Interaction of Aromatic Residues of Proteins with Nucleic Acids. Binding of Oligopeptides to Copolynucleotides of Adenine and Cytosine†

Jean-Claude Maurizot,* Guy Boubault, and Claude Hélène

ABSTRACT: The binding of the peptide Lys-Trp-Lys to various single-stranded copolymers of adenine and cytosine has been investigated using circular dichroism (CD) and fluorescence measurements. Two types of complexes are formed, both involving electrostatic interactions between lysyl residues and phosphate groups. The fluorescence quantum yield of the first complex is identical with that of the free peptide. The other complex involves a stacking of the polynucleotide bases with the tryptophan residue whose fluorescence is quenched. The binding of the peptide leads to a conformational change of the copolymers as shown by the CD variations. The fluorescence and CD data have been analyzed according to the model involving the two types of complexes. The values of the binding constants have been studied as a function of the cytosine content of the copolymers. Analysis of the stacking process in term of nearest neighbor frequency demonstrates that this binding is sequence dependent and is favored in AA sequence as compared with AC, CA, and CC sequence. Thus this simple tripeptide is able to distinguish between various base sequences in a single-stranded nucleic acid.

The binding of small peptides to nucleic acids has been widely used to obtain information on the specific interactions involved in the association of nucleic acids with proteins. A particular attention was focused on those peptides bearing an aromatic amino acid whose aromatic ring could play a special role in the recognition phenomenon through stacking interaction with the nucleic acid bases (Hélène & Dimicoli, 1972; Dimicoli & Hélène, 1974a,b; Brun et al., 1975; Durand et al., 1975; Toulmé et al., 1974; Toulmé & Hélène, 1977; Novak & Dohnal, 1973, 1974; Gabbay et al., 1972, 1973, 1976a,b; Standke & Brunnert, 1975). In previous works from our laboratory we have shown that the tripeptide Lys-Trp-Lys binds to DNA (Toulmé et al., 1974; Toulmé & Hélène, 1977) and to single-stranded polynucleotides (Brun et al., 1975; Durand et al., 1975) according to a two-step model (eq 1)

$$\text{Lys-Trp-Lys} + \text{nucleic acid} \overset{K_1}{\rightleftharpoons} \text{complex I} \overset{K_2}{\rightleftharpoons} \text{complex II} \quad (1)$$

Both complexes involve electrostatic interactions between the phosphate groups of the nucleic acid and the lysyl residues and α-amino group of the peptide. The fluorescence quantum yield of complex I is identical with that of the free peptide. Complex II involves a stacking of nucleic acid bases with the aromatic amino acid whose fluorescence is completely quenched. In a previous paper (Brun et al., 1975) we have shown that the value of K_2, which represents the ratio of the concentrations of stacked and unstacked complexes is much smaller for double-stranded than for single-stranded conformations of nucleic acids. For example, $K_2 = 4.9$ for denatured *E. coli* DNA as compared with 0.3 for native DNA. Thus in a nucleic acid with single-stranded and double-stranded regions the peptide will preferentially interact with the single-stranded region. This was demonstrated in the case of DNA submitted to ultraviolet irradiation (Toulmé et al., 1974; Toulmé & Hélène, 1977).

The next problem to solve was to determine whether the binding of Lys-Trp-Lys to single strands involved a base-sequence specificity. One way of studying this problem is to use homopolynucleotides such as poly(A), poly(C), poly(U) . . . However, it is likely that the formation of complex II involves more than one base either because tryptophan is "inserted" between two bases or because its interaction with one base leads to the unstacking of this base with one of its neighbors. In each of these cases the study of the interaction of the peptide with an homopolynucleotide will give information on only one type of sequence since every base has the same environment in the polymer. To try to overcome this difficulty we have used statistical copolynucleotides of adenine and cytosine with different compositions. These polymers were chosen because it was known that poly(A) and poly(C) are in a single-stranded stacked conformation at neutral pH (Van Holde et al., 1965; Brahms et al., 1966, 1967a) and it seemed very likely that the copolymers will adopt the same type of conformation since no hydrogen bonding has been reported between adenine and cytosine at neutral pH. A study of the interaction of the peptide Lys-Trp-Lys with copolymers should yield information on the specificity of interaction of the peptide with various base sequences. We have used circular dichroism and fluorescence techniques to analyze the binding processes. The first technique allowed us to follow conformational changes of the polynucleotide upon peptide binding whereas fluorescence gave information on peptide behavior. These two methods were therefore complementary.

