was maximum for poly(rA)·poly(dT). The maximum negative contribution at 260 nm was observed for poly(dA)·poly(dT).

The difference spectra of KWGK recorded in the presence of single-stranded polynucleotides, poly(rA), poly(dA), or poly(dT), are shown in Figure 2. The basic features of the spectra were similar to those observed with double-stranded polynucleotides. The positive peak was centered around 300 nm, and negative bands were centered at 280 and 288 nm and at 250–260 nm. The positive peak decreased in the order poly(dT) > poly(rA) > poly(dA). Its amplitude was comparable to that observed with double-stranded polynucleotides (Figure 1). The negative bands were much more intense with single strands than with double helices.

Fluorescence Studies. As previously reported (Montenay-Garestier et al., 1982, 1983), the fluorescence of KWGK is quenched upon binding to polynucleotides. The extent of quenching depends on the nature of the polynucleotide. Analysis of fluorescence quenching data according to eq 2 (see Materials and Methods) requires that the stacked complex $(PN)_2$ (eq 1) has its fluorescence completely quenched whereas complex $(PN)_1$ has the same fluorescence quantum yield as the free peptide. If this assumption is correct, the fluorescence lifetime of the peptide should remain unchanged when bound to polynucleotides. In order to test this assumption, fluorescence decays were recorded for the free peptide and its complexes with poly(dA)·poly(dT), poly[d(A-T)], and poly(rA)·poly(dT). In all cases a good fit of the fluorescence decay curves was obtained with a sum of two exponential contributions according to eq 3.

$$I(t) = a_1 \exp(-t/\tau_1) + a_2 \exp(-t/\tau_2) \quad (3)$$

These two components were previously ascribed to two conformers of the peptide with different tryptophan environments (Montenay-Garestier et al., 1982, 1983). The fluorescence quantum yield of the peptide should be proportional to the average fluorescence lifetime $\langle\tau\rangle$ defined by eq 4.

$$\langle\tau\rangle = (a_1\tau_1 + a_2\tau_2)/(a_1 + a_2) \quad (4)$$

The two lifetimes (τ_1 and τ_2) and their respective contributions (a_1 and a_2) as well as the average lifetime $\langle\tau\rangle$ (ac-

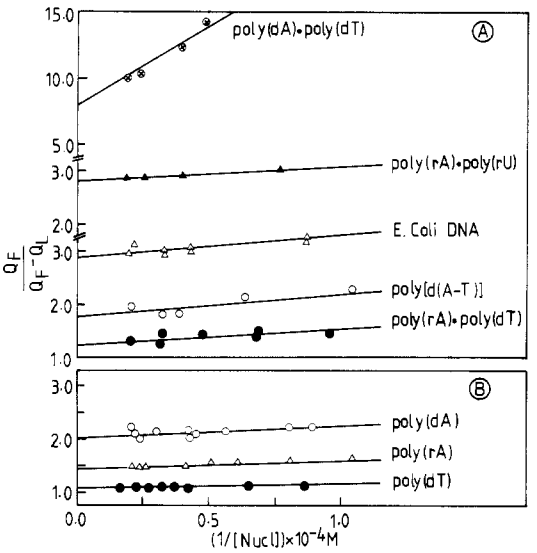

FIGURE 3: Analysis of fluorescence quenching data for the binding of KWGK to various polynucleotides according to eq 2. (A) Poly(dA)•poly(dT) (⊗); poly(rA)•poly(rU) (▲); *E. coli* DNA (△); poly[d(A-T)] (○); poly(rA)•poly(dT) (●). (B) Poly(dA) (○); poly(rA) (△); and poly(dT) (●). The fluorescence measurements were carried out in 1 mM NaCl, 0.2 mM EDTA, and 1 mM sodium cacodylate, pH 7.0, at 5 °C.

cording to eq 4) are given in Table I (χ^2 values are the sum of the squares of the weighted residuals). The relative quantum yields (Q/Q_F) are given in the last column of Table I. The results of Table I show that the lifetimes were not significantly affected upon binding of KWGK to the polynucleotides. The only exception was poly(rA)•poly(dT) for which the two lifetimes, τ_1 and τ_2, as well as the average lifetime $\langle\tau\rangle$, were slightly decreased. However the fluorescence of the peptide was strongly quenched (≈80%) when bound to poly(rA)•poly(dT), making it more difficult to obtain accurate data (as a matter of fact the χ^2 value was much higher). Also the residual fluorescence of the polynucleotide (which is about 25 times more concentrated than the peptide) affected the analysis of the fluorescence decay profiles in highly quenched samples even though its contribution to the steady-state intensities was low (<5%). The results presented in Table I were obtained after substraction of the polynucleotide fluorescence.

The results of Table I show that KWGK binding to poly(dA)•poly(dT) and poly[d(A-T)] and very likely also to poly(rA)•poly(dT) could be analyzed according to eq 1 and 2. A comparison of the relative fluorescence quantum yields of the bound versus the free peptide (Q_B/Q_F) and the average lifetime $\langle\tau\rangle$ demonstrated that part of the bound peptide was completely quenched [complex $(PN)_2$] and that the fluorescent complex $(PN)_1$ exhibited the same fluorescence lifetime—and therefore the same quantum yield—as the free peptide.

Binding Constants of KWGK–Polynucleotide Complexes. The binding constants K_1 and K_2 of the peptide–polynucleotide complexes were determined with fluorescence quenching data as explained under Materials and Methods. Plots of $Q_F/(Q_F - Q_L)$ versus $[N]^{-1}$ for KWGK in the presence of various polynucleotides are shown in Figure 3. The K_1 and K_2 values together with the overall association constants [$K = K_1(1 + K_2)$] are presented in Table II for the polynucleotides and for *E. coli* DNA. The K_2 values were obtained with good accuracy from an extrapolation of the plots shown in Figure 3 while K_1 values were obtained from the slope $(K_1K_2)^{-1}$ and the extrapolated K_2 values. It should be noted that the slopes of these plots were quite small, which caused a large uncertainty in K_1 values. However, we were more interested in comparing

Table II: Equilibrium Constants K_1 and K_2 and Overall Association Constants [$K = K_1(1 + K_2)$] for the Binding of KWGK to Single- and Double-Stranded Polynucleotides Containing rA, rU, dA, and dT[a]

polynucleotide	$K_1 \times 10^{-4}$ (M^{-1})	K_2	$K \times 10^{-4}$ (M^{-1})
single stranded			
poly(dA)	6.2	0.95	12.0
poly(rA)	2.6	2.3	8.6
poly(rU)	*b*	5.0	*b*
poly(dT)	*b*	8.6	*b*
E. coli DNA (denatured)	*b*	5.2	*b*
double stranded			
poly(dA)•poly(dT)	1.1	0.14	1.25
poly[d(A-T)]	1.5	1.3	3.45
poly(rA)•poly(dT)	0.8	4.0	4.0
poly(rA)•poly(rU)	10.0	0.5	15.0
E. coli DNA	3.9	0.5	5.9

[a] These values were determined from fluorescence quenching data with eq 2 under the following experimental conditions: t = 5 °C; buffer = 1 mM NaCl, 0.2 mM EDTA, and 1 mM sodium cacodylate, pH 7.0; concentration range = (KWGK) (0–7) × 10^{-6} M and (polynucleotide) (1–5) × 10^{-4} M. [b] The slopes of the plots according to eq 2 were too small to allow an accurate determination of K_1.

K_2 values, which measure the extent of stacking. More accurate K_1 values could be obtained at higher ionic strengths, but this was not investigated in further detail.

