AN INVESTIGATION OF COMPLEXES OF SOME MODEL TRIPEPTIDES CONