The Role of Tyrosine in the Association of Proteins and Nucleic Acids

SPECIFIC RECOGNITION OF SINGLE-STRANDED NUCLEIC ACIDS BY TYROSINE-CONTAINING PEPTIDES*

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Oligopeptides containing tyrosyl, lysyl, and alanyl residues bind to polynucleotides and nucleic acids as shown by proton magnetic resonance, fluorescence spectroscopy, and difference absorption spectroscopy. Proton magnetic resonance data indicate that stacking of tyrosyl residues with nucleic acid bases takes place only in single-stranded structures (such as poly(A) or denatured DNA). Stacking interactions lead to a quenching of tyrosine fluorescence. However, the tyrosyl fluorescence of the peptides is quenched in their complexes with both single-stranded and double-stranded nucleic acids. A comparison of the behavior of homologous peptides containing Tyr, methoxytyrosine, and Phe leads to the conclusion that hydrogen bonding of tyrosine with bases or phosphates is not involved in the investigated complexes. An energy transfer mechanism from tyrosine to nucleic acid bases is proposed to account for fluorescence quenching in oligopeptide complexes with double-stranded DNAs.

Due to the specificity of its stacking interaction for single-stranded nucleic acid structures, tyrosine might be involved through such interactions in the selective recognition of single strands by proteins.

Interactions between proteins and nucleic acids play a central role in living systems by controlling every step in the expression of the cellular genome. In many cases, proteins bind to highly specific nucleic acid base sequences, e.g. interactions between repressors and operators, RNA polymerases and promoters, or restriction endonucleases and DNA restriction sites. In other cases, e.g. in tRNA-aminoclyl-tRNA synthetase complexes it is very likely that the tRNA bases interacting with the enzyme are located at different positions on the tRNA molecules instead of being contiguous in the sequence as in the previous examples. Other proteins have a structure specificity rather than base specificity. For example, single strand binding proteins such as the proteins coded by gene 32 of phage T4 or by gene 5 of phage fd and other helixdestabilizing proteins which are involved in replication, recombination, and repair of DNA bind strongly (and most often cooperatively) to single-stranded nucleic acids.

In all cases, the formation of a protein-nucleic acid complex requires interactions between amino acid side chains and nucleic acid chemical groups (1). Electrostatic interactions between positively charged side chains (Lys, Arg) and negatively charged phosphate groups are likely to be involved in every protein-nucleic acid complex. A source of specificity in the selective recognition of nucleic base sequences by proteins is provided by hydrogen-bonding interactions, especially those involving amino acid side chains which have the capability of forming simultaneously two hydrogen bonds with a nucleic acid base or base pair (2-5). Aromatic amino acids may play a special role due to their ability to form stacked complexes with nucleic acid bases. It has already been shown that tryptophyl residues of oligopeptides can give rise to stacking interactions with bases in single-stranded and double-stranded nucleic acids (6-9). However, stacking is strongly favored in single strands, and this introduce a specificity of Trp containing peptides for single-stranded nucleic acids (8, 10). This led us to propose a model in which tryptophyl residues of proteins might be used to anchor the protein along single strands (11).

Tyrosyl side chains represent a more complicated case since they can give rise to both stacking and hydrogen-bonding interactions. We have already shown that a tripeptide such as Lys-Tyr-Lys makes use of stacking interactions to bind to single-stranded polynucleotides but not to double-stranded DNA (12). We report here a comparative study of the binding of oligopeptides containing tyrosyl residues to single-stranded poly(A) and double-stranded native DNA. Several methods have been used to investigate complex formation, namely, proton magnetic resonance, fluorescence spectroscopy, and difference absorption spectroscopy. Emphasis is put on the interactions which involve the tyrosyl side chain, comparing tyrosine and its O-methyl derivative to investigate the possible role of hydrogen-bonding interactions. The role of tyrosyl residues of proteins in complex formation with nucleic acids is discussed as well as the specificity that these residues can confer with respect to single strand binding.

MATERIALS AND METHODS

Compounds—All amino acids1 were obtained from Merck. Except where otherwise stated, they were of the L configuration. Commercial reagents were of the highest available purity. For thin layer chromatography, precoated silica gel plates from Eastman Kodak were used. Microgranular carboxymethylcellulose was purchased from Whatman.

Except lysyl-tyrosyl-lvaline, obtained from Mann Research Laboratories in the diacetate hemihydrate form, all the oligopeptides used hereafter were synthesized in our laboratory.

Escherichia coli DNA was isolated and purified in our laboratory, and the sample used in proton magnetic resonance experiments was sonicated until its sedimentation coefficient was decreased to 7.3. Polyadenylic acid was obtained from Miles.