Experimental Section

Materials. The polynucleotides used in this study were prepared from adenosine and cytidine diphosphate with *Escherichia coli* polynucleotide phosphorylase. This enzyme was obtained according to the method of Williams & Grunberg-Manago (1964). The reaction mixture for the synthesis contained the following components: 2.5×10^{-3} M $MgCl_2$, 6×10^{-2} M Tris buffer at pH 9.0, 0.01 M total nucleotide diphosphates, and the appropriate amount of enzyme

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in a total volume of 20 mL. The reaction was carried out in a thermostated bath at 37 °C. Circular dichroism spectra were measured and used to follow the course of reaction. Aliquots (20 μL) of the reaction mixture were diluted with 3 mL of double-distilled water, and the amplitude of circular dichroism was rapidly measured. This value increased because of the difference of signal between the monomers and the polymer until a plateau was reached. When the circular dichroism signal attained its maximum value, the reaction was stopped by chilling. The polymers were purified by several treatments with phenol followed by extensive dialysis.

The average length of the various copolymers was determined from end residue analysis. The nucleoside and nucleotides obtained after total alkaline hydrolysis were separated on a small column of DEAE-Sephadex A-25 using a salt gradient, and their ratio was determined spectrophotometrically. The length of the copolymers was always larger than 80 residues (molecular weight 25 000).

Molar extinction coefficients and base ratios of all copolynucleotides were determined from the UV spectra of alkaline hydrolysates of the polymers (Loring, 1955). A sample of each copolymer was hydrolyzed at 37 °C in 0.3 N KOH for 24 h. The hydrolysate was then adjusted to pH 7 by addition of HCl and the UV spectrum measured and analyzed.

Solutions were made in a buffer containing NaCl (1 mM), sodium cacodylate (1 mM), and EDTA (0.2 mM) at pH 7.

The peptide L-lysyl-L-tryptophyl-L-lysine was purchased from Schwarz/Mann and used without further purification.

Fluorescence Experiments. Fluorescence measurements were performed with a modified Jobin-Yvon spectrofluorometer (Brun et al., 1975). Difference absorption experiments have shown that the binding of Lys-Trp-Lys to polynucleotides induces changes in the absorption spectrum. The excitation wavelength was chosen at the isosbestic wavelength (292 nm).

The analysis of fluorescence data was done as previously described (Brun et al., 1975). The method can be summarized as follows. According to eq 1 the overall fluorescence quantum yield (ϕ) of a solution containing the peptide and a nucleic acid is given by

$$\phi = \phi_F \left(\frac{[B] + [C_I]}{[B]_0} \right) \quad (2)$$

where ϕ_F is the fluorescence quantum yield of the free peptide, and [B], $[C_I]$, and $[B]_0$ are the concentrations of free peptide, complex I, and total peptide. Complex II is not fluorescent. Complex I has the same fluorescence quantum yield as the free peptide (see Brun et al., 1975; and introductory section). At low peptide concentration, i.e., when the number of occupied sites (r) is low as compared with the total number of sites (n), the overall fluorescence quantum yield tends toward a limit (ϕ_L) such that eq 3 is obeyed

$$\frac{\phi_F}{\phi_F - \phi_L} = \frac{1 + K_2}{K_2} + \left(\frac{1}{K_1 K_2} \right) \left(\frac{1}{[P]_0} \right) \quad (3)$$

where $[P]_0$ is the nucleic acid concentration. At constant $[P]_0$ extrapolation toward $[B_0] = 0$ of plots of ϕ vs. $[B]_0$ gives the values of ϕ_L. Then a plot of $\phi_F/(\phi_F - \phi_L)$ against $1/[P]_0$ gives straight lines and from the slope and y axis intercept, the values of K_1 and K_2 can be obtained.

