INTERACTION OF OLIGOPEPTIDES CONTAINING AROMATIC AMINO ACIDS WITH NUCLEIC ACIDS. FLUORESCENCE AND PROTON MAGNETIC RESONANCE STUDIES

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Received 21 July 1972

1. Introduction

A study of interactions between amino acids and nucleic acid bases is required to explain the nature and specificity of recognition of nucleic acids by enzymes and proteins and to shed some light on the origin of the genetic code. We previously reported that aromatic amino acids could form complexes with nucleic acid bases either in frozen aqueous solutions [1, 2] or in concentrated fluid solutions [3]. More recently, evidence has been provided for the interaction of nucleotides with immobilized amino acids [4]. A study of complex formation between tyramine [2] or tryptamine [5] and nucleic acids in single-stranded or double-stranded conformations demonstrated a direct interaction of the phenol or indole rings with the purine and pyrimidine bases. Proton magnetic resonance studies provided evidence for intercalation of the indole ring between the bases of single-stranded poly A [2, 5, 6] and of double-stranded DNA [7]. This led us to propose that aromatic amino acids could be involved in the binding of enzymes or proteins to nucleic acids by direct interaction with the bases [8]. Such a proposal was also made in the case of tryptophan upon indirect evidence based upon changes in the denaturation temperature of DNA [9]. A study of the binding of oligopeptides containing aromatic amino acids to nucleic acids was undertaken to provide evidence for this direct interaction with the bases. In order to increase the affinity of the oligopeptides for nucleic acids, the aromatic amino acid was linked to lysine residues. The techniques of fluorescence and proton magnetic resonance were used to investigate the binding process.

2. Experimental

Most of the experimental conditions can be found in earlier publications [2, 3, 5]. The oligopeptides were purchased either from Schwarz-Mann (Lys Trp Lys, Trp Lys, Lys Tyr Lys) or from Cyclo Chemical Corporation (Lys Tyramide, Lys Lys Lys). Calf thymus DNA samples used in the PMR experiments were sonicated until the sedimentation coefficient was decreased to about 7. All measurements were carried out in a buffer containing 1 mM Na cacodylate and 1 mM NaCl at pH 7. The temperature was maintained at 20°. Tryptophan-containing peptides were excited at 295 nm and fluorescence intensity measured at 370 nm. For tyrosine-containing peptides, the corresponding wavelengths were 275 and 310 nm. Proton magnetic resonance experiments were carried out in D_2O with a Brüker HFX 90 MHz spectrometer. Chemical shifts were measured with respect to an external reference (HMS).

3. Results

3.1. *Fluorescence measurements*

The fluorescence of the aromatic ring (Trp or Tyr) of the oligopeptides Lys Trp Lys, Trp Lys, Lys Tyr Lys and Lys Tyr amide is quenched in the presence of DNA or poly A to a greater extent than already observed with tyramine [2] or tryptamine [5]. Using the method previously described [5], it is concluded that the fluorescence quantum yield of the oligopeptides bound to poly A is reduced to zero. In this case,

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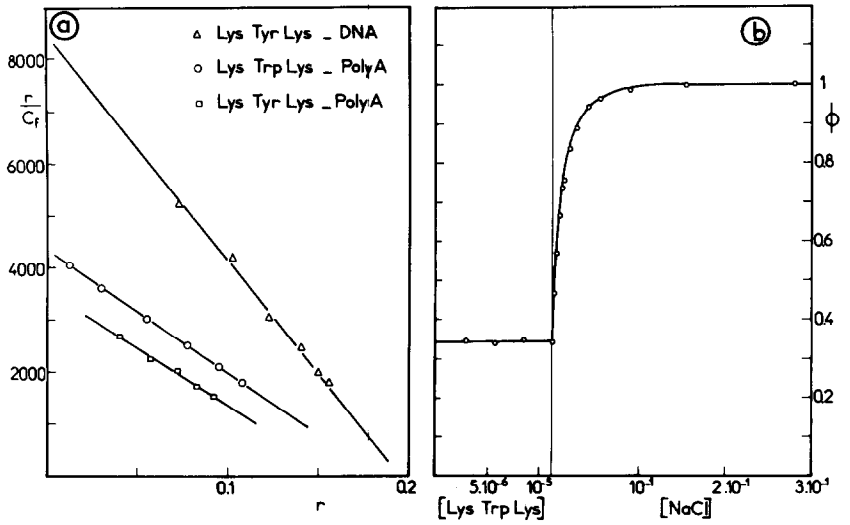

Fig. 1. a) Scatchard plots according to eq. (1) for the binding of Lys Tyr Lys and Lys Trp Lys to poly A and DNA at pH 7. The concentration of bound peptide was calculated from the apparent fluorescence quantum yield assuming that only the free peptide is fluorescent. b) Ionic strength effect on the binding of Lys Trp Lys to poly A. The overall fluorescence quantum yield is measured relative to that of the free peptide.

the fluorescence results can then be analyzed according to the Scatchard relationship:

$$\frac{r}{C_f} = K\,(n - r) \qquad (1)$$

where r is the concentration of peptide bound per phosphate, n is the number of binding sites per phosphate, K is the association constant and C_f the concentration of free peptide. Plots according to equation (1) are straight lines (fig. 1a) and the values of K and n can be determined (table 1).