Peptide Synthesis—All the oligopeptides were prepared by stepwise addition of amino acid residues using the classical dicyclohexyl-
carbodiimide method, at low temperature, with chloroform as solvent. The lysine derivatives were Nps-Lys(OH), DCHA, \(^2\) Nps-Lys(OMe), and Nps-Lys(N-HET). The alanine derivative was Nps-Ala-OH, DCHA, and the tyrosine derivatives were Nps-Tyr(OH), Nps-Tyr(OMe), and Nps-Tyr(OMe)-OH, DCHA. In order to determine the role of the terminal amino and carboxyl groups in complex formation with nucleic acids the terminal carboxylic acid of the oligopeptides was blocked by ethyl amide formation and the NH-terminal group by acetylation.

All the protected oligopeptides and the intermediate compounds showed a single spot when subjected to thin layer chromatography. The greatest deviation in the elementary analysis between the observed and calculated values did not exceed 0.2% for carbon, 0.2% for hydrogen, 0.1% for nitrogen, 0.3% for oxygen, and 0.2% for sulfur.

**Peptide Purification**—After deprotection by HBr/acetic acid and deacetylation of the tyrosyl residue by 0.2 M hydroxylamine, the oligopeptides were purified by preparative chromatography using a carboxymethylcellulose column in the Li form, pre-equilibrated with 0.2 M LiCl at pH 5.0 and eluted with a linear LiCl gradient from 0.2 M to 0.8 M LiCl. Flow rate was 30 ml/h. For desalting, the pooled fractions corresponding to the pure oligopeptide were diluted with water until a concentration of 0.05 M LiCl was reached so that each peptide was then quantitatively adsorbed at the top of a carboxymethylcellulose column pre-equilibrated with 0.05 M LiCl. The column was washed free of salt. Afterwards the peptide was eluted with 0.05 M HCl, and the material absorbing at 275 nm was collected and lyophilized. The residue was then taken up three times with anhydrous acetone in which LiCl is soluble. Then the oily residue was dissolved in water and lyophilized.

**Fluorescence**—Fluorescence measurements were made with a Jobin Yvon spectrophotometer modified in our laboratory to correct for lamp fluctuations as described elsewhere (8). The excitation wavelength, sodium chloride was added to the peptide-nucleic acid complex up to 0.5 M NaCl where the apparent decrease of the peptide fluorescence intensity is then entirely due to the screening effect of the nucleic acid since any complex is dissociated under these ionic strength conditions (8).

The solutions were prepared in a 1 mM sodium cacodylate buffer containing 1 mM sodium chloride and 0.3 mM EDTA adjusted to pH 6.0 for DNA solutions and to pH 7.0 for poly(A) solutions.

**Proton Magnetic Resonance**—Proton magnetic resonance experiments were carried out in D$_2$O with a Bruker WH 90 spectrophotometer working in the Fourier transform mode and equipped with a variable temperature probe. Chemical shifts were measured with respect to an external reference (HMDSS).

Measurements were made with respect to an external reference (HMDSS). The concentrations of the peptide and of the nucleic acid were 2 \(\times\) 10$^{-3}$ M and 2 \(\times\) 10$^{-3}$ M respectively. A total of 1500 spectra for oligopeptides and poly(A) complexes and of 2500 spectra for DNA complexes were currently used. The resonance positions of the tyrosyl protons in all investigated peptides are given in Table I.

**RESULTS AND DISCUSSION**

**Difference Absorption Spectra**—The addition of tyrosine-containing peptides to both poly(A) and DNA is accompanied by a change in the absorption spectrum of the mixture as compared to the sum of the absorption spectra of the two separated components. In Fig. 1 are shown the difference spectra obtained with different peptides having related sequences Lys-X-Lys where X = Tyr, Tyr(OMe), Phe, Trp, and Ala. The three peptides having a phenyl ring give rise to the same kind of difference spectra characterized by a positive band around 265 nm and a negative one around 250 nm. Since phenylalanine does not absorb above 260 nm, the difference spectrum above 260 nm clearly represents a change in poly(A) absorption. Phe and Tyr peptides give quite similar difference spectra although they have different absorption spectra and different extinction coefficients. This is a strong argument for ascribing the main contribution of the difference spectra to a change in the absorption spectrum of poly(A).

The peptide Lys-Ala-Lys which does not have any aromatic ring gives a difference spectrum lacking the positive band at 265 nm but still induces a negative band around 250 nm. This peptide binds to poly(A) by electrostatic interactions only. The difference spectrum, therefore, represents the change in the absorption of poly(A) due to the electrostatic binding of Lys-X-Lys assuming that all peptides of this family make use of the same electrostatic bonds. The positive contribution at 265 nm observed with Lys-Tyr-Lys and Lys-Phe-Lys is thus very likely the result of a destacking of adenine bases in poly(A) due to an interaction with the aromatic phenyl ring of tyrosine or phenylalanine.

In the case of Lys-Trp-Lys absorption changes due to the tryptophyl residue are clearly indicated by the bands at 265 m-n observed with Lys-Tyr-Lys and Lys-Phe-Lys is thus very likely the result of a destacking of adenine bases in poly(A) due to an interaction with the aromatic phenyl ring of tyrosine or phenylalanine.

