Interactions of Aromatic Residues of Proteins with Nucleic Acids. II. Proton Magnetic Resonance Studies of the Binding of Tyramine and Tyrosine-Containing Peptides to Poly(adenylic acid) and Deoxyribonucleic Acid†

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ABSTRACT: The binding of tyramine, of its O-methylated derivative (*p*-methoxyphenethylamine), and of oligopeptides containing tyrosine residues (Lys-Tyr-Lys, Lys-Tyr-NH_2) to single-stranded poly(adenylic acid) (poly(A)) and double-stranded calf-thymus DNA has been studied using proton magnetic resonance. Important upfield shifts of the tyrosine ring protons are observed in complexes with poly(A) and denatured DNA as a result of stacking with nucleic acid bases. This stacking induces changes in the conformation of poly(A) which can be followed by proton magnetic resonance. Upfield shifts are much smaller in complexes with native DNA except for *p*-methoxyphenethylamine. An important broadening of the resonance lines occurs in DNA complexes even when only small upfield shifts are observed. The contribution of electrostatic forces is demonstrated by the ionic strength and pH dependence of binding. The results obtained by proton magnetic resonance are compared to those obtained by other physical methods. It is concluded that tyrosine rings can have two types of direct interactions with nucleic acid bases involving stacking and hydrogen bonding, respectively. Competition between these two types of interactions depends both on the environment of the tyrosine ring and on the nucleic acid structure. These results are discussed with respect to the role that would be played by tyrosine residues in the binding of proteins and enzymes to nucleic acids.

In the preceding paper, we have shown that the indole ring of tryptophyl residues of oligopeptides is able to form stacked complexes with bases both in single-stranded poly(adenylic acid) (poly (A)) and in double-stranded DNA (Dimicoli and Hélène, 1974). From previous infrared studies (Sellini *et al.*, 1973), it was concluded that the indole NH group would not form hydrogen bonds to nucleic acid bases. On the contrary, infrared and proton magnetic resonance (pmr) investigations have shown that tyrosine can form strong hydrogen-bonded complexes with nucleic acid bases involving the hydroxyl group of the phenol ring (Sellini *et al.*, 1973). Therefore, tyrosine residues of proteins might be involved in at least two types of interactions in the formation of nucleic acid–protein complexes: stacking of the phenol ring with purine and pyrimidine bases and hydrogen bonding of the hydroxyl group to acceptor positions (bases or phosphates) on the nucleic acid. In order to obtain evidence for these types of interactions, we have studied the binding to poly(A) and DNA of tyramine, its O-methylated derivative (*p*-methoxyphenethylamine), and two oligopeptides containing tyrosyl residues (Lys-Tyr-Lys and Lys-Tyr-NH_2). The technique of nuclear magnetic resonance was used to study the environment of the protons of bound molecules. The magnetic anisotropy of nucleic acid bases (ring current effects), especially of purines, will lead to a change in chemical shifts of neighboring protons. In particular, a stacking with the tyrosine ring will induce upfield shifts of the ring proton resonances (Pople *et al.*, 1959). Previous studies in our laboratory have shown that the tyrosine ring fluorescence is quenched upon binding to poly(A) and DNA (Hélène *et al.*, 1971). Conformational changes are induced in the nucleic acid as shown by circular dichroism studies (Hélène *et al.*, 1971). Preliminary investigations also demonstrated upfield shifts of the tyramine proton resonances in the presence of poly(A). We have extended these investigations to *p*-methoxyphenethylamine and Tyr-containing peptides as well as to DNA complexes. Oligopeptides containing lysyl residues (in addition to tyrosine) were chosen because electrostatic interactions with nucleic acid phosphate groups should enhance the binding strength. A preliminary report of these studies already appeared (Hélène and Dimicoli, 1972).

Materials and Methods

Poly(A) was purchased from Miles and used as the potassium salt. Sonicated calf-thymus DNA was a gift from Dr. Sicard. Solutions were made in a buffer containing 1 mM sodium cacodylate, 1 mM NaCl, and 0.2 mM EDTA. Tyramine and *p*-methoxyphenethylamine were obtained from Aldrich. The oligopeptides L-lysyl-L-tyrosine-α-L-lysine (Lys-Tyr-Lys) and L-lysyl-L-tyrosinamide (Lys-Tyr-NH_2) were purchased from Schwarz/Mann and Cyclo Chemical Corporation, respectively.

Pmr measurements were carried out with a Brüker HFX 90-MHz spectrometer and chemical shifts were measured with respect to an external reference (hexamethyldisiloxane). Corrections for changes in bulk susceptibility with temperature and ionic strength were done by investigating the resonance lines of an internal *tert*-butyl alcohol sample.