The binding of the oligopeptides to DNA appears to involve several types of complexes. For example, a comparison of centrifugation and fluorescence quenching experiments demonstrates that fluorescence is quenched in only a part of Lys Trp Lys–DNA complexes. A complete analysis of the binding process in terms of association constants and number of binding sites must await a more detailed study.

The binding of oligopeptides to either poly A or DNA is strongly dependent upon ionic strength. The overall fluorescence quantum yield increases with NaCl concentration as a result of complex dissociation. The absorbance of poly A or DNA at the excitation wavelength does not change when NaCl concentration increases from 10^{-3} M to 3×10^{-1} M. The plateau obtained at high concentration (fig. 1b) was therefore used to account for the screening effect of the nucleic acid in the determination of relative fluorescence quantum yields. This was found to be more convenient than other methods previously used [5].

The oligopeptides Lys Tyr Lys and Lys Lys Lys do not absorb at 295 nm where Trp is excited. So their binding to poly A (or DNA) can be conveniently investigated by studying the induced dissociation of the complex Lys Trp Lys – Poly A (or DNA). This dissociation is accompanied by an increase of the overall

Table 1

Association constants and number of binding sites per phosphate for the binding of oligopeptides to poly A at pH 7 (5×10^{-4} M phosphate/l) in a buffer containing 1 mM sodium cacodylate and 1 mM sodium chloride.

Oligopeptide	K ($\times 10^{-4}$) M^{-1}	n
Lys Trp Lys	2.4	0.18
Lys Tyr Lys	2.2	0.16

Values are obtained from Scatchard plots deduced from fluorescence measurements (see fig. 1).

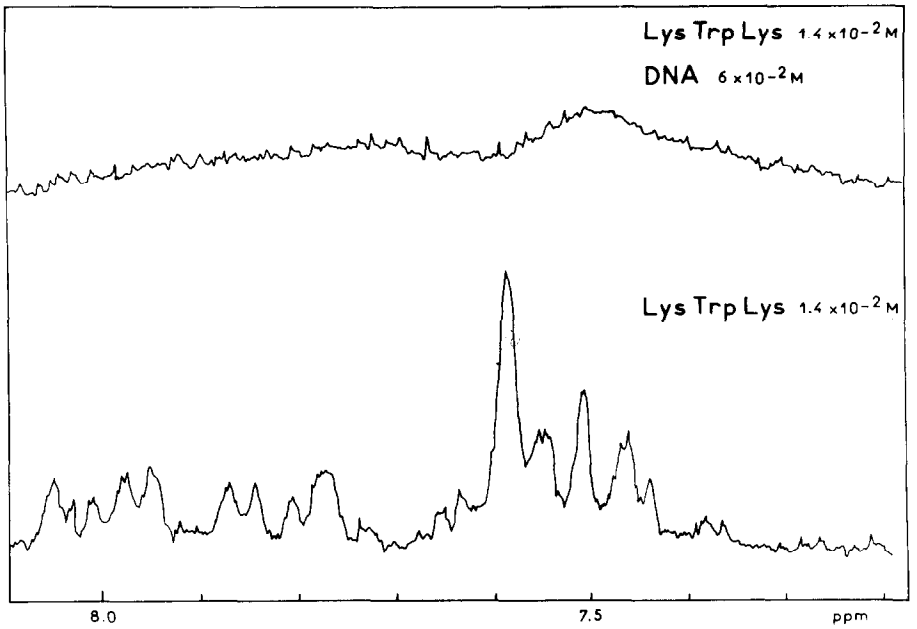

Fig. 2. PMR spectra of the aromatic protons of Lys Trp Lys (1.4×10^{-2}M) in the absence (lower spectrum) and in the presence (upper spectrum) of DNA (6×10^{-2}M).

fluorescence quantum yield of the tryptophan residue. In the case of Lys Tyr – poly A complex the same value of the association constant is obtained under direct excitation at 275 nm and from competition experiments.

