INTERACTION BETWEEN THE PEPTIDE LYSYL-TRYPTOPHANYL-LYSINE AND COPOLYNUCLEOTIDES OF ADENINE AND URACIL: SELECTIVITY OF INTERACTION

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Received 8 November 1977

1. Introduction

Particular attention has been focused on the binding of small peptides bearing an aromatic amino acid to nucleic acids since these aromatic rings could play a special role in the recognition phenomenon through stacking interaction with the nucleic acid bases [1–14]. The tripeptide LysTrpLys binds to DNA [6,7] and to single stranded polynucleotides [4,5] according to a two-step model (1):

$$\text{LysTrpLys + nucleic acid} \underset{}{\overset{K_1}{\rightleftharpoons}} \text{Complex I} \overset{K_2}{\rightleftharpoons} \text{Complex II} \qquad (1)$$

Both complexes involve electrostatic interactions between the phosphate groups of the nucleic acid and the amino groups of the lysyl residues of the peptide. In complex I the fluorescence quantum yield of tryptophan is identical to that of the free peptide. Complex II involves a stacking of the tryptophanyl ring with the nucleic acid bases and the fluorescence of the peptide is completely quenched. The value of K_2, which represents the ratio of the concentrations of stacked to unstacked complexes, is much smaller for double stranded than for single stranded nucleic acids [4,6,7].

We report here results on the interaction of LysTrpLys with copolynucleotides of adenine and uracil. Such a study was undertaken to determine whether the binding of the peptide to single strands involved a base sequence specificity. Previous experiments had shown that the constant K_2 was larger for poly(U) (K_2 4) than for poly(A) (K_2 2.3) [4]. It was of interest to follow the evolution of K_2 when the content of U increased in a copoly(AU) since one could think that the interaction of LysTrpLys involved more than one base and would thus be sequence dependent.

2. Experimental

2.1. *Materials*

The polynucleotides were prepared from adenosine and uridine diphosphate with *Escherichia coli* polynucleotide phosphorylase. They were purified by several treatments with phenol followed by extensive dialysis. Their compositions were determined by alkaline hydrolysis (0.3 N KOH) followed by neutralization and analysis of the absorption spectrum. These experiments also allowed us to calculate their molar extinction coefficients.

Solutions were made in a buffer containing NaCl (1 mM), sodium cacodylate (1 mM) and EDTA (0.2 mM) at pH 7. The peptide LysTrpLys was purchased from Schwarz-Mann.

2.2. *Fluorescence measurements*

Fluorescence measurements were done with a modified Jobin-Yvon spectrofluorimeter [4]. The analysis of fluorescence data was done as in [4].

2.3. *Circular dichroism experiments*

CD spectra were obtained with a Jouan II dichrograph in thermostated cells at 0°C.

3. Results

In the buffer used for this study the temperature of denaturation of poly(A).poly(U) is 23°C and the kinetics of double-strand formation is very slow (several days). One can think that in this buffer the poly(AU) with 10%, 20%, 30% of U are in single-stranded conformation. To avoid pairing of adenine and uracil, samples were prepared at a temperature higher than the T_m of poly(A).poly(U) and used immediately. At higher ionic strength formation of double-stranded and triple-stranded regions with hydrogen bonds between adenine and uracil may occur. This induces a change in the CD spectrum of the polynucleotides which can be used to check the single-strandedness of poly(AU) under the experimental conditions used to study peptide binding.

3.1. *CD experiments*

Addition of the peptide LysTrpLys to poly(AU) leads to a decrease of both the positive and negative bands of the CD spectrum (fig.1). Upon addition of salt this effect is reversed. However the intensities obtained are not those of poly(AU) alone but those obtained when salt is added to poly(AU) (fig.1). The addition of peptide produce a very small shift of the crossing point between the base line and the CD spectrum whereas the addition of the salt reverses this effect when added to the mixture (polynucleotide + peptide).

These experiments demonstrate that the peptide LysTrpLys interacts with the poly(A,U) and that the effect is similar to that observed when it binds to poly(A) [5]. There is almost no effect of the content of uracil on the relative decrease of the positive band of the CD spectrum.

3.2. *Fluorescence experiments*

Fluorescence measurements were used to determine the binding parameters of LysTrpLys to poly(AU) according to scheme (1). Analysis of fluorescence data was done as in [4] (fig.2). The values of K_1 and K_2 are presented in table 1 together with those of poly(A) and poly(U) for comparison.

The presence of uracil drastically affects the binding constant K_1 corresponding to the formation of a purely electrostatic complex. 20% uracil in a poly(AU) leads to a decrease of K_1 by a factor two,