A large difference in the K_2 values was observed between alternating and nonalternating A•T-containing double-stranded polynucleotides. The K_2 value was almost 10 times higher for poly[d(A-T)] than for poly(dA)•poly(dT). However, the K_1 values were quite similar (1.5×10^4 M^{-1} and 1.1×10^4 M^{-1}, respectively). Comparison of poly(rA)•poly(dT) with poly(dA)•poly(dT) showed that the K_2 value was much higher (about 30 times) for the ribo–deoxyribopolymer hybrid than for the deoxyribo duplex without important changes in the K_1 values. The ribo polymer duplex poly(rA)•poly(rU) had an intermediate K_2 value as did *E. coli DNA*, which contains approximately 50% A•T base pairs. However, the K_1 value for poly(rA)•poly(rU) appeared to be much higher than that of the other double-stranded polynucleotides, suggesting a better electrostatic interaction of the peptide with the poly(rA)•poly(rU) structure. A similar behavior was previously observed with the tripeptide Lys-Trp-Lys (Brun et al., 1975).

For single-stranded polynuleotides, the K_2 value was highest for poly(dT) and then decreased in the following order: poly(dT) > *E. coli* denatured DNA > poly(rU) > poly(rA) > poly(dA). It is about 10 times easier to stack the tryptophyl ring with bases in poly(dT) than in poly(dA). The difference between poly(rU) and poly(rA) is much less (≈2 times). These differences reflect—at least in part—the stacking properties of the bases themselves (see Discussion).

Discussion

The formation of a complex between the peptide Lys-Trp-Gly-Lys (KWGK) and nucleic acids induces changes in the absorption spectra of both peptide and nucleic acid and these changes are reflected in the difference spectra shown in Figures 1 and 2 for double-stranded and single-stranded polynucleotides, respectively. The positive peak developed at 295–300 nm is due to the stacking of indole and bases as already observed in stacked monomers (Dimicoli & Hélène, 1971). The negative peaks at 280 and 288 nm are due to changes in the environment of the aromatic residue of the peptide. As a matter of fact, the positive band at 295–300 nm and the negative bands at 280–288 nm seem to reflect a red shift of the tryptophan absorption spectrum arising from stacking with nucleic acid bases (Toulmé & Hélène, 1980).

The fine structure seen in the difference absorption spectrum around 280 nm corresponds to the absorption spectrum of tryptophan. This indicates that the main contribution in this region arises from the tryptophyl ring and not from the bases. The broad negative band at 250–260 nm arises from conformational changes in the polynucleotide structure. The amplitude of the positive band at 295–300 nm can be taken as a measure of the extent of tryptophan stacking with the bases (Toulmé & Hélène, 1980) at least with adenine and thymine (uracil) bases whose main absorption is at shorter wavelengths. It is clear from Figure 1 that poly(rA)·poly(dT) forms a stronger stacked complex with KWGK as compared to other A·T-containing duplexes. The amplitude of the 295-nm band decreases in the order poly(rA)·poly(dT) > poly[d(A-T)] > DNA (*E. coli*) > poly(dA)·poly(dT). The ability of KWGK to form *stacked* complexes with single-stranded polynucleotides decreases in the order poly(dT) > poly(rA) > poly(dA) (Figure 2).

The amplitude at the negative band at 250–260 nm can be taken as a measure of the changes in nucleic acid conformation (Toulmé & Hélène, 1977). With both single-stranded and double-stranded polynucleotides the maximum of the negative band at 250–260 nm corresponds to the absorption maximum of the polynucleotide. The sign of this absorption change indicates that binding of KWGK to polynucleotides induces an unstacking of the bases as already demonstrated by proton magnetic resonance (Dimicoli & Hélène, 1974).

The order of stacking efficiency qualitatively estimated from absorption changes, and especially the amplitude of the positive band at 295–300 nm, is corroborated by the quantitative analysis carried out with fluorescence data. Fluorescence quenching and fluorescence lifetime measurements indicate that the peptide KWGK binds to double-stranded A·T-containing polynucleotides according to a two-step mechanism described by eq 1. The purely electrostatic complex $(PN)_1$ has a fluorescence quantum yield and lifetime identical with that of the free peptide. Complex $(PN)_2$ involves not only electrostatic interactions but also a stacking of the tryptophyl ring with the bases. Such a stacking is known from monomer studies to lead to complete quenching of the tryptophan and base fluorescence (Montenay-Garestier & Hélène, 1968, 1971).