Circular Dichroism Measurements. CD spectra were obtained with a Jouan II dichrograph in thermostated cells at 0 °C. The CD data are plotted in units of M^{-1} cm^{-1}. The analysis of CD data was done as previously described (Durand et al., 1975, 1976). The decrease of the CD signal of the polynucleotide upon addition of peptide is measured at various

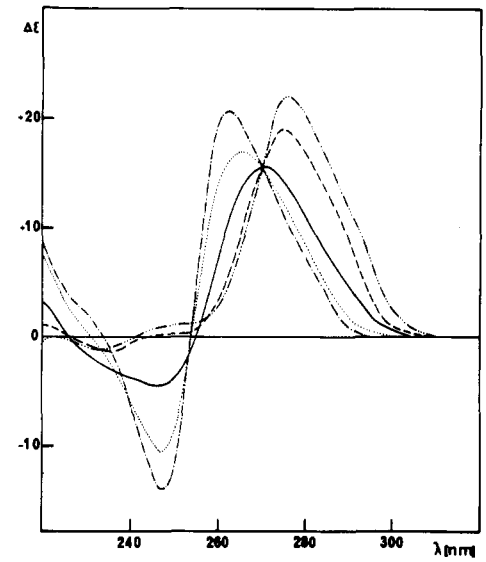

FIGURE 1: CD spectra of the various poly(A,C) in a pH 7 buffer containing 1 mM NaCl, 1 mM sodium cacodylate, 0.2 mM EDTA. Temperature 1 °C. (- - -) Poly(A); (· · · ·) poly(A,C) 27% C; (—) poly(A,C) 56% C; (- - -) poly(A,C) 90% C; (- · · · ·) poly(C).

concentrations of polynucleotide. The same relative decrease $\Delta\epsilon/\Delta\epsilon_0$ corresponds to the same value of $r = ([C_I] + [C_{II}])/P_0$, whatever the concentration of polymer $[P]_0$. The total concentration of peptide $[B]_0$ can be expressed as

$$[B]_0 = [B] + r[P]_0 \quad (4)$$

The values of $X = [B]_0/[P]_0$ corresponding to the same value of the relative decrease of the CD signal are related to the concentration $[P]_0$ by eq 5

$$X = r + [B]/[P]_0 \quad (5)$$

Plots of X vs. $1/[P]_0$ give the values of r and [B]. As already discussed in previous papers (Durand et al., 1975, 1976), the values of r calculated by this method correspond to the sum of all complexes whatever their respective effects on the CD spectrum of the polymer, as far as the distribution of ligands between complex I and complex II is independent of $[P]_0$.

Results

Conformation of the Poly(A,C). The CD spectra of the various poly(A,C) are shown in Figure 1, and the optical properties are summarized in Table I. These optical properties and the thermodynamic behavior of these copolymers have been studied and will be published separately. The main conclusions of this study can be summarized as follows.

(1) The distribution of bases (adenine and cytidine) in the copolynucleotides is statistical as shown by a CD analysis of nearest neighbor frequency (Maurizot & Boubault, in preparation).

(2) Under the experimental conditions used in this study (neutral pH and temperature of 0 °C), the copolymers are in a single-stranded stacked conformation with a stability similar to that of poly(A) or poly(C).

Changes in the CD Spectrum of Poly(A,C) Induced by Peptide Binding. Previous results (Durand et al., 1975) have shown that the binding of Lys-Trp-Lys to poly(A) induces a conformational change of the polynucleotide, which is reflected by a marked change of its CD spectrum. Figure 2 shows the effect of an equimolar concentration of Lys-Trp-Lys on the CD spectrum of a poly(A,C) containing 40% of cytidine. There is a large decrease of the positive band of the spectrum and a smaller decrease of the negative one. Difference between CD

TABLE I: Spectroscopic Parameters of the Various Poly(A,C) and of the Difference CD Spectra between Poly(A,C) in the Absence and in the Presence of an Equimolar Concentration of Lys-Trp-Lys.

	Absorption		Circular dichroism				Difference circular dichroism			
% C	ϵ_{260nm} (M^{-1} cm^{-1})	λ_{max} (nm)	λ_1 (nm)	λ_c (nm)	λ_2 (nm)	d^+/d^-	λ_1 (nm)	λ_c (nm)	λ_2 (nm)	d^+/d^-
0	10 100	256, 5	247, 5	253, 5	262, 5	1, 48	245	252	260	1, 90
15	9 700	256, 8	247, 5	253, 5	263	1, 51	245	252	260	1, 85
27	9 300	257	247, 5	253, 75	265	1, 70	245	252	261, 5	1, 90
40	8 800	258	247, 5	255	267	2, 05	246	252	262	1, 95
56	7 850	260	246, 5	255	271	3, 45	247	255	265	1, 85
68	7 000	262, 5	242, 5	255	274	5, 40	248	258	265	1, 90
90	6 100	267, 5			276	20				
100	5 700	268			276	∞				

[a] λ_1 and λ_2 are the maximum wavelengths of the negative and positive bands in the CD spectra; λ_c is the wavelength of the crossing point; d^+/d^- is the ratio between positive and negative band.