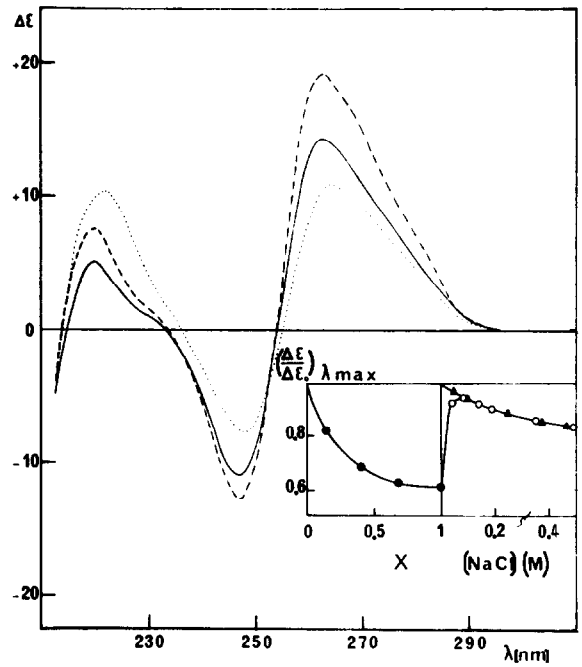

Fig.1. Left: CD spectra of poly(AU) with 10% uracil in a buffer 1 mM NaCl, 1 mM Na cacodylate, 0.2 mM EDTA, pH 7.0 (– – – –); in presence of 0.5 M NaCl (——); with an equimolar concentration of LysTrpLys (. . . .). Temp. 1°C. Inset: Effect of the addition of LysTrpLys (• • •) then of NaCl (o o o) on the CD signal of poly(AU) with 10% uracil. Effect of the addition of NaCl (▼ ▼ ▼) on the same polymer alone. X is the ratio of the concentration of LysTrpLys to that of copolymer.

and the K_1 value for a polymer with 30% uracil is close to that of poly(U). On the contrary one does not notice any change in the value of K_2 corresponding to the ratio between the concentration of the two types of complexes.

The behaviour of K_1 and K_2 indicates that their variations are not linearly related to the content of uracil in the polymer. This behaviour is similar to that observed in the interactions of poly(AC) with the same peptide [15]. This indicates that the binding properties of the peptide depend on the base sequence either because the peptide interacts with more than one base, or because its interaction with one base depends of the neighbouring bases.

The variation of constant K_1 corresponding to the formation of the electrostatic complex probably

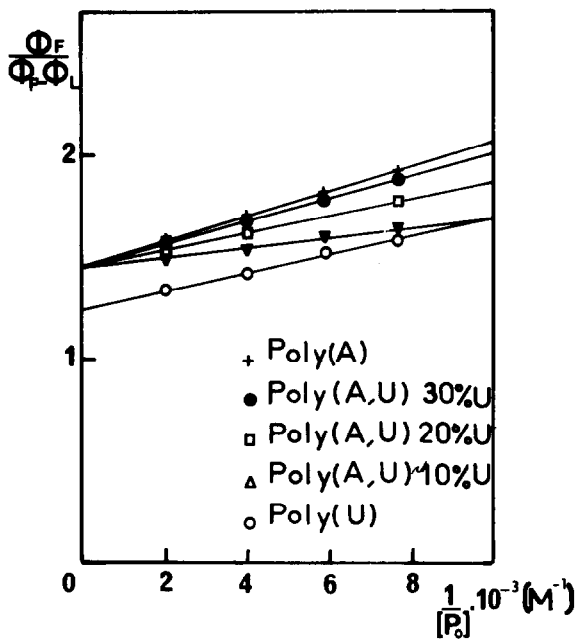

Fig.2. Fluorescence analysis of the binding of LysTrpLys, to poly(A), poly(U) and various poly(AU). The relationship used for the analysis is

$$\frac{\Phi_F}{\Phi_F-\Phi_L} = 1 + \frac{1}{K_2} + \frac{1}{K_1}\left(\frac{1}{P_0}\right)$$

where Φ_F is the quantum yield of the free peptide and Φ_L the limit of the overall quantum yield of the peptide in presence of a concentration (P_0) of polynucleotide when the concentration of peptide tends toward 0.

reflects the variation of the electrostatic potential of the polymer. The presence of uracil bases in the polynucleotide is likely to change the respective location of phosphate groups in their vicinity due to the weaker stacking tendency of uracil as compared to adenine.

As done in the case of a series of copolymers of adenine and cytosine [15] one can try to interpret our results with the hypothesis that the stacking interaction involves two neighbouring bases. There will be four types of binding sites for the peptide LysTrpLys in a poly(AU): AA, UU, UA and AU. The constant K_2 obtained with a given poly(A,U) will be a combination of constants $K_{2(AA)}$, $K_{2(UU)}$, $K_{2(UA)}$ and $K_{2(AU)}$ corresponding to each of these doublets. The values $K_{2(AA)}$ and $K_{2(UU)}$ are those obtained for poly(A) and poly(U), respectively. If one makes the assumption that the doublets AU and UA give the same K_2 value, the lack of variation for the poly(AU) with low contents of U (< 30%) can be explained only if the value of $K_{2(AU)}$ is very similar to that of $K_{2(AA)}$. Details of such calculation are given in [15].

The similarity between the value of $K_{2(AA)}$ and $K_{2(AU)}$ means that a sequence AU behaves like a sequence AA as far as the stacking interaction is concerned. Since $K_{2(UU)}$ is higher than $K_{2(AU)}$ and $K_{2(AA)}$ this implies that complex II will be found preferentially in UU sequences. In another study we have shown that in sequences containing adenine and cytosine the value of K_2 for AC or CA is identical to that of CC. This study demonstrates that the order of interaction for the stacked complex in a single stranded polynucleotide is:

UU > AA, AU, UA > AC, CA, CC

Table 1
Binding parameters of LysTrpLys to the various poly(A,U)

% Uracil	$K_1 \times 10^{-4}$ (M^{-1})	K_2
0	1.8	2.3
10	1.1	2.3
20	0.9	2.3
30	0.75	2.3
100	0.55	4

4. Conclusions

The formation of complex II is favoured in single-stranded DNA as compared to double-stranded DNA [6,7]. The results reported here demonstrate that in single-stranded polynucleotides the tryptophanyl residue of a small peptide can distinguish between different base sequences. This supports the idea that aromatic amino acid residues can contribute to the selective recognition of a nucleic acid by a protein.

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