In the case of Lys-Trp-Lys absorption changes due to the tryptophyl residue are clearly indicated by the bands at 265 nm (positive) and at 288 and 290 nm (negative). A strong negative band is observed around 250 nm analogous to that obtained with the other peptides. The negative contribution due to the tryptophyl residue between 270 and 290 nm is probably cancelling most of the positive contribution seen with the other peptides containing an aromatic residue.

The Lys-Trp-Lys-poly(A) difference spectrum would thus be the superposition of three contributions: a change in the poly(A) absorption due to electrostatic binding, and to adenine destacking induced by the aromatic residue, and a change in the absorption spectrum of the tryptophyl residue.

The binding to DNA of peptides containing aromatic residues induces much smaller changes in absorption spectra. In Fig. 2 are compared the difference spectra obtained with Lys-

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*Note: The text is a fragment of a scientific paper discussing the interactions of peptides with nucleic acids, focusing on the differences in absorption spectra and the contributions of aromatic and amino acid residues. The specific data and methods used are detailed in the referenced tables.*

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**TABLE I**

<table>
<thead>
<tr>
<th>Peptides</th>
<th>(\delta (H_a))</th>
<th>(\delta (H_b))</th>
<th>J</th>
<th>(\Delta \delta (H_a - H_b))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lys-Tyr-Lys</td>
<td>7.48</td>
<td>7.14</td>
<td>8.50</td>
<td>0.34</td>
</tr>
<tr>
<td>Lys-Tyr-Lys-NHEt</td>
<td>7.44</td>
<td>7.13</td>
<td>8.46</td>
<td>0.31</td>
</tr>
<tr>
<td>Ac-Lys-Tyr-Lys-NHEt</td>
<td>7.43</td>
<td>7.13</td>
<td>8.50</td>
<td>0.30</td>
</tr>
<tr>
<td>Lys-Ala-Tyr-Ala-Lys-NHEt</td>
<td>7.45</td>
<td>7.11</td>
<td>8.50</td>
<td>0.33</td>
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<tr>
<td>Lys-Tyr(OMe)-Lys</td>
<td>7.43</td>
<td>7.12</td>
<td>8.46</td>
<td>0.31</td>
</tr>
<tr>
<td>Lys-Tyr(OMe)-Lys-OMe</td>
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<td>7.27</td>
<td>8.77</td>
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</tr>
<tr>
<td></td>
<td>7.55</td>
<td>7.27</td>
<td>8.82</td>
<td>0.28</td>
</tr>
</tbody>
</table>

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*The abbreviations used are: Nps, nitrophenylsulfenyl.*

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**FIG. 1.** Difference absorption spectra of poly(A) complexes with Lys-Trp-Lys (---O---), Lys-Tyr-Lys (---), Lys-Tyr(OMe)-Lys (---O---), Lys-Phe-Lys (---), and Lys-Ala-Lys (---). Concentrations were 2 \(\times\) 10$^{-3}$ M for poly(A) and 4 \(\times\) 10$^{-3}$ M for each peptide.

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*Figures and tables are placeholders as the actual data and figures are not included in the text.*

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*Note: The table provides chemical shifts for the tyrosine aromatic protons of investigated peptides, along with other relevant data such as mole fraction (J) and difference in chemical shift (\(\Delta \delta\)).*
Specific Interactions of Peptides with Nucleic Acids

Although there is clearly a change in the absorption spectra of the mixtures with DNA, these changes are small and difficult to ascribe unambiguously to DNA or to the peptide. Both components probably contribute to the difference spectrum. In Fig. 2 it should be noted that the addition of $7 \times 10^{-5}$ M Lys-Tyr-Lys to $2 \times 10^{-4}$ M poly(A) induces only a 2% change in the absorption spectrum at the maximum (265 nm). Fluorescence experiments with tyrosine-containing peptides which are reported below have been carried out with an excitation wavelength of 276 nm. They have not been corrected for a change in absorbance of tyrosine in the complexes. From the difference spectra reported here it is clear that these changes are not very important and can be neglected even in the case of poly(A).

In the case of Lys-Trp-Lys complexes, excitation was chosen at the isosbestic wavelength (292 nm, see Fig. 1). In DNA-Lys-Trp-Lys complexes a small change in the absorption spectrum of both DNA and peptide was observed (data not shown). The characteristic bands due to the tryptophyl residue and the isosbestic point at 292 nm (shown in Fig. 1 in the case of the poly(A) complex) are still observed in DNA complexes.

These results show that the change in the environment of the tryptophyl residue is similar in both types of complex.