Association constants for the binding to poly A and DNA of the oligopeptides investigated are about one order of magnitude higher than those obtained for the binding of the aromatic amines tyramine and tryptamine [2, 5, 8]. This is ascribed to the enhanced electrostatic interaction due to lysine residues whose essential contribution is demonstrated by the ionic-strength dependence of the binding.

The fluorescence of Trp and Tyr residues could be quenched as a result of interactions not only with bases [1, 2, 5] but also with phosphate groups [10, 11]. Proton magnetic resonance measurements which are described below provide evidence for a direct interaction with bases in several cases.

3.2. *Proton magnetic resonance measurements*

The proton resonances of the aromatic ring and, to a lesser extent, of the CH_2 (β) group of tryptophan residues are displaced upfield in the presence of both poly A and DNA (fig. 2). These upfield shifts are very likely due to the magnetic anisotropy of neighboring nucleic acid bases (mainly the purines in DNA) and they indicate that the indole ring of tryptophan residues in Lys Trp Lys and Trp Lys is stacked with the bases in poly A and also in DNA. The resonance peaks of the aromatic protons are markedly broadened in the DNA complexes (fig. 2) whereas those of the aliphatic protons are not. This probably reflects the more restricted mobility of the aromatic ring in the complex as compared to other parts of the oligopeptide. The linewidth of the CH_2 resonance peak of citrate ions is not affected by DNA, eliminating viscosity as a source of broadening. Upfield shifts and line broadening depend on the relative concentration of peptide and DNA. As expected, they increase when the peptide concentration decreases at constant DNA concentration. Fast exchange occurs on the PMR time scale between the free and bound peptide since only one resonance peak is observed for every proton and since integration experiments show that there is no loss of proton resonance intensity upon binding.

The phenol ring of tyrosine residues is also stacked with adenine bases in poly A as indicated by an upfield

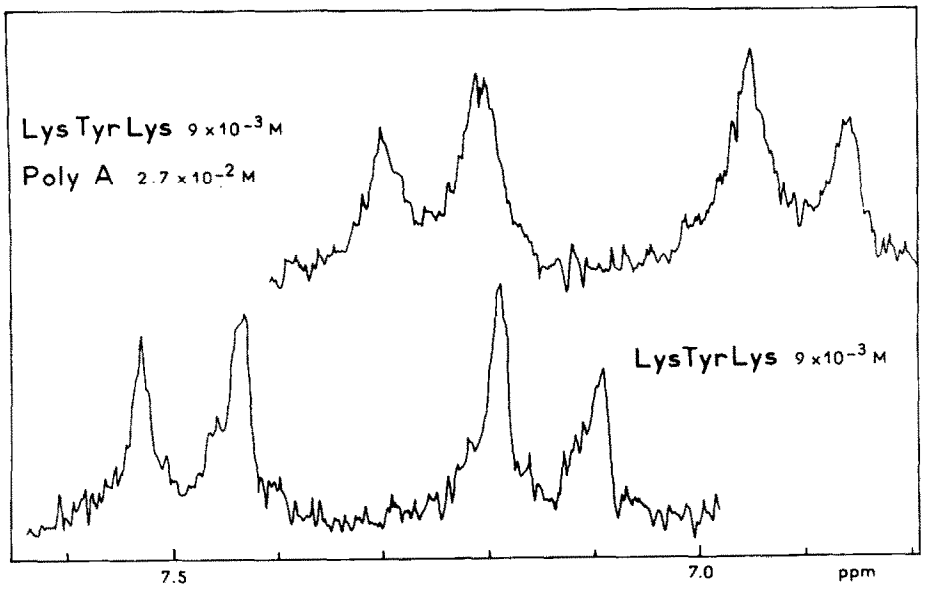

Fig. 3. PMR spectra of the aromatic protons of Lys Tyr Lys (9×10^{-3}M) in the absence (lower spectrum) and the presence (upper spectrum) of poly A (2.7×10^{-2}M).