As previously reported (Brun et al., 1975; Maurizot et al., 1978), the stacking of tryptophyl residues of peptides with bases in single-stranded polynucleotides depends of the base stacking properties of the polynucleotide. The order of decreasing K_2 values (Table II) correlates with the amplitude of the positive absorption band around 300 nm (Figure 2). Stacking of the indole ring of tryptophan with nucleic acid bases in a polynucleotide requires an unstacking of the bases. This is expected to be much easier in unstacked polypyrimidines, poly(dT) and poly(rU), as compared to stacked polypurines, poly(dA) and poly(rA). However, other contributions might be involved such as the local deformation of the polynucleotide backbone induced by electrostatic binding of the lysyl residues. It should be noted that single-strand binding proteins all have a strong preference for poly(dT) as compared to other single-stranded polynucleotides (Coleman & Oakley, 1980).

The results of fluorescence data analysis according to eq 2 presented in Table II indicate that stacking is strongly preferred in alternating poly[d(A-T)] as compared to nonalternating poly(dA)·poly(dT) and that the strongest stacking is observed in the hybrid poly(rA)·poly(dT). A similar behavior was previously reported for intercalating agents. For

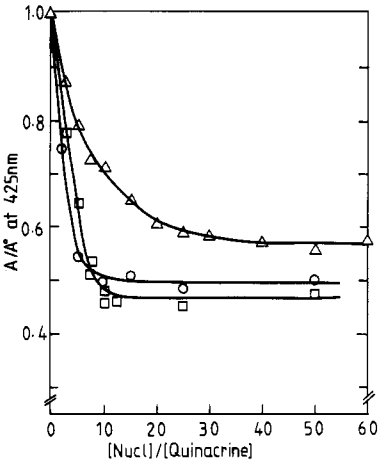

FIGURE 4: Plot of relative change in absorbance of quinacrine at 425 nm (A/A^0) versus the molar ratio of polynucleotide to quinacrine (5×10^{-6} M) in 10 mM sodium cacodylate and 0.2 mM EDTA, pH 7.0, at 20 °C for poly(dA)·poly(dT) (△), poly[d(A-T)] (○), and poly(rA)·poly(dT) (□).

example, ethidium bromide binding decreases in the order poly(rA)·poly(dT) > poly[d(A-T)] ≫ poly(dA)·poly(dT) (Bresloff & Crothers, 1981). A strong preference of intercalation for alternating poly[d(A-T)] as compared to nonalternating poly(dA)·poly(dT) was also reported for tilorone (Sturm et al., 1981; Sturm, 1982), daunomycin (Chaires, 1983, 1986), and propidium iodide (Wilson et al., 1985). We also observed a similar behavior for the binding of quinacrine, which is known to intercalate its acridine ring between base pairs (Wilson & Lopp, 1979; Baldini et al., 1981). Figure 4 shows titration curves recorded from the changes in absorption spectra of quinacrine upon binding to poly[d(A-T)] and poly(dA)·poly(dT). A quantitative analysis of quinacrine binding to DNA was previously reported (Wilson & Lopp, 1979). We did not engage ourselves in a quantitative analysis of quinacrine binding to A·T-containing polynucleotides, but Figure 4 clearly shows that quinacrine can be added to the list of intercalating agents that strongly prefer alternating poly[d(A-T)] as compared to nonalternating poly(dA)·poly(dT).

The nonalternating double-stranded polynucleotide poly(dA)·poly(dT) exhibits a series of peculiar properties. The number of base pairs per turn (10.1) is the lowest among all investigated sequences (Strauss et al., 1981). The fiber X-ray data were interpreted as arising from a heteronomous structure with the poly(dA) chain assuming an A-type conformation and the poly(dT) chain a B-type conformation (Arnott et al., 1983). Such a structure has received experimental support in solution from Raman spectroscopic studies (Jolles et al., 1985) even though the NMR data seem to indicate a fairly standard B conformation (Sarma et al., 1985; Behling & Kearns, 1986). Electrophoretic anomalies have been observed in DNA fragments containing successive A·T base pairs with all A's on the same strand (Wu & Crothers, 1984; Koo et al., 1986; Hagerman, 1986). This result has been ascribed to a bending of DNA at sections of nonalternating A·T base pairs resulting from a difference in conformation of the $(dA)_n$·$(dT)_n$ stretches.