Proton Magnetic Resonance—Proton magnetic resonance is a powerful technique to investigate both stacking and hydrogen-bonding interactions. Since all our measurements have been made in D2O solutions only nonexchangeable protons could be observed. This means that, e.g., the hydroxyl group of tyrosyl side chains could not be followed. Stacking interactions lead to upfield shifts of proton resonances of the aromatic rings. On the basis of PMR measurements it has already been reported that tyrosyl residues of oligopeptides can form stacked complexes with single-stranded nucleic acids such as poly(A) or denatured DNA (12, 13). In the case of double-stranded DNA, small upfield shifts of tyrosyl protons were observed in a few cases. This might depend on the sequence of the oligopeptide, but there is always some uncertainty regarding the perfect double-strandedness of DNA in these experiments since sonicated DNA is used to reduce the viscosity of the solution. In former experiments we used the tripeptide Lys-Tyr-Lys. This tripeptide did not give rise to any upfield shift of the Tyr protons when bound to native DNA. However, this could have been due to the constraints imposed by the electrostatic interactions involving the lysyl side chains and the NH2-terminal amino group of the peptides. This last group is very important for the binding of unprotected peptides as shown by the pH dependence of binding (6, 8). We have now investigated the binding of several Tyr-containing peptides having either a free or a blocked NH2-terminal group or Ala residues in between the lysyl and tyrosyl residues.

As shown in Fig. 3, the same qualitative behavior was observed with all investigated peptides. In the presence of single-stranded poly(A), an upfield shift of the tyrosyl resonances was observed. On the contrary, no upfield shift could be observed with double-stranded native DNA independently of the oligopeptide sequence and of the protection of the NH2-terminal group. Only broadening of the resonance lines was observed, indicating that the peptide was bound to DNA. No loss of integrated intensity was observed in the presence of DNA, indicating that the absence of change in chemical shift could not be due to a slow exchange between free molecules and bound peptides whose spectrum would be broadened beyond detection (as is the PMR spectra of DNA itself). Moreover, quantitative analysis of the binding data (see below) indicated that more than 90% of the peptide should be bound under the PMR experimental conditions. This again excluded slow exchange as an explanation for the absence of upfield shifts.

As shown in Table II, blocking the terminal carboxyl by an ethylamide group increased the upfield shifts, whereas blocking the terminal amino group by acetylation decreased the upfield shifts. These results reflect the role of electrostatic interactions in the binding of peptides to nucleic acids.

Under given concentration conditions, the magnitude of the upfield shifts observed with single-stranded poly(A) should depend on the percentage of bound peptides (in the limit of fast exchange between free and bound peptides) and on the probability that a bound peptide has its tyrosyl side chain stacked with adenine bases. Peptides containing an apparent (net) charge of +3 were expected to bind more strongly than those containing a net charge of +2 due to an increased electrostatic contribution. They also gave rise to higher upfield shifts (compare, e.g., acetylated and nonacetylated peptides in Table II).

However, the concentration dependence of the upfield shifts reported in Fig. 4 for Ac-Lys-Ala-Tyr-Ala-Lys-NHEt shows that even at the lowest concentration of the peptide ($5 \times 10^{-4}$ M) where nearly all the peptide should be bound (see fluorescence results below) the maximum upfield shift obtained with the acetylated peptide never reaches that observed with the nonacetylated derivative. This is a strong argument for a different structure in the complexes of the two derivatives.

Fluorescence and PMR data for the binding of oligopeptides to nucleic acids have been interpreted by a two-step model (8). According to this model bound peptide molecules exist in two different structures: an "outside" structure and a stacked structure. In the former the aromatic ring does not interact with the nucleic acid bases, while in the second structure the aromatic ring is stacked with bases. The results reported above (Fig. 4) strongly suggest that the probability of stacking per bound peptide is higher in the complex which involves a peptide with a free amino group than that in involving the acetylated derivative.

If the temperature of the DNA-peptide mixture was raised, there was no shift of the tyrosyl protons with respect to the free peptide until melting of the DNA double helix began to take place. Then the tyrosyl protons were progressively shifted upfield (Fig. 5). When the sample heated to 359 K was rapidly cooled down to 293 K, a large upfield shift of the

![Fig. 2. Difference absorption spectra of Lys-Tyr-Lys complexes with poly(A) (-----) and DNA (--.--), absorption spectrum of the peptide Lys-Tyr-Lys (-----), at 2 \times 10^{-4} M. The concentration of poly(A) and DNA was 2 \times 10^{-4} M. The peptide concentration was 7 \times 10^{-5} M in the case of poly(A) and 1.9 \times 10^{-4} M in the case of DNA.](image-url)
tyrosyl protons was observed (Fig. 5). Under these conditions most of the DNA was single-stranded, and tyrosine was able to stack with bases as observed in the case of poly(A). At high temperature (359 K) the upfield shift was less important than at 293 K due to the fact that less complex was formed as a result of a decrease of the association constant when the temperature was raised.

One experiment done with total transfer RNA revealed an important upfield shift of the tyrosyl proton resonances indicating that tyrosine did stack with bases in the single-stranded regions of the RNA molecule.