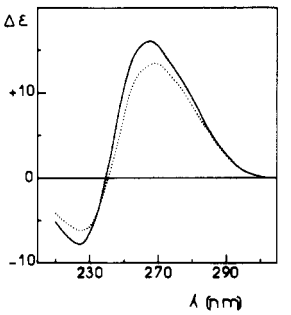

FIGURE 2: CD spectrum of poly(A,C) 40% of C, alone (5×10^{-4} M) (—) and in equimolar concentration with Lys-Trp-Lys (- - - -). Experimental conditions are similar to those of Figure 1.

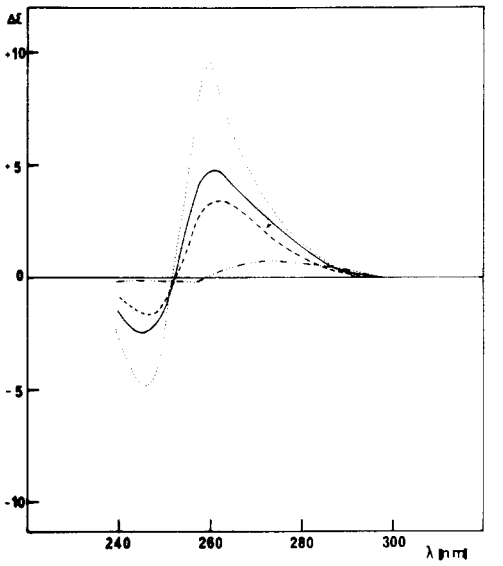

FIGURE 3: CD difference spectra between poly(A,C) alone and in the presence of equimolar concentration of peptide. Same conditions as in Figure 1; (· · · ·) 0% C (poly(A)); (—) 27% C; (- - -) 40% C; (- · · · ·) 90% C.

spectra recorded in the absence and in the presence of an equimolar amount of peptide are shown on Figure 3 and spectroscopic parameters related to these spectra are given in Table I. With the exception of the polymer with the highest content of cytidine (90%) and poly(C), all the difference spectra are of similar shape with a positive and negative band and are similar to the difference spectrum obtained when Lys-Trp-Lys binds to poly(A). The intensity of the difference spectra decreases as the cytidine content increases and no information could be obtained on poly(A,C) with a large content of cytidine since almost no change could be observed in their CD spectra. This might reflect the fact that for an equimolar mixture less peptide is bound when the cytidine content increases and/or that the effect of one bound molecule is less important. Quantitative analysis reported below will show that both phenomena contribute to the observed changes.

Quantitative Analysis of the CD Results. In previous papers we have presented a method which allowed the calculation of binding parameters from the CD variation of the polynucleotide as a function of peptide and polynucleotide concentrations (Durand et al., 1975). This method was applied to poly(A,C) with less than 50% of cytidine since the decrease of the CD spectrum for larger content of cytidine was too small to permit any computation. The binding process was analyzed according to the method of Scatchard, plotting r/C_F against r where $r = ([C_I] + [C_{II}])/P_0$ and $C_F = B_0 - ([C_I] + [C_{II}])$ (Figure 4 and Table II). As pointed out in previous papers the binding constant obtained by this method is an overall binding constant taking into account all types of complexes. In the scheme described for poly(A) (eq 1), this overall constant K is related to constants K_1 and K_2 by the relationship

$$K = K_1(1 + K_2) \quad (6)$$

There is a large decrease of the apparent binding constant when the content of cytidine increases, whereas there is no change in the apparent number of binding sites. However, it should be noted that the analysis was made only for small values of r ($r < 0.1$) and the Scatchard plots may show curvature for larger values of r as previously shown in the case of poly(A) (Durand et al., 1975).