Results

Binding of Tyramine and p-Methoxyphenethylamine to Poly(A) and DNA. In order to determine the possible role of the hydroxyl group of tyrosine in the binding of tyrosine-

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containing peptides to nucleic acids, we first compared the behavior of tyramine and of its *O*-methylated derivative, *p*-methoxyphenethylamine. The proton resonances of tyramine and *p*-methoxyphenethylamine are shifted to higher fields in the presence of either poly(A) in its single-stranded conformation (pD 7) or DNA in its native double-stranded structure or in its denaturated state (Table I, Figure 1). The

TABLE I: Changes in Chemical Shifts (in Hz at 90 MHz) of Proton Resonances of Tyramine and Its *O*-Methyl Derivative (1.8×10^{-2} M) in the Presence of Poly(A) and Native and Denatured DNA.[a]

		H_m	H_o	CH_3
Poly(A)	Tyramine	14.0	14.8	
2.9×10^{-2} M pD 7.3 $T = 299°K$	*p*-Methoxyphenethylamine	19.1	23.8	14.1
Native DNA	Tyramine	3.5	2.7	
2.5×10^{-2} M pD 6.5 $T = 298°K$	*p*-Methoxyphenethylamine	7.5	7.6	4.1
Denatured DNA	Tyramine	8.9	9.3	
2.5×10^{-2} M pD 6.5 $T = 298°K$	*p*-Methoxyphenethylamine	16.1	18.5	9

[a] H_m and H_o are protons in meta and ortho positions with respect to the OH (or OCH_3) group.

largest upfield shifts are observed for the ring protons (H_m and H_o refer to the protons in position meta and ortho, respectively, with respect to the OH or OCH_3 group). The upfield shifts decrease in the order $H_o \geq H_m > CH_3 > CH_2(\beta) > CH_2(\alpha)$, *i.e.*, they decrease when going farther away from the phenyl ring. A broadening of all the resonance lines is also observed, particularly in the case of *p*-methoxyphenethylamine.

The observed upfield shifts depend on the relative concentration of amine and nucleic acid. At constant nucleic acid concentration, the upfield shifts of the amine protons increase when the amine concentration decreases. Extrapolation to zero amine concentration (Figure 2) gives the values reported in Table II for the changes ($\overline{\Delta\delta}$) in chemical shifts. In the preceding paper (Dimicoli and Helene, 1974), the extrapolated upfield shifts ($\overline{\Delta\delta}^*$) were shown to represent the differences in chemical shifts between the free and bound molecules or an average over the different types of complexes that could exist

$$\overline{\Delta\delta}^* = \frac{\sum_i K_i n_i \Delta\delta_i}{\sum_i K_i n_i} \qquad (1)$$

where K_i is the association constant and n_i is the number of binding sites per phosphate for complex of type i; $\Delta\delta_i$ is the difference in chemical shift between free and bound molecules in complex i. This relation was derived under the assumption that $K_i n_i P_0 \gg 1$, where P_0 represents the concentration of the nucleic acid expressed in moles of phosphate/liter.

Examination of the $\overline{\Delta\delta}^*$ values reported in Table II reveals the following features. (i) Upfield shifts are smaller for tyramine complexes than for those of its O-methylated derivative, especially in the case of DNA. (ii) Upfield shifts are much smaller for DNA than for poly(A) complexes, especially in the case of tyramine. It must be noticed (Table I) that denatured DNA gives rise to much higher upfield shifts than native DNA. (iii) All values are smaller than those obtained with tyramine–AMP complexes (calculated under the assumption of 1:1 complex formation; see Hélène *et al.*, 1971). (iv) $\overline{\Delta\delta}^*$ values for different protons of the phenol ring are similar.

Interaction of the negatively charged phosphate groups with the phenol ring would certainly result in an upfield shift of the ring proton resonances due to an increase of electron density. However, these effects would not be expected to be as high as those reported in Tables I and II (with the possible exception of tyramine–DNA complexes). The observed up-

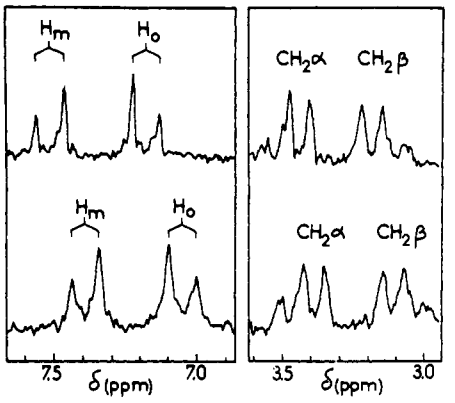

FIGURE 1: Proton magnetic resonance spectra of 10^{-2} M tyramine in the absence (upper spectrum) and the presence (lower spectrum) of 1.3×10^{-2} M poly(A) at 302°K; pD 7.4. H_m and H_o refer to protons in positions meta and ortho, respectively, with respect to the hydroxyl group.