In order to determine whether the -OH group of the tyrosyl side chain was involved in the binding of the peptides to poly(A) and DNA, two O-methylated peptides, Lys-Tyr(OMe)-Lys and Lys-Tyr(OMe)-Lys-OMe, were synthesized. The p-methoxyphenylalanine ring was clearly stacked with bases when these peptides were bound to poly(A) as indicated by the large upfield shifts of the aromatic proton resonances (including the -OCH₃ group). However, with double-stranded DNA the upfield shifts were very small (see Fig. 3 and Table III). A similar conclusion was reached when tyrosine was replaced by phenylalanine. A large upfield shift of the proton resonance was observed in the complex with poly(A), whereas only a very small shift was obtained in the complex with DNA (Table III). Therefore, it appears that the absence of upfield shifts of the tyrosyl protons in complexes of DNA with the Tyr-containing peptides that we have investigated is not due to hydrogen bonding which would strongly compete with stacking but that this results from the intrinsic inability of the tyrosyl ring to stack with bases in double-stranded DNA.

Fluorescence—We have already reported that the fluorescence of Lys-Trp-Lys was quenched upon complex formation with single-stranded poly(A) and DNA (8, 9). The extent of quenching was much higher, however, with single strands than with double strands. This was ascribed to the increased probability of stacking interactions in the former case. As a matter of fact, there was good correlation between tryptophan fluorescence quenching and stacking with bases (as measured by PMR).
TABLE II
Change in chemical shifts (upfield, in parts per million) of the aromatic proton resonances of Tyr-containing peptides (2 mM) in the presence of 20 mM poly(A), pH 7.0

<table>
<thead>
<tr>
<th>Peptides</th>
<th>∆δ (Hₐ)</th>
<th>∆δ (Hₖ)</th>
<th>∆δ (Me)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lys-Tyr-Lys</td>
<td>0.28</td>
<td>0.30</td>
<td></td>
</tr>
<tr>
<td>Lys-Tyr-Lys-NHEt</td>
<td>0.34</td>
<td>0.32</td>
<td></td>
</tr>
<tr>
<td>Ac-Lys-Tyr-Lys-NHEt</td>
<td>0.23</td>
<td>0.21</td>
<td></td>
</tr>
<tr>
<td>Lys-Ala-Tyr-Ala-Lys-NHEt</td>
<td>0.30</td>
<td>0.32</td>
<td></td>
</tr>
<tr>
<td>Ac-Lys-Ala-Tyr-Ala-Lys-NHEt</td>
<td>0.20</td>
<td>0.19</td>
<td></td>
</tr>
<tr>
<td>Lys-Tyr(OMe)-Lys-NHEt</td>
<td>0.34</td>
<td>0.42</td>
<td>0.28</td>
</tr>
<tr>
<td>Lys-Tyr(OMe)-Lys-OMe</td>
<td>0.40</td>
<td>0.45</td>
<td>0.23</td>
</tr>
</tbody>
</table>

The problem appears much more complex with peptides containing tyrosine or phenylalanine. Most of the fluorescence of these peptides was quenched when they bound to DNA as well as to poly(A) even though PMR results clearly showed that stacking did not take place in DNA complexes (Table III).

It can be seen in Table IV that the fluorescence of acetylated peptides is much less quenched by poly(A) than that of the nonacetylated analogues. These results can be compared to those obtained by PMR (Table II). It can be seen that there is a good correlation between fluorescence quenching and the upfield shifts of the tyrosyl proton resonances.

Such a comparison is not possible for DNA complexes since no upfield shifts were observed (see above). However, the results reported in Table IV show that the difference between acetylated and nonacetylated peptides is much less in the case of DNA complexes than in that of poly(A) complexes. It can even be in the opposite direction; compare Lys-Tyr-Lys-NHEt and Ac-Lys-Tyr-Lys-NHEt, on one hand, and Lys-Ala-Tyr-Ala-Lys-NHEt and Ac-Lys-Ala-Tyr-Ala-Lys-NHEt, on the other hand.

If one now compares the results obtained with poly(A) and DNA one can see that fluorescence quenching is always higher in poly(A) than in DNA complexes of nonacetylated peptides, whereas the reverse holds for acetylated peptides.

Fluorescence lifetime measurements were made difficult due to the important quenching superimposed on the large screening effect of the nucleic acid at the excitation wavelength. In the absence of any information on fluorescence lifetime we analyzed fluorescence data according to the simplest scheme

\[ P + N = (PN) \]

where \( P \) is the free peptide of initial concentration \([P_0] \) and \( N \) the nucleic acid of initial concentration \([N_0] \) (moles of phosphates/liter). The complex \((PN)\) was assumed to have a fluorescence quantum yield \( \phi_c \) and the free peptide a quantum yield \( \phi_f \).

At low degree of saturation, i.e., \( P_0 \ll N_0 \), Equation 1 leads to Equation 2:

\[ \phi_f - \phi_c = \phi_f \left(1 + \frac{1}{K N_0} \right) \]

FIG. 4. Upfield shifts of tyrosine protons of Ac-Lys-Ala-Tyr-Ala-Lys-NHEt as a function of peptide concentration in the presence of 2 x 10⁻⁴ M poly(A).