This quantitative analysis also gives the relative decrease of the intensity of the CD signal as a function of r. As for poly(A) a linear relationship is found according to the equation

$$\Delta\epsilon/\Delta\epsilon_0 = 1 - \alpha r \quad (7)$$

The value of α which is a measure of the perturbation affecting the CD spectrum upon binding of one peptide molecule shows a large decrease as the cytidine content increases (Figure 4). Thus both the binding constant and the effect of bound peptide decrease and contribute to the observed effect for an equimolar mixture of poly(A,C) and Lys-Trp-Lys (see above).

Fluorescence Studies. The fluorescence of the tryptophan residue of Lys-Trp-Lys is quenched in the presence of poly(A,C). Data were analyzed according to eq 3 (Figure 5). The

TABLE II: Binding Parameters of Lys-Trp-Lys to the Various Poly(A,C) Determined from CD and Fluorescence Measurements.

	Circular dichroism		Fluorescence		
% C	K ($\times 10^{-4}$ M^{-1})	α	K_1 ($\times 10^{-4}$ M^{-1})	K_2	$K_1(1 + K_2)$ ($\times 10^{-4}$ M^{-1})
0	5.6	1.47	1.8	2.3	5.9
15	3.6	1.05	0.85	1.95	2.5
27	2.4	0.80	0.86	1.77	2.4
40	0.8	0.60	0.53	1.45	1.3
56		0.29	0.48	1.15	0.9
68			0.50	1	1
100			0.50	1.06	1

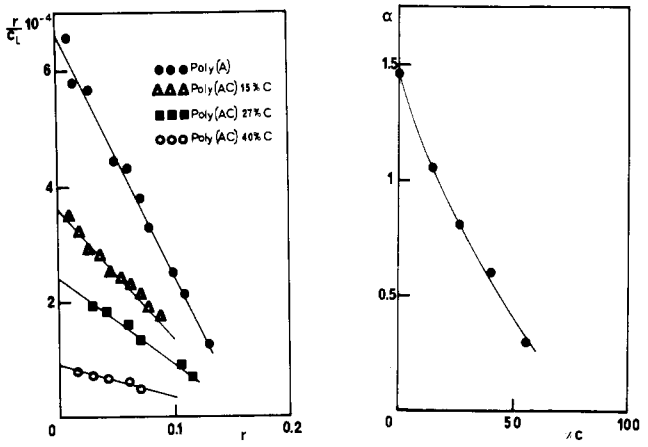

FIGURE 4: (Left) Scatchard plots for the binding of Lys-Trp-Lys to several poly(A,C); (●) poly(A); (△) poly(A,C), 15% C; (■) poly(A,C) 27% C; (○—○) poly(A,C) 40% C. Data were obtained from CD measurements. The lines correspond to least-squares analysis of the data. (Right) Influence of the base content of the copolynucleotide on the value of α (see eq 7).

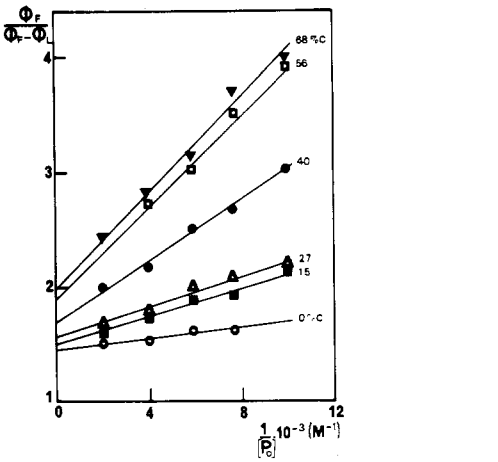

FIGURE 5: Fluorescence analysis of the binding of Lys-Trp-Lys to various poly(A,C). Same conditions as in Figure 2. The curve for poly(C) is similar to that for poly(A,C), 68% C.

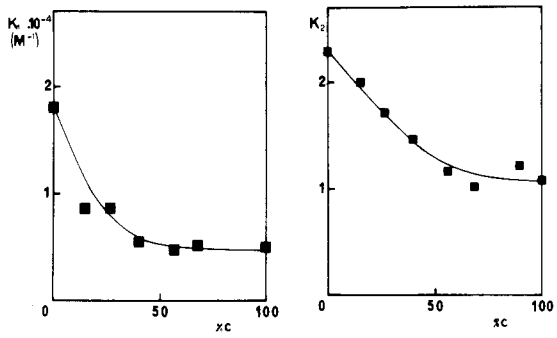

FIGURE 6: Variations of association constants for the binding of Lys-Trp-Lys to poly(A,C) as a function of cytidine content. (Left) K_1; (Right) K_2.

fact that the plots presented on Figure 5 are straight lines is a strong argument for the validity of eq 1 in the case of the poly(A,C).