TABLE II: Upfield Shifts (in Hz) at 90 MHz of the Ring Protons of Tyramine, Its *O*-Methylated Derivative, and Oligopeptides Containing Tyrosine Residues Extrapolated to Zero Concentration in the Presence of Poly(A) and DNA.[a]

	Poly(A)			DNA		
	H_m	H_o	CH_3	H_m	H_o	CH_3
Tyramine	22.5 (26)	23.5 (30)		6	5.5	
p-Methoxyphenethylamine	30.5	38	25.0	18.5	19.0	11
Lys-Tyr-NH_2	25	26		9	8	
Lys-Tyr-Lys	27	29.5		2.5	2.5	

[a] The concentration of the nucleic acid is 2.5×10^{-2} M. The pD is 6.9 in the case of poly(A) and 6.5 in the case of DNA. Measurements were performed at 298°K with poly(A) and at higher temperatures (315°K) with DNA because line broadening prevents accurate determination of chemical shifts at lower temperatures in the latter case. The values in parentheses in the case of tyramine represent the values obtained at 277°K. The upfield shifts of tyramine H_m and H_o protons in tyramine–AMP complexes are 45 and 41 Hz, respectively (values calculated on the basis of 1:1 complex formation; see Hélène *et al.*, 1971).

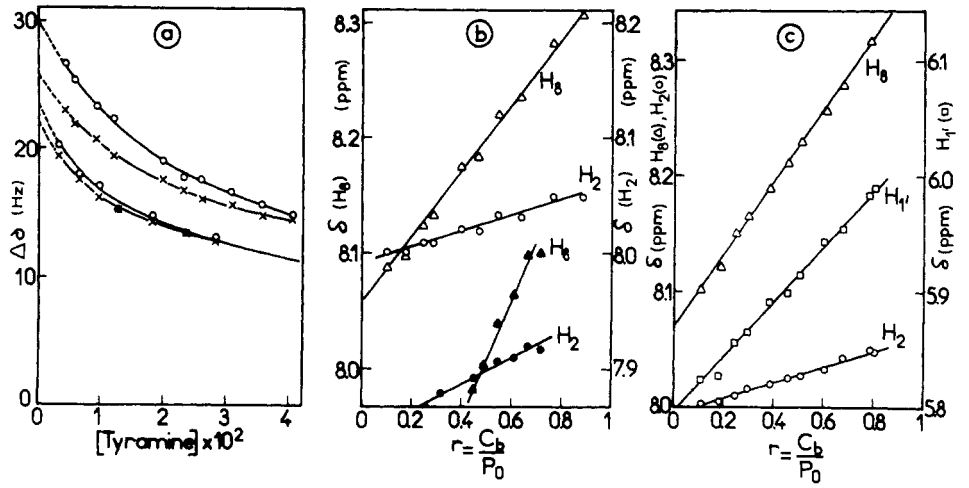

FIGURE 2: (a) Concentration dependence of the upfield shifts ($\Delta\delta$) of the aromatic H_o (○) and H_m (X) protons of tyramine in the presence of 2.9×10^{-2} M poly(A) at 277°K (upper curves) and at 298°K (lower curves). The curves have been extrapolated to zero tyramine concentration. (b) Chemical shifts (δ) of H_8 (Δ,▲) and H_2 (○,●) adenine protons of poly(A) *vs.* the ratio r of bound tyramine molecules (C_b) and polymer phosphate concentrations. C_b was determined from the results reported in a and from eq 2; $P_0 = 2.9 \times 10^{-2}$ M. Open symbols refer to 298°K and full symbols to 277°K. (c) Chemical shifts of H_8 (Δ), H_2 (○), and $H_{1'}$ (□) adenine protons of 2.9×10^{-2} M poly(A) at 298°K *vs.* the ratio r of the concentrations of bound *p*-methoxyphenethylamine (C_b) and polymer phosphate (P_0).

field shifts are thus very likely due to the magnetic anisotropy of nucleic acid bases.

That electrostatic interactions are important in the binding of the phenol derivatives investigated is demonstrated by an important decrease of the upfield shifts and of line widths when the ionic strength increases. It has already been shown by fluorescence and circular dichroism measurements that increasing the ionic strength leads to complex dissociation (Hélène *et al.*, 1971).

When the temperature of the solution is raised, the broadening of the resonance lines of the amine molecules decreases. The upfield shifts of the amine proton resonances do not change with temperature in a monotonous way (Figure 3). In the presence of poly(A), these upfield shifts are increasing

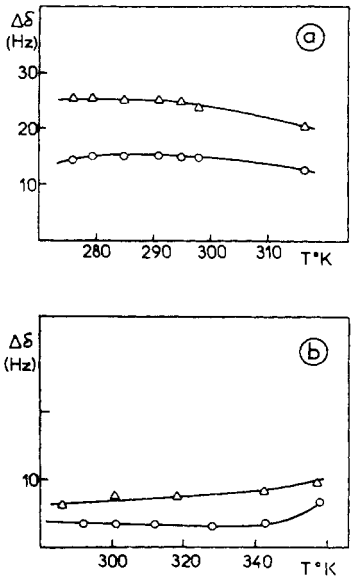

FIGURE 3: Temperature dependence of the upfield shift of the aromatic proton H_m of tyramine (○) and *p*-methoxyphenethylamine (Δ) in the presence of (a) 2.9×10^{-2} M poly(A) and (b) 5×10^{-2} M calf-thymus native DNA. Amine concentrations are: (a) 1.3×10^{-2} M and (b) 1.8×10^{-2} M.