FIG. 5. NMR spectra of the aromatic protons of Lys-Tyr-Lys (2 x 10⁻³ M) in the presence of DNA (2 x 10⁻³ M) at different temperatures. The lowest spectrum corresponds to the sample heated to 359 K and cooled down to 293 K.

TABLE III
Comparison of upfield shifts observed in the presence of 2 x 10⁻⁴ M poly(A) or DNA with different peptides (2 x 10⁻³ M) containing a tyrosyl or a phenylalanyl residue

Measurements were made at pH 7 in the case of poly(A) and pH 6 in the case of DNA. HMDS was used as an internal reference.

<table>
<thead>
<tr>
<th>Peptides</th>
<th>Poly(A)</th>
<th>DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lys-Tyr-Lys</td>
<td>Hₐ 0.28</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Hₖ 0.30</td>
<td>0.00</td>
</tr>
<tr>
<td>Lys-Tyr(OMe)-Lys</td>
<td>Hₐ 0.34</td>
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<td></td>
<td>Hₖ 0.32</td>
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<tr>
<td>Lys-Ala-Tyr-Ala-Lys-NHEt</td>
<td>Hₐ 0.34</td>
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<td>Hₖ 0.32</td>
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<td>Lys-Tyr(OMe)-Lys-OMe</td>
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<td></td>
<td>Hₖ 0.23</td>
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<td>Lys-Phe-Lys</td>
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<td>0.045</td>
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<tr>
<td>Lys-Phe-Lys-OMe</td>
<td>0.32</td>
<td>0.055</td>
</tr>
</tbody>
</table>
Specific Interactions of Peptides with Nucleic Acids

where $\phi_c$ is the overall (apparent) fluorescence quantum yield of the peptide in the presence of the nucleic acid extrapolated to zero concentration as indicated in Fig. 6 in the case of Lys-Phe-Lys and Fig. 7 for Lys-Tyr-Lys. At each nucleic acid concentration the screening effect was taken into account by measuring the fluorescence intensity after dissociation of the complexes at high ionic strength (see Figs. 6 and 7 and Ref. 8). Plots of $\phi_c/\phi_{c-F}$ versus $[N_0]$ were straight lines as required by Equation 2 (Fig. 8). The results obtained according to Equations 1 and 2 are summarized in Table V for two peptides (Lys-Tyr-Lys and Lys-Tyr(OMe)-Lys) and different nucleic acids.

**Mechanism of Tyrosine Fluorescence Quenching**—The fluorescence quantum yield of the complex ($\phi_c$) depends on the nucleic acid. For double-stranded nucleic acids the value of $\phi_c$ is much smaller for poly(G)-poly(C) than for poly(A)-poly(U). The percentage of fluorescence quenching of tyrosine-containing peptides ($\phi_{c-F}$) is very high when these peptides bind to nucleic acids. A comparison of these fluorescence results with PMR data reported above clearly demonstrates that fluorescence quenching by DNA cannot be due to stacking of the tyrosyl side chains with bases. In order to determine whether hydrogen bonding of the $-OH$ group of tyrosine was at the origin of fluorescence quenching, the behavior of $O$-methylated peptide Lys-Tyr(OMe)-Lys was investigated. As shown in Tables IV and V, its fluorescence quenching was not due to stacking of the nonmethylated peptide. This high quenching could not be correlated with the very low efficiency of stacking (if any) demonstrated by PMR measurements (see above). In the case of double-stranded DNA, it appears that fluorescence quenching is not due either to stacking or to hydrogen-bonding interactions of tyrosine with bases.

**TABLE IV**

Relative fluorescence quantum yields ($\phi_c/\phi_{c-F}$) of peptide complexes with poly(A) and E. coli DNA

<table>
<thead>
<tr>
<th>Peptides*</th>
<th>Poly(A)</th>
<th>E. coli DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lys-Tyr-Lys</td>
<td>0.21</td>
<td>0.30</td>
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<tr>
<td>Lys-Tyr(OMe)-Lys</td>
<td>0.13</td>
<td>0.33</td>
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<tr>
<td>Lys-Tyr-Lys-NHEt</td>
<td>0.21</td>
<td>0.38</td>
</tr>
<tr>
<td>Ac-Lys-Tyr-Lys-NHEt</td>
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<td>0.34</td>
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<td>0.29</td>
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<tr>
<td>Ac-Lys-Ala-Tyr-Ala-Lys-NHEt</td>
<td>0.46</td>
<td>0.39</td>
</tr>
</tbody>
</table>

* The results reported for the first two peptides were obtained from Equation 2 between $[N_0]$ to 0. For the other four peptides $\phi_c/\phi_{c-F}$ was estimated from fluorescence data obtained at only one poly(A) concentration, namely, $5 \times 10^{-4}$ M after extrapolation to $[P_0] = 0$. These values are, therefore, slightly overestimated as compared to those for the first two peptides.