The value of K_1 decreases rapidly until the polymer contains more than about 50% of cytidine. Then it levels off at the value found for poly(C). Since the results of the CD study reported above indicated that the number of binding sites was similar for the various copolymers (Figure 4), the decrease of K_1 reflects primarily the change in electrostatic binding.

The value of the constant K_2 is the ratio of the concentrations of stacked and externally bound peptides. In the case of poly(C) the molecules of peptides are equally distributed between the two types of complexes ($K_2 \simeq 1$), whereas in the case of poly(A) there is more peptide interacting with the bases. The variation of constant K_2 with the content of cytidine is roughly similar to that of constant K_1 (Figure 6).

There is a good agreement between the value of the overall binding constant obtained from CD measurement and that obtained from fluorescence experiments (Table II). The fluorescence analysis demonstrates that the variation of the apparent binding constant K is due to the decrease of both K_1 and K_2 [$K = K_1(1 + K_2)$].

Discussion

That the peptide Lys-Trp-Lys binds to the various poly(A,C) is clearly demonstrated by the change of the circular dichroism spectra of the polynucleotides and by the quenching of the peptide fluorescence. Experimental data were analyzed according to the two-step model already proposed for poly(A) and DNA.

$$\text{Poly(A,C)} + \text{Lys-Trp-Lys} \rightleftharpoons \text{complex I} \rightleftharpoons \text{complex II}$$

The very good agreement between the binding constants obtained from circular dichroism measurements (which give information on the polynucleotide) and those obtained from fluorescence experiments (which provide information on the peptide) strongly supports this model.

Our results clearly show that when the cytidine content of the polymer increases there is a decrease of all binding constants (K_1, K_2, K) as well as a decrease of the average effect (α) of one bound peptide molecule on the circular dichroism spectrum of the polynucleotides. The values of all these parameters decrease nonlinearly with cytidine content. The binding properties are thus depending on the base sequence either because the aromatic amino acid interacts with more than one base or because if it interacts with only one base this interaction modifies the environment of the neighboring bases. We will now examine the behavior of the different binding parameters.

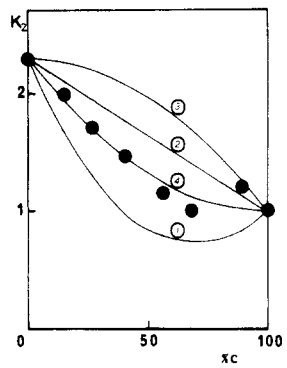

FIGURE 7: Variation of K_2 calculated according to the different assumptions described in the text. (1) $K_{2(AC)} = 0$; (2) $K_{2(AC)} = (K_{2(AA)} + K_{2(CC)})/2$; (3) $K_{2(AC)} = K_{2(AA)}$; (4) $K_{2(AC)} = K_{2(CC)}$; (●—●) experimental points.

The constant α is a measure of the relative decrease of the CD signal when one peptide binds to the polynucleotide. It thus reflects the conformational change of the polymer induced by peptide binding. In a previous paper (Durand et al., 1975) we have compared the effects of Lys-Trp-Lys and Lys-Ala-Lys on poly(A) conformation. It was concluded that the main conformational change was due to the formation of complex II. Thus one could think that the decrease of α with the increase in cytidine content just reflects the decrease of complex II vs. complex I. In fact this is not the case since the percentage of complex II $[K_2/(1 + K_2)]$ decreases much less than α. For example, in a copolymer containing 56% of cytidine the value of α is five times smaller than that for poly(A) (0.29 as compared with 1.47), whereas $K_2/(1 + K_2)$ shows only a small change (0.53 as compared with 0.70). If one takes into account only those molecules of Lys-Trp-Lys which are involved in complex II, the effect of one bound molecule decreases when the content of cytidine increases. This may reflect either that the sensitivity to deformation of poly(A) is higher than that of poly(C) or that the binding of Lys-Trp-Lys to poly(A) requires a conformational change to allow the indole ring to interact with adenine bases, whereas this would not be necessary in the case of poly(C).