up to about 290°K and then decreasing. In the case of DNA, an increase of the upfield shifts is observed especially when the double-stranded structure begins to melt. As reported in Table I, the observed upfield shifts are higher with denatured than with native DNA at the same temperature of 298°K. The temperature dependence of the upfield shifts must arise from a competition between different processes: change in the association constants with temperature, change in the nucleic acid structure, and change in the relative amounts of different types of complexes. Obviously, stacking with bases will be favored when the polymer structure is less rigid (denatured DNA, poly(A) at moderately high temperatures, etc.). The increase in upfield shifts of *p*-methoxyphenethylamine in the presence of DNA when the temperature increases could be related to the known change of DNA structure at premelting temperatures (Paleček and Frič, 1972; Studdert *et al.*, 1972). The rigidity of the poly(A) structure decreases when the temperature increases from 273 to 290°K as shown by the change in area of the poly(A) proton resonance peaks (McDonald *et al.*, 1965) and by the change in the radius of gyration (Inners and Felsenfeld, 1970). Stacking of adenine bases decreases when the temperature is raised (Brahms and Van Holde, 1967) and this may also favor stacking of adenine bases with the tyrosine ring.

The total concentration of bound molecules (C_b) can be calculated from the ratio of the observed upfield shift ($\Delta\delta$) to the extrapolated shift ($\overline{\Delta\delta}^*$) and the initial concentration C_0 by eq 2. This assumes that rapid exchange occurs between the

$$C_b = (\Delta\delta/\overline{\Delta\delta}^*)C_0 \qquad (2)$$

free and bound molecules. This assumption is supported by the fact that only one resonance is observed for each proton and that the peak area does not change with polymer concentration. Also, the association constants calculated from fluorescence data (Hélène *et al.*, 1971) are such that the lifetime of the complex is much shorter than $1/\overline{\Delta\delta}^*$ if association is controlled by diffusion. Equation 2 also assumes that eq 1 is valid, that is, $KnP_0 \gg 1$. This assumption will be tested *a posteriori*. A binding isotherm [$C_b = f(C_0)$] can be obtained at constant nucleic acid concentration and analyzed according to

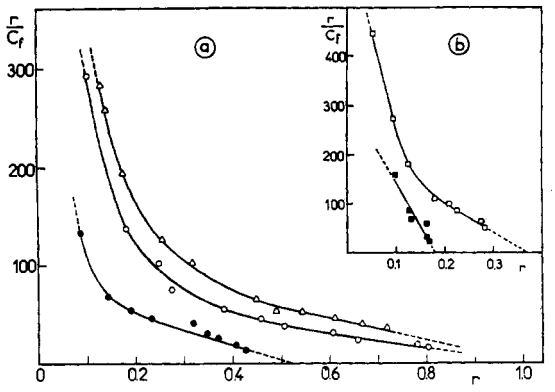

FIGURE 4: (a) Scatchard plots for the binding of *p*-methoxyphenethylamine to poly(A) (○) and native DNA (●) and for the binding of tyramine to poly(A) (Δ) at 298°K, pD 6.9. The nucleic acid concentration was constant: 2.5×10^{-2} and 2.9×10^{-2} M for DNA and poly(A), respectively. The concentration of bound amine was determined according to eq 2. (b) Scatchard plots for the binding of Lys-Tyr-NH_2 to poly(A) (□) and to DNA (■) at 298°K, pD 6.9. Nucleic acid concentrations were constant and equal to 2.9×10^{-2} and 2.5×10^{-2} M for poly(A) and DNA, respectively. The concentration of bound peptide was determined from eq 2.

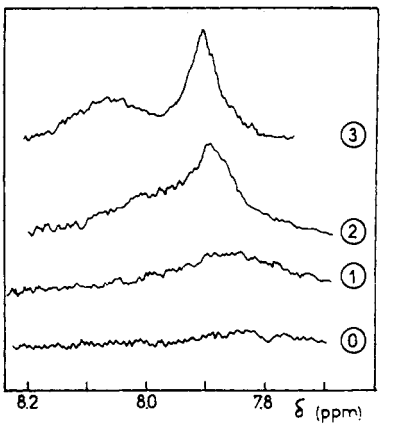

FIGURE 5: Pmr spectrum at 277°K of 5.6×10^{-2} M poly(A) (H_2 at high field and H_8 at low field) in the presence of increasing concentrations of tyramine: (0) without tyramine; (1) 4.1×10^{-3} M; (2) 2.0×10^{-2} M; (3) 3.1×10^{-2} M.