**TABLE V**

Relative fluorescence quantum yields of peptide complexes ($\phi_c/\phi_{c-F}$) and association constants ($K$) for the binding of Lys-Tyr-Lys and Lys-Tyr(OMe)-Lys to different polynucleotides (DNA, poly(A), poly(U), poly(G), poly(C)) with bases.

<table>
<thead>
<tr>
<th>Peptides</th>
<th>Poly(A)</th>
<th>Poly(U)</th>
<th>Poly(G)</th>
<th>Poly(C)</th>
<th>E. coli DNA</th>
<th>M. L. DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lys-Tyr-Lys</td>
<td>0.215</td>
<td>0.195</td>
<td>0.285</td>
<td>0.135</td>
<td>0.30</td>
<td>0.275</td>
</tr>
<tr>
<td>Lys-Tyr(OMe)-Lys</td>
<td>1.5</td>
<td>1.35</td>
<td>3.9</td>
<td>7.25</td>
<td>3.6</td>
<td>5.0</td>
</tr>
</tbody>
</table>

Experiments were performed at 2°C in the buffer described in the legend of Table IV. Fluorescence data were analyzed according to Equation 2 (see Fig. 8).

Fig. 7. A, change in fluorescence intensity of Lys-Tyr-Lys (excitation 276 nm, emission 305 nm) in the presence of poly(A) at different concentrations. $R$ is the ratio of the fluorescence intensities of the peptide in the presence and in the absence of poly(A). B, dissociation of the complexes at high ionic strength (same buffer as in Fig. 1). $R$ represents the screening effect of poly(A) at the excitation wavelength (276 nm). This screening effect increases with poly(A) concentration. 4.6 $\times 10^{-5}$ M (○), $2.6 \times 10^{-4}$ M (□), $1.8 \times 10^{-6}$ M (●), and $1.9 \times 10^{-4}$ M (Δ).

Fig. 8. Analysis of fluorescence data according to Equation 2 for the binding of Lys-Tyr-Lys (a) and Lys-Tyr(OMe)-Lys (b) to different polynucleotides (DNA, poly(A), poly(U), poly(G), poly(C)). Same experimental conditions as in Fig. 1.
poly(A) does involve stacking interactions (see PMR results above). Such a stacking leads to tyrosine fluorescence quenching as shown by the study of mixed aggregates formed in frozen aqueous solutions (14). It is thus clear that stacking interactions do contribute to fluorescence quenching in the case of single strands and that binding should include more than one type of complex as already proposed in the case of Lys-Trp-Lys (8, 9). Equation 1 should thus be rewritten as

\[ P + N \rightarrow \text{(PN)} \rightarrow \text{(PN')} \]

where (PN) is a purely electrostatic complex of fluorescence quantum yield \( \phi' \) (tyrosine does not interact with bases in this complex, but its fluorescence quantum yield is reduced as a result of, e.g., energy transfer to nucleic acid bases, see below). (PN') is a stacked complex whose fluorescence quantum yield is zero. Equation 2 still holds if \( \phi' \) is replaced by \( \phi' \) versus \( (N_0)^{-1} \) but \( K_2 \) and \( \phi' \) cannot be determined independently. Fluorescence lifetime measurements would be required to determine \( \phi' \).

Phosphate monoanions are known to quench the fluorescence of tyrosine although less than phosphate diamions (15). This quenching was previously attributed to proton transfer in the excited state from tyrosine to phosphate ions with concomitant formation of the nonfluorescent tyrosine anion. Methylation of the hydroxyl group should prevent such an excited state reaction. As a matter of fact we have verified that phosphate mono- and diamions do not quench the fluorescence of the methoxyphenyl group even at high concentrations (Table VI). Moreover, recent experiments have shown that phosphate diesters (such as dimethylphosphate) do not quench tyrosine fluorescence. Nevertheless the fluorescence of the O-methylated peptide is quenched in DNA complexes (Tables IV and V).

One could contemplate the possibility that upon binding to nucleic acids, the investigated peptides undergo a conformational change which could bring the phenol ring in close contact with a quenching group inside the peptide. However, most of the deactivation processes leading to tyrosine fluorescence quenching do imply the hydroxyl group and should not occur with O-methyltyrosine. Quenching by the carbonyl group of the peptide bonds is not expected to lead to such high quenching in nucleic acid complexes as compared to the free peptide. Also one would expect this mechanism to depend on the peptide sequence. This is not the case (compare, e.g., Lys-Tyr-Lys and Lys-Ala-Tyr-Ala-Lys).