The binding constant K_1 which corresponds to the formation of the electrostatic complex decreases when the cytidine content of the copolynucleotides increases. All these polynucleotides have single-stranded ordered conformation with base stacking. The change in the electrostatic constant probably reflects the change in the electrostatic potential of the polynucleotide. This electrostatic potential depends on the geometry of the phosphate groups. Recent data have shown that the geometries of the single-stranded helical conformation of poly(C) and of poly(A) are different (Arnott et al., 1976; Saenger et al., 1975). It can be calculated that the distance between two adjacent phosphate groups is 5.3 Å in poly(A), whereas it is 6.2 Å in poly(C). Such a difference might explain the large decrease of K_1 when going from poly(A) to poly(C).

Also the relative location of phosphate groups is important in allowing the formation of electrostatic bonds with the positively charged amino groups of the peptide. A change in phosphate-to-phosphate distances might require a larger deformation of the peptide and therefore be energetically unfavorable in poly(C) as compared with poly(A).

In complex II there is an interaction of the tryptophyl residue of the peptide with bases. If this interaction involved only one base, K_2 should be linearly related to the cytidine content in the copolymer. This is not what is experimentally observed. One can try to interpret our results with the hypothesis that the stacking interaction involves two neighboring bases. There will be four types of sites for the peptide Lys-Trp-Lys in a poly(A,C): AA, CC, AC, and CA. Constant K_2 will be a combination of constants $K_{2(AA)}$, $K_{2(CC)}$, $K_{2(AC)}$, and $K_{2(CA)}$ corresponding to each of these doublets. $K_{2(AA)}$ and $K_{2(CC)}$ are the values obtained with poly(A) and poly(C), respectively. If one makes the assumption that the doublets AC and CA give the same K_2 values, the average value of constant K_2 for a copolynucleotide is given by eq 8:

$$K_2 = K_{2(AA)}(1 - f)^2 + K_{2(CC)}f^2 + 2K_{2(AC)}f(1 - f) \quad (8)$$

where f is the fraction of cytidine in the copolymer; $(1 - f)^2$, f^2, $f(1 - f)$ are the fractions of each of the doublets if one is dealing with random copolymers. The values of $K_{2(AA)}$ and $K_{2(CC)}$ are respectively 2.3 and 1, and several assumptions can be made concerning $K_{2(AC)}$ (Figure 7).

(i) $K_{2(AC)} = 0$: this would mean that there is no interaction with the doublets AC or CA or that the peptide needs two similar bases to form complex II.

(ii) $K_{2(AC)} = (K_{2(AA)} + K_{2(CC)})/2$: each base of the doublet contributes in a similar way to the interaction with the peptide.

(iii) $K_{2(AC)} = K_{2(AA)}$: adenine would be the dominant base of the doublet and the interaction with AC is similar to that of poly(A).

(iv) $K_{2(AC)} = K_{2(CC)}$: cytidine would be dominant.

There is a very good fit between the curve calculated using this last assumption and the experimental values. This indicates that the stacking interaction with the doublets AC and CA is similar to that with CC. It is not possible to make a distinction between AC and CA and different values of K_2 may correspond to these two doublets. However, the average value must be similar to that of poly(C). These results provide evidence for a specificity if the stacking interaction of the peptide Lys-Trp-Lys with the sequence AA in the poly(A,C). Many factors are involved in the formation of complex II: the stacking interaction which exists between the bases before complex formation has to be disrupted; stacking interaction of the tryptophyl ring with one or two bases is introduced instead. There are no large differences between the stacking energies of the bases (Brahms et al., 1967b; Davis & Tinoco, 1968). The stacking energy of the indole ring may be larger with adenine than with cytidine due to the larger size of the purine ring, but the respective orientation of the two rings which must play an important role is unknown. As the value of K_2 depends on the balance between these different factors, it is difficult to determine the major contribution to the stability of the complexes.

Conclusion

This study demonstrates that the peptide Lys-Trp-Lys binds to copolymers of adenine and cytidine according to the scheme

$$\text{Lys-Trp-Lys} + \text{poly(A, C)} \rightleftharpoons \text{complex I} \rightleftharpoons \text{complex II}$$

Complex II involves stacking interactions between bases and the tryptophan residue. In this type of complex the peptide binds in such a way that at least two bases are involved. Our results can be quantitatively analyzed assuming that the polynucleotide is similar to a collection of dimers. This is in agreement with the current view concerning the conformation of single-stranded polynucleotides. Of course this is an approximation and the interaction with larger fragments of the sequence might also be involved.