Scatchard (1949) plotting r/C_f *vs.* r (where $r = C_b/P_0$ and $C_f = C_0 - C_b$). This representation does not give straight lines (Figure 4). Nevertheless, the first part of the plot shows that the assumption $KnP_0 \gg 1$ can certainly be considered as valid. Therefore, the values reported in Table II represent the differences in chemical shifts between free and complexed molecules (averaged over all possible types of complexes) (eq 1). The curvature of the Scatchard plots may have different origins. The presence of several types of complexes, as reported above, contributes to this behavior. But, even in the presence of only one type of complex involving electrostatic interactions, a curvature should be expected because the electrostatic potential of the nucleic acid molecule decreases as the binding proceeds (and thus the binding strength decreases).

Stacking interactions of the phenol and adenine rings can be demonstrated by following poly(A) proton resonances in the presence of tyramine and its O-methylated derivative. At a low temperature (277°K), the poly(A) pmr spectrum is severely broadened and this is accompanied by a decrease in peak area (McDonald *et al.*, 1965). At 277°K, addition of tyramine or of *p*-methoxyphenethylamine leads to an increase in peak area as shown in Figure 5. Simultaneously, the resonance lines are narrowed and shifted to lower fields. This behavior is quite similar to what is observed upon increasing the temperature of the pure poly(A) solution or upon adding indole derivatives (Razka and Mandel, 1971; Dimicoli and Hélène, 1974). The loss of peak area of poly(A) at low temperatures is due to the rigidity of the polymer whose rotational relaxation is slow. Increasing the temperature or inserting an aromatic ring between two consecutive bases will induce a flexibility of the polymer at the points where the bases are not stacked and therefore a decrease of the rigidity is expected. The temperature at which peak areas reach the value calculated from the known poly(A) concentration is about 300°K for poly(A) alone under our experimental conditions. This temperature is decreased in the presence of tyramine and its O-methylated derivative as expected from the results reported above.

At temperatures higher than 300°K, addition of tyramine and *p*-methoxyphenethylamine induces a downfield shift of the polymer proton resonances and this is accompanied by a narrowing of the resonance lines (whereas peak area is not modified). The chemical shifts of the poly(A) protons vary linearly with the concentration of bound molecules calculated from eq 2 (Figure 2). This indicates that the effects of bound molecules are additive from the point of view of the magnetic environment of adenine bases. It must be noticed that the linearity of the plots shown in Figure 2 does not depend on the extrapolated values ($\overline{\Delta\delta}^*$) for the upfield shifts of the amine aromatic ring protons. Only the slope of the straight lines will change. The additivity of bound molecule effects on the poly(A) pmr spectrum is not affected therefore by the accuracy of the extrapolation procedure. The behavior of the poly(A) pmr spectrum can be described by the effect of each bound amine molecule per phosphate on the chemical shifts of the polymer protons (see Table III). The values reported in Table III may also be considered as representing the difference between the chemical shifts of free poly(A) and a hypothetical poly(A) molecule having one amine molecule bound per phosphate. These values can be compared to those obtained with indole derivatives (tryptamine and 5-methoxytryptamine) reported in the preceding paper (Dimicoli and Hélène, 1974).

TABLE III: Changes in Chemical Shifts (in Hz at 90 MHz) of Poly(A) Protons per Bound Amine or Peptide Molecule and per Phosphate at pD 6.9 and 298°K.[a]

	H_8	H_2	$H_{1'}$
Tyramine 277°K	−45	−10	
Tyramine 298°K	−26	−5.5	−18
p-Methoxyphenethylamine	−29	−6.5	−21.5
Lys-Tyr-NH_2	−63	−16.5	−47
Lys-Tyr-Lys	−60	−15.5	−48

[a] Poly(A) concentration is 2.9×10^{-2} M. The values are deduced from the plots of $\Delta\nu$ *vs.* r where $\Delta\nu$ is the change in chemical shift of poly(A) protons in the presence of a concentration (C_b) of bound amine or peptide and $r = C_b/P_0$ is calculated from eq 2 using the $\overline{\Delta\delta}^*$ values reported in Table II. Measurements were performed at two different temperatures in the case of tyramine (277 and 298°K).

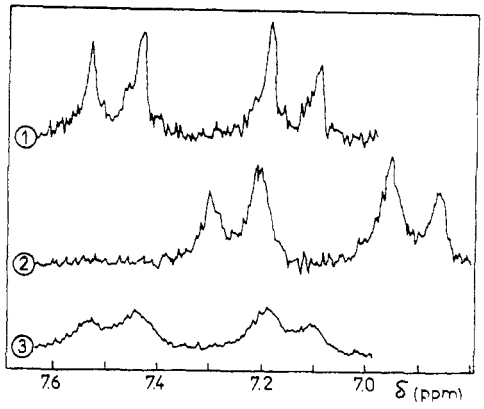

FIGURE 6: Pmr spectra at 298°K and pD 6.9: (1) Lys-Tyr-Lys, 9 × 10^{-3} M; (2) Lys-Tyr-Lys, 9 × 10^{-3} M + poly(A), 2.7 × 10^{-2} M; (3) Lys-Tyr-Lys, 1.8 × 10^{-2} M, + DNA, 6 × 10^{-2} M.