The most satisfactory phenomenon which could explain tyrosine fluorescence quenching thus appears to be energy transfer to nucleic acid bases. Förster critical distances \( R_0 \) have been calculated (16). \( R_0 \) represents the distance at which the probability of transfer is equal to the sum of all other deactivation probabilities, i.e., on the average, 50% of the excited tyrosines will transfer their energy to bases. These calculations show that tyrosine is able to transfer its singlet excitation energy to nucleobases with \( R_0 \) values ranging from 10 to 24 Å (Table VII). The reverse transfer process (from bases to tyrosine) is very inefficient due to the very short singlet lifetimes of the bases and to the poor overlap between base fluorescence spectra and tyrosine absorption. When one of the peptides investigated here is bound electrostatically to DNA the tyrosyl side chains will be at distances from the bases which are within the range of calculated \( R_0 \) values. This means that energy transfer should be very efficient in the peptide-DNA complexes. Since \( R_0 \) is larger for G-C than for A-U base pairs this might explain why fluorescence quenching is higher with poly(G)-poly(C) than with poly(A)-poly(U) (Table VII). The same mechanism (energy transfer to nucleic acid bases) is likely to account for the high quenching of phenylalanine fluorescence in Lys-Phe-Lys-poly(A) or DNA complexes.

**Conclusion**—The results presented above clearly show that tyrosyl residues of peptides form stacked complexes with nucleic acid bases when the nucleic acid is single-stranded. No stacking was observed by proton magnetic resonance in the case of double-stranded DNA. This is in disagreement with some of the results reported by Gabbay et al. (13) who observed upfield shifts when tyrosine-containing peptides interacted with DNA. Although we have no explanation for this apparent discrepancy it should be noted that the PMR experiments of Gabbay et al. (13) were carried out at much higher DNA concentrations and that the upfield shifts were much smaller with native than with denatured DNA.

Our results also show that the specificity of stacking interactions with single strands does not depend on the sequence of the peptide. Notice particularly that this is not limited to the case of tyrosyl residues which have lysyl residues as nearest neighbors in the peptide sequence. The presence of alanyl residues in between tyrosine and lysine does not change the specificity of the stacking interaction for single strands. Stacking interactions of tyrosine with nucleic acid bases were shown earlier (14) to lead to tyrosine fluorescence quenching. Comparison of PMR and fluorescence data demonstrates that tyrosine fluorescence quenching may occur even when stacking interactions do not take place. The possible role of hydrogen-bonding interactions in fluorescence quenching was eliminated by a comparison with peptides containing O-methylated tyrosine or phenylalanine. The most likely explanation is that tyrosyl residues which are close enough to nucleic acid bases can transfer their excitation energy to bases. This transfer will lead to fluorescence quenching of the donor (tyrosine) without any appearance of a sensitized fluorescence since nucleic acid bases do not emit fluorescence at room temperature (their fluorescence quantum yield is 3 orders of magnitude lower than that of tyrosine). The same holds true in the case of phenylalanine fluorescence quenching.

The results presented above with oligopeptides do not ex-

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### Table VI

Effect of phosphate mono- and diamions on fluorescence of tyrosine and p-methoxyphenethylamine

<table>
<thead>
<tr>
<th>pH</th>
<th>Tyramine</th>
<th>p-Methoxyphenethylamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl (0.5 M)</td>
<td>6.0</td>
<td>1.0</td>
</tr>
<tr>
<td>NaH2PO4 (0.5 M)</td>
<td>4.6</td>
<td>0.6</td>
</tr>
<tr>
<td>NaH2PO4 (0.25 M)</td>
<td>9.1</td>
<td>0.06</td>
</tr>
<tr>
<td>NaH2PO4 (0.25 M) + NaCl</td>
<td>6.8</td>
<td>0.20</td>
</tr>
</tbody>
</table>

### Table VII

Critical Förster distances \( (R_0) \) in Ångströms for energy transfer from tyrosine to nucleosides in water at 293 K and in ethanol at 77 K, assuming fluorescence quantum yields of 0.14 and 0.425 at 293 and 77 K, respectively (see Ref. 16).

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Guanosine</th>
<th>Cytidine</th>
<th>Thymidine</th>
<th>Uridine</th>
<th>Adenosine</th>
</tr>
</thead>
<tbody>
<tr>
<td>293 K</td>
<td>15.7</td>
<td>15.2</td>
<td>14.6</td>
<td>12.1</td>
<td>10.3</td>
</tr>
<tr>
<td>77 K</td>
<td>23.6</td>
<td>22.2</td>
<td>21.8</td>
<td>20.1</td>
<td>20.0</td>
</tr>
</tbody>
</table>
elude that hydrogen bonding of tyrosyl side chains to nucleic acid bases might be involved in protein–nucleic acid complexes. However, they show that tyrosine fluorescence quenching does not imply a direct interaction of tyrosine with bases or phosphate groups on the nucleic acid.

REFERENCES
2. Brukov, V. I., and Bushuev, V. N. (1977) Biofizika 22, 26–31
The role of tyrosine in the association of proteins and nucleic acids. Specific recognition of single-stranded nucleic acids by tyrosine-containing peptides.
R Mayer, F Toulme, T Montenay-Garestier and C Helene

J. Biol. Chem. 1979, 254:75-82.

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