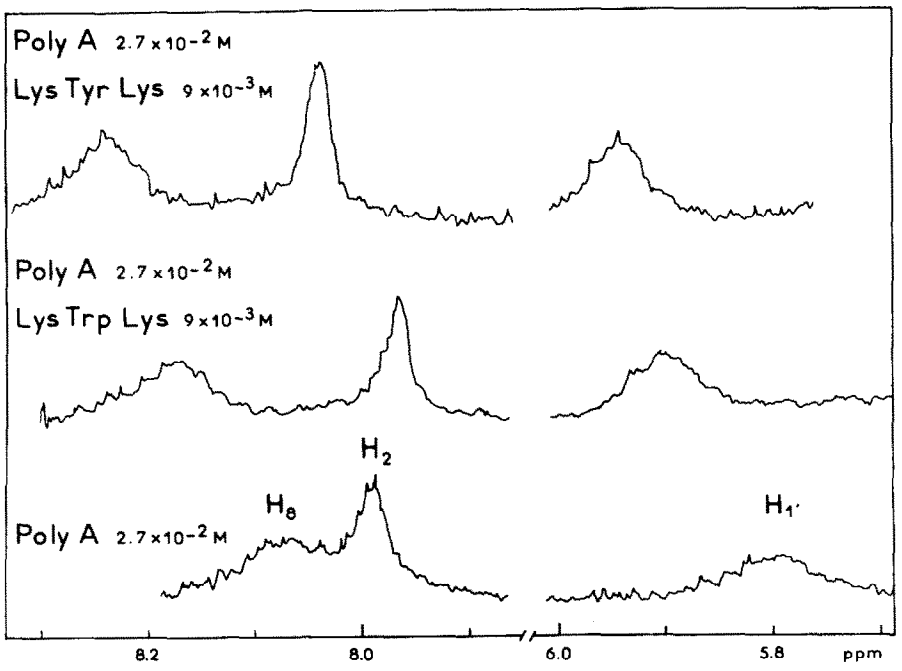

Fig. 4. PMR spectra of poly A (2.7×10^{-2}M) in the absence and the presence of Lys Trp Lys and Lys Tyr Lys.

shift of its proton resonances (fig. 3). In the complexes with DNA, the ring proton resonances of Lys Tyr amide are slightly shifted upfield and broadened whereas those of Lys Tyr Lys are only broadened but not shifted at all (although the association constant of Lys Tyr Lys is slightly higher than that of Lys Tyr amide). This would indicate either that the tyrosine ring essentially intercalates between pyrimidine bases which have a small magnetic anisotropy as compared to purines or, more likely, that the tyrosine ring of Lys Tyr Lys does not intercalate between bases.

Intercalation of the indole and phenol rings between adenine bases in single-stranded poly A is also demonstrated by the change that is observed in the PMR spectrum of poly A. In the presence of Lys Tyr Lys, the H_2 and H_8 resonance peaks of the adenine bases are shifted downfield and narrowed. This is due to a decrease in adenine–adenine interactions as already observed when tyramine binds to poly A [2]. The ring-current effect of the phenol ring is smaller than that of the adenine ring. This is not so in the case of the indole ring. As already reported in the case of tryptamine or serotonin [5], the H_2 resonance is slightly shifted upfield whereas the H_8 resonance undergoes a downfield shift (fig. 4).

4. Discussion

The results presented above demonstrate that the aromatic ring of tryptophan residues is able to interact with nucleic acid bases in oligopeptide–nucleic acid complexes. The indole ring intercalates between bases and forms stacked complexes both in single-stranded poly A and in double-stranded DNA. This result is also consistent with the observation that triplet–triplet energy transfer occurs from nucleic acid bases to the indole ring in frozen solutions of the complexes (C. Helene, to be published). Such a transfer is known to require a good overlap of the π electron clouds of the energy donor and acceptor.

The phenol ring of tyrosine is able to form stacked complexes with adenine bases in single-stranded poly A. But in Lys Tyr Lys–DNA complexes, tyrosine does not appear to intercalate between base pairs. However, the tyrosine fluorescence is quenched in these complexes. This suggests that the phenol ring of Lys Tyr Lys is involved in some type of interaction with either the DNA bases (e.g. hydrogen bonding of the hydroxyl group to the N 7 position of purines which is accessible in the large groove of the double helix) or with phosphate groups which are known to quench tyrosine fluorescence [11]. However, competition between different types of binding of the tyrosine ring could be observed depending on the particular environment of the tyrosine residue in the oligopeptide. For example, PMR experiments suggest that intercalation occurs to a certain extent in the complexes of DNA with Lys Tyr amide but not with Lys Tyr Lys.

These results demonstrate that a particular role could be played by the aromatic residues of proteins and enzymes interacting with nucleic acid, e.g., in the case of histone and non-histone proteins of chromatin or in aminoacyl-tRNA synthetase–tRNA interactions [12]. A specific role has been already attributed to a tryptophan residue in position 52, adjacent to a lysine residue, in the interaction between the protein and RNA components of tobacco mosaic virus [13].

A study of complex formation between oligopeptides containing aromatic amino acids and nucleic acids or polynucleotides of different base compositions should allow us to determine the specificity of interaction of the aromatic residues with the bases. The role of the secondary and tertiary structure of tRNA's on the binding of these oligopeptides is also under investigation.

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