Among the different available sequences in a poly(A,C) the stacking interaction of Lys-Trp-Lys decreases in the order AA

> AC and CC. A similar study with copolymers of adenine and uracil in single-stranded conformation has shown that the interaction is favored in UU sequence (Maurizot et al., 1978). Thus we have the following order of stacking interactions of the tryptophyl residue of Lys-Trp-Lys: UU > AU, AA > AC,CC.

Previous studies have shown that the peptide Lys-Trp-Lys has a selectivity of interaction with respect to the structure of the nucleic acid. It binds much more strongly to single stranded than to double stranded nucleic acids (Toulmé et al., 1974; Toulmé & Hélène, 1977). The present study shows that in single-stranded conformation there is also a selectivity of interaction with respect to the base sequence. This demonstrates that an aromatic amino acid such as tryptophan may participate in the selective recognition of nucleic acids by proteins.

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References

Arnott, S., Chandrasekaran, R., & Leslie, A. G. W. (1976) *J. Mol. Biol. 106,* 735–748.

Brahms, J., Michelson, A. M., & Van Holde, K. E. (1966) *J. Mol. Biol. 15,* 467–488.

Brahms, J., Maurizot, J. C., & Michelson, A. M. (1967a) *J. Mol. Biol. 25,* 465–480.

Brahms, J., Maurizot, J. C., & Michelson, A. M. (1967b) *J. Mol. Biol. 25,* 481–495.

Brun, F., Toulmé, J. J., & Hélène, C. (1975) *Biochemistry 14,* 558–563.

Davis, R. C., & Tinoco, I., Jr. (1968) *Biopolymers 7,* 223–242.

Dimicoli, J. L., & Hélène, C. (1974a) *Biochemistry 13,* 714–723.

Dimicoli, J. L., & Hélène, C. (1974b) *Biochemistry 13,* 724–730.

Durand, M., Maurizot, J. C., Borazan, H. N., & Hélène, C. (1975) *Biochemistry 14,* 563–569.

Durand, M., Borazan, H. N., Maurizot, J. C., Dimicoli, J. L., & Hélène, C. (1976) *Biochimie 58,* 395–402.

Gabbay, E. J., Sanford, K., & Baxter, C. S. (1972) *Biochemistry 11,* 3429–3435.

Gabbay, E. J., Sanford, K., Baxter, C. S., & Kapicok, L. (1973) *Biochemistry 12,* 4021–4029.

Gabbay, E. J., Adawadkar, P. D., & Wilson, W. D. (1976a) *Biochemistry 15,* 146–151.

Gabbay, E. J., Adawadkar, P. D., Kapicok, L., Pearce, S., & Wilson, W. D. (1976b) *Biochemistry 15,* 152–157.

Hélène, C., & Dimicoli, J. L. (1972) *FEBS Lett. 26,* 6–10.

Loring, H. S. (1955) in *The Nucleic Acids* (Chargaff, E., & Davidson, J. N., Eds.) pp 191–199, Academic Press, New York, N.Y.

Maurizot, J. C., Boubault, G., & Hélène, C. (1978) *FEBS Lett. 88,* 33–36.

Novak, R. L., & Dohnal, J. (1973) *Nature (London), New Biol. 243,* 155–157.

Novak, R. L., & Dohnal, J. (1974) *Nucleic Acids Res. 1,* 753–766.

Saenger, W., Reicke, J., & Suck, D. (1975) *J. Mol. Biol. 93,* 529–534.

Standke, K. H. C., & Brunnert, H. (1975) *Nucleic Acid Res. 2,* 1839–1849.

Toulmé, J. J., & Hélène, C. (1977) *J. Biol. Chem. 252,* 244–249.

Toulmé, J. J., Charlier, M., & Hélène, C. (1974) *Proc. Natl. Acad. Sci. U.S.A. 71,* 3185–3188.

Van Holde, K. E., Brahms, J., & Michelson, A. M. (1965) *J. Mol. Biol. 12,* 726–739.

Williams, F. R., & Grunberg-Manago, M. (1964) *Biochim. Biophys. Acta 89,* 66–89.