The resonances of the H_8 and $H_{1'}$ protons of poly(A) are shifted downfield by the same amount but the H_2 proton resonance undergoes a downfield shift in the presence of phenol derivatives whereas an upfield shift was observed with indole derivatives. As discussed previously (Dimicoli and Hélène, 1974), the change in chemical shifts of poly(A) protons is the net result of different effects: (i) a downfield shift ($\Delta\nu^P$) due to a destacking of adenine bases induced by the interaction of these bases with the phenol ring; (ii) an upfield shift due to the ring current effect of the phenol ring on neighboring adenine bases; (iii) a downfield shift due to electrostatic interactions with phosphate groups. The magnetic anisotropy of the phenol ring is smaller than that of the indole ring (Giessner-Prettre and Pullman, 1971). The upfield shift contribution is thus expected to be smaller for phenol than for indole derivatives. This is observed for the H_2 proton which therefore must be in the region of high magnetic anisotropy of the phenol or indole ring. The stacking of adenine bases in poly(A) increases when the temperature is lowered as shown by an increased upfield shift of the base protons (Jardetzky, 1964; McTague *et al.*, 1964; McDonald *et al.*, 1965). Therefore, the downfield shift contribution due to destacking of adenine bases (i) is expected to increase when the temperature decreases. As shown in Table III, the downfield shifts of the poly(A) proton resonances markedly increase when the temperature is lowered from 298 to 277°K.

Binding of Peptides Containing Tyrosyl Residues to Poly(A). The binding of Lys-Tyr-NH_2 and Lys-Tyr-Lys to single-stranded poly(A) at pD 7 is accompanied by important upfield shifts of the tyrosine ring protons (Table IV; Figure 6) and to a lesser extent of the Tyr $CH_2(\beta)$ and $CH(\alpha)$ groups. The $CH(\alpha)$ resonance of the first (Lys) residue undergoes a downfield shift (Table IV). As already observed for tryptophan-containing peptides (Dimicoli and Hélène, 1974), these results are ascribed to a stacking of the phenol ring with the nucleic acid bases (upfield shifts) and to an increase of the pK of the terminal ND_3^+ group of the oligopeptide due to electrostatic binding to phosphate groups. The neutralization of this group is accompanied by an upfield shift of the $CH(\alpha)$ resonance of the Lys(1) residue. The pK increase will shift the corresponding titration curve toward higher pH values. Therefore, at constant pD a downfield shift of the $CH(\alpha)$ resonance will be observed. These results are thus quite similar to those obtained with tryptophan-containing peptides (Dimicoli and Hélène, 1974).

The importance of electrostatic interactions between the positive charges of the oligopeptides and the negative charges of the nucleic acid phosphate groups is demonstrated by the ionic strength dependence of the upfield shifts of the tyrosine ring protons (Table V). The change of the pK value of the terminal ND_3^+ group of the peptide (as reported above) demonstrates the role of this group in electrostatic interactions. Also, the upfield shifts of the aromatic proton resonances decrease when the pD is raised.

As already observed in the case of tyramine and its O-methylated derivative in the presence of poly(A) (Figure 3), when the temperature is raised the upfield shifts of the tyrosine ring protons do not vary in a monotonous way. An increase in upfield shifts is first observed at low temperatures followed by a decrease.

At constant poly(A) concentration, the upfield shifts of the tyrosine ring protons increase when the peptide concentration decreases. Extrapolation to zero concentration gives the values ($\overline{\Delta\delta^*}$) reported in Table II. Analysis of the binding isotherm according to Scatchard does not lead to straight lines as already observed with Trp-containing peptides (Dimicoli and Hélène, 1974) (Figure 4). However, the assumption $KnP_0 \gg 1$ is certainly valid at low peptide concentration and therefore the extrapolated $\overline{\Delta\delta^*}$ values represent the difference in chemical shifts between the free and the bound peptide (or an average overall bound species, eq 1). These values are quite comparable to those obtained with tyramine (Table II).

The binding of Tyr-containing peptides to poly(A) induces a downfield shift of the poly(A) proton resonances. These downfield shifts vary linearly with the concentration of bound peptide molecules calculated from eq 2 (Figure 7). The slope of the straight lines thus obtained represents the downfield shift of the poly(A) proton resonances per bound peptide and per phosphate (Table III). These values are much higher than those obtained with tyramine. However, it must be noticed that the number of binding sites as determined from Scatchard plots (Figure 4) is $n \simeq 1$ for tyramine and its O-methylated derivative and $n \simeq 0.4$ for Lys-Tyr-NH_2 and Lys-Tyr-Lys. Thus, to obtain the difference in chemical shifts between free poly(rA) and a saturated hypothetical polymer, the values reported in Table IV have to be multiplied by n. It can be observed that these differences in chemical shifts are quite similar for all the tyrosine derivatives investigated.