A quantitative analysis of daunomycin (Chaires, 1986) and propidium iodide (Wilson et al., 1985) binding to poly(dA)·poly(dT) revealed a cooperative binding behavior. The final propidium intercalation complex seems to be similar to that observed with alternating poly[d(A-T)]. However, the initial interactions are significantly different. Even though the exact details of the cooperative step are not known yet, differences in hydration of the two polymers might be re-

sponsible for the observed effects (Jones et al., 1986). A striking observation is that the initial step for daunomycin and propidium iodide binding to poly(dA)·poly(dT) is characterized by a positive enthalpy and a positive entropy whereas in all other double-stranded polynucleotides binding is enthalpy driven (Chaires, 1986; Wilson et al., 1985). A similar result was reported for the nonintercalating agent netropsin (Marky et al., 1985) even though in this case binding to poly(dA)·poly(dT) was as strong as with poly[d(A-T)]. Other groove-binding ligands such as hydroxystilbamidine do not discriminate between the alternating and nonalternating A·T-containing polynucleotides (Festy et al., 1975). We have observed a similar behavior for the nonintercalating agent (diamidinophenyl)indole (DAPI) (A. Bêré, T. Montenay-Garestier, C. Hélène, unpublished results).

The results reported in this study for the tetrapeptide KWGK were all obtained at low peptide/phosphate ratio. This is a prerequisite condition for using eq 2 to analyze fluorescence quenching data (Brun et al., 1975). Therefore, the K_2 values reported in Table II refer to initial binding of the peptide to the polynucleotides. An analysis at higher saturation of the lattice is complicated by the superimposition of different complex structures (Ramstein, 1978). The absorption changes in the 250–260-nm range (Figure 1) strongly suggest that base-pair stacking in poly(dA)·poly(dT) is more strongly affected by KWGK binding than in the other polynucleotides. This is in agreement with the conformational change postulated to account for cooperative binding (Wilson et al., 1985). The *tert*-butyl ester of the tetrapepide Lys-Trp-Gly-Lys was used in these experiments because higher binding constants were achieved at low ionic strength due to the removal of the terminal negative charge. This allowed us to analyze stacking interactions with a better accuracy (as measured by K_2 values; see eq 1 and 2). The presence of the *tert*-butyl ester did not significantly perturb stacking of the tryptophyl residue as compared, e.g., to the tripeptide Lys-Trp-Lys. It should be noted that stacking of the tryptophyl residues with nucleic acid bases was less efficient for the peptide Lys-Gly-Trp-Lys than for Lys-Trp-Gly-Lys, which behaved similarly to Lys-Trp-Lys (Montenay-Garestier et al., 1982, 1983).

The results reported in Table II clearly indicate that the tryptophyl residue of the peptide at low binding density strongly prefers to stack with the bases in alternating poly[d(A-T)] rather than in the nonalternating poly(dA)·poly(dT). The RNA–DNA hybrid poly(rA)·poly(dT) is preferred to the alternating double-stranded deoxyribopolynucleotide. It was previously shown that this double-stranded polymer adopted a B-like conformation in fibers (Zimmerman & Pfeiffer, 1981). The order of stacking efficiency is in good agreement with that observed with intercalating agents (see discussion above). Nonintercalating agents such as netropsin, hydroxystilbamidine, or DAPI bind equally well to alternating and nonalternating A·T-containing polynucleotides even though a thermodynamic analysis reveals a difference in the binding mode.

From the results reported here it appears that the tryptophyl residue of a simple oligopeptide behaves as an intercalating agent. Even though the exact details of the structure at the intercalation site might be quite different from that of a "true" intercalator, the strong discrimination between alternating and nonalternating A·T base pairs might play a role in the recognition of base-pair sequences by the proteins involved in gene regulation. It should be noted that previous studies with tyrosine-containing peptides have indicated that tyrosine *does not stack* with bases in *double-stranded* polynucleotides but engages strong stacking interactions with bases in single-stranded polynucleotides (Dimicoli & Hélène, 1974b; Hélène & Maurizot, 1981). Therefore, tyrosine provides a better discrimination than tryptophan between single-stranded and double-stranded structures. But tryptophan "intercalation" in double-stranded structures might contribute to the discrimination between base-pair sequences.

Registry No. KWGK, 86370-03-8; poly(dA)·poly(dT), 24939-09-1; poly[d(A-T)], 26966-61-0; poly(rA)·poly(dT), 27156-07-6; poly(rA)·poly(rU), 24936-38-7; poly(rA), 24937-83-5; poly(rU), 27416-86-0; poly(dA), 25191-20-2; poly(dT), 25086-81-1; Trp, 73-22-3; adenine, 73-24-5; thymine, 65-71-4.

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Deoxyribonuclease I Sensitivity of the Ovomucoid–Ovoinhibitor Gene Complex in Oviduct Nuclei and Relative Location of CR1 Repetitive Sequences†

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ABSTRACT: The location of CR1 middle repetitive sequences within or near the boundaries of the ovalbumin DNase I sensitive domain has suggested that CR1 sequences may play a role in defining transition regions of DNase I sensitivity in hen oviduct nuclei. We have examined this apparent relationship of CR1 sequences and transitions of chromatin structure by determining the DNase I sensitivity in oviduct nuclei of a 47-kilobase region that contains five CR1 sequences and the transcribed ovomucoid and ovoinhibitor genes. We find that three of the CR1 sequences occur within a broad transition region of decreasing DNase I sensitivity downstream of the ovomucoid gene. Another CR1 is in a region of decreased DNase I sensitivity within the ovoinhibitor gene. The fifth CR1 sequence is in a DNase I sensitive region between the two genes but which is less sensitive to DNase I digestion than the region immediately upstream from the ovomucoid gene. Thus, the CR1 sequences occur within regions of reduced relative DNase I sensitivity, suggesting that CR1s could facilitate the formation of a chromatin conformation that is less sensitive to DNase I digestion. Unexpectedly, the noncoding strand of sequences within and immediately adjacent to the 5′ end of the actively transcribed ovomucoid and ovalbumin genes was less sensitive to DNase I digestion than their respective coding strands.

It is well documented that actively transcribed genes in chromatin are in a DNase I sensitive conformation (Weisbrod, 1982). The DNase I sensitive conformation can extend beyond the transcription unit. For example, the related ovalbumin, X, and Y genes are all contained within a 100-kilobase (kb)[1] DNase I sensitive domain in oviduct nuclei (Lawson et al., 1982). At the boundaries of the domain, there is a gradual transition to a DNase I resistant conformation. The DNase I sensitive conformation appears to be unique to those tissues in which the gene is expressed (Weintraub & Groundine, 1976; Lawson et al., 1982). Furthermore, the acquisition of a DNase I sensitive state appears to precede gene expression in some developmental programs (Stalder et al., 1980; Storb et al., 1981).

In order to gain further insight into the differentiation process, we have been interested in identifying those sequences that could play a role in defining the boundaries of DNase I sensitive domains in oviduct nuclei. A possible candidate for such a regulatory sequence is the CR1 repetitive sequence. Of the three CR1 sequences in the ovalbumin domain, all occur within or near the transition regions of DNase I sensitivity (Stumph et al., 1983). Moreover, a CR1 sequence downstream of the GAPDH domain is in a region of decreased DNase I sensitivity that may correspond to the boundary of a neighboring domain (Alevy et al., 1984). There are, however, no CR1 sequences within the GAPDH domain. CR1 sequences are about 300–500 bp in length (Stumph et al., 1984) and are present at 1500–7000 copies per haploid chicken genome (Stumph et al., 1981). Two regions of about 100 and 400 nucleotides, respectively, are particularly well conserved among

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[1] Abbreviations: kb, kilobase(s); Tris-HCl, tris(hydroxymethyl)-aminomethane hydrochloride; EDTA, ethylenediaminetetraacetic acid; Hepes, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; bp, base pair(s); GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

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