The poly(A) downfield shifts are decreased when the ionic strength increases and this decrease parallels that of the tyrosine ring protons (Table V).

Binding of Peptides Containing Tyrosyl Residues to DNA. The binding of Lys-Tyr-NH_2 to double-stranded DNA is accompanied by small upfield shifts of the tyrosine ring protons. The line widths of these proton resonances are markedly in-

TABLE IV: Change in Chemical Shifts of Different Protons of Lys-Tyr-NH_2 and Lys-Tyr-Lys in the Presence of 2.75 × 10^{-2} M Poly(A).[a]

	H_m(Tyr)	H_o(Tyr)	CH(α) (Lys 1)
Lys-Tyr-NH_2	+22.0	+21.3	−9.8
Lys-Tyr-Lys	+20.7	+21.4	−7.3

[a] Concentration 9 × 10^{-3} M at pD 6.9 and T = 298°K (plus and minus signs indicate upfield and downfield shifts, respectively).

TABLE V: Ionic Strength Effects upon the Changes in Chemical Shifts of the Tyrosine Ring and Poly(A) Protons.[a]

	Lys-Tyr-NH_2					Lys-Tyr-Lys				
	Tyr Protons		Poly(A) Protons			Tyr Protons		Poly(A) Protons		
[NaCl] (M)	H_m	H_o	H_8	H_2	$H_{1'}$	H_m	H_o	H_8	H_2	$H_{1'}$
0.001	22.0	22.9	−10.3	−4.0	−7.0	23.1	24.1	−11.3	−4.1	−10.7
0.07	16.7	16.5	−8.7	−2.4	−6.4	15.4	16.3	−9.5	−2.8	−6.8
0.14	12.8	12.1	−6.9	−1.4	−4.8	10.7	11.1	−6.9	−1.6	−5.1
0.26	7.2	7.6	−4.1	−0.2	−3.2	5.6	6.8	−4.5	−0.7	−3.3

[a] Poly(A) concentration was 3.6×10^{-2} M. The concentration of peptides was 7.5×10^{-3} and 9×10^{-3} M for Lys-Tyr-NH_2 and Lys-Tyr-Lys, respectively; $T = 298$°K; pD 6.9 (a minus sign indicates a downfield shift).

creased. At constant Lys-Tyr-Lys concentration, addition of DNA leads to a progressive broadening without any important upfield shift (Figure 6). Broadening is not due to an increase of viscosity since the resonance lines of citrate ions introduced in the DNA sample are not broadened.

The small upfield shifts of the tyrosine protons do not markedly depend on temperature until DNA begins to denature (at about 345°K) which leads to an increase in upfield shifts.

The absence of important upfield shifts of tyrosine protons in the presence of DNA is not due to a weak binding of the oligopeptides. This can be shown by a competition experiment. The binding of 5-methoxytryptamine to DNA is accompanied by important upfield shifts of the indole ring proton resonances. Addition of Lys-Trp-Lys or Lys-Tyr-Lys at the same concentration leads to downfield shifts of the 5-methoxytryptamine protons indicating that these two oligopeptides are equally effective in competing with the amine molecule for binding to DNA.

At constant DNA concentration, the upfield shifts of tyrosine protons increase when the peptide concentration decreases. Extrapolation to zero peptide concentration gives the values reported in Table II which are much smaller than in the case of poly(A). The determination of these values is not very accurate (especially for Lys-Tyr-Lys) due to the weakness of the observed effects.

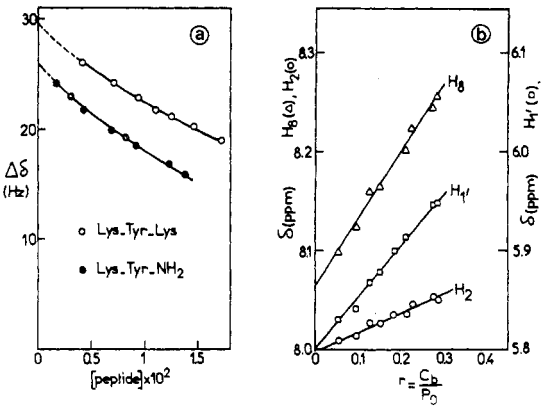

FIGURE 7: (a) Concentration dependence of the upfield shifts of the H_o tyrosine proton in the presence of poly(A) at 298°K and pD 6.9; concentration of poly(A) 3.75×10^{-2} M in the case of Lys-Tyr-Lys (○) and 2.9×10^{-2} M in the case of Lys-Tyr-NH_2 (●). (b) Change in chemical shifts of poly(A) protons *vs.* the ratio r of the concentrations of bound Lys-Tyr-NH_2 (C_b) and poly(A) phosphate (P_0). P_0 was constant and equal to 2.9×10^{-2} M. C_b was determined from the results reported in Figure 7a and from eq 2.

Discussion

The binding of tyramine and of oligopeptides containing tyrosine residues to single-stranded poly(A) and double-stranded DNA involves several types of complexes. All the molecules investigated possess positive charges which give electrostatic interactions with phosphate groups as shown by the ionic strength and pD dependence of binding. The behavior of the aromatic phenol ring will then depend on several parameters such as the structure of the nucleic acid or the environment of the tyrosine ring. Stacking of the tyrosine ring with bases is favored in single-stranded poly(A) and denatured DNA as compared to double-stranded native DNA (see Tables I and II). The upfield shifts of the tyrosine ring protons in the presence of DNA increase in the order Lys-Tyr-Lys < tyramine < Lys-Tyr-NH_2. This might reflect a change in the structure of the stacked tyrosine–base complexes. However, the relative values for the different protons of the tyrosine ring (H_o and H_m) do not change appreciably indicating that the structural parameters are not very different. Therefore, the relative amount of stacked complexes changes with the environment of the tyrosine ring. Different values of upfield shifts for several peptides in the presence of DNA were also reported by Gabbay *et al.* (1972). However, their measurements were performed at only one peptide concentration so that this might reflect only a difference in the relative concentrations of bound peptides. The comparison made here (Table II) refers to the values of the upfield shifts extrapolated to zero peptide concentration which represent the difference in chemical shift between free and complexed (100%) molecules.

The tyrosine ring can give at least two types of interactions with nucleic acid constituents: stacking with bases and hydrogen bonding to bases, ribose or phosphates. The fluorescence of the tyrosine ring is expected to be quenched upon stacking or hydrogen bonding to bases (Feitelson, 1964; Hélène *et al.*, 1971). Centrifugation experiments demonstrate that only part of the bound peptides have their fluorescence quenched. This means that an important fraction of bound molecules does not involve either stacking or hydrogen bonding of the tyrosine ring with bases. Methylation of the tyramine hydroxyl group leads to an enhancement of the upfield shifts of the aromatic ring protons. This enhancement is particularly important in the case of native and denatured DNA. Note that the methyl group resonance is upfield shifted indicating that this group is situated in the region of high magnetic anisotropy of the bases. Since methylation of the OH group prevents hydrogen bonding, the enhancement of upfield shifts can be interpreted as indicating that hydrogen bonding of the OH group to some acceptor group on the

nucleic acid maintains part of the bound molecules outside the double helix. The observation of an important broadening of Tyr resonance lines in the presence of DNA indicates a restricted mobility of the tyrosine ring in the complexes. A study of O-methylated peptides would be necessary to demonstrate the role of hydrogen bonding of the tyrosine hydroxyl group in peptide binding to DNA. It must be noticed that hydrogen bonding of the tyrosine ring to nucleic acid bases in chloroform solutions has been demonstrated by infrared and pmr studies (Sellini *et al.*, 1973).

The conformational changes of the nucleic acid induced by the interaction of the tyrosine ring could be important in protein–nucleic acid complexes. In the case of poly(A) such a conformational change is demonstrated by the downfield shifts of the poly(A) proton resonances. These downfield shifts should be compensated in part by the ring current effect of the tyrosine ring stacked with adenine bases. The H_2 adenine resonance is particularly sensitive to this ring current effect which is smaller than that of the indole ring (Dimicoli and Hélène, 1974). This suggests that stacking involves mainly the six-membered ring of the adenine bases. In the case of DNA, the binding of tyrosine-containing peptides leads to a decrease of the amplitude of the DNA circular dichroism spectrum as already observed with tyramine (Hélène *et al.*, 1971, and unpublished results).

Tyrosine residues may participate in protein–nucleic acid complex formation. Both stacking and hydrogen bonding might be involved. These residues may distinguish between single-stranded and double-stranded regions of the nucleic acid if stacking interactions are involved. The tyrosine fluorescence of staphylococcal nuclease is quenched upon binding of the inhibitor thymidine 3′,5′-diphosphate (pTp) (Cuatrecasas *et al.*, 1967). This might reflect a direct interaction of some of the tyrosine residues with the thymine ring. Crystallographic studies, however, have shown that although Tyr-106 and thymine rings lie parallel to each other in the active site their relative position is not indicative of an efficient stacking interaction (Arnone *et al.*, 1971). The crystal structure of puromycin reveals a stacking interaction of the adenine and *p*-methoxyphenylalanine rings (Sundaralingam and Arora, 1972). Studies of interactions between monomers (tyrosine and purine or pyrimidine bases) have provided evidence for both stacking (Hélène *et al.*, 1971) and hydrogen bonding (Sellini *et al.*, 1973) interactions. Fluorescence, circular dichroism, and proton magnetic resonance studies should help clarify the role of tyrosine in the binding of proteins to nucleic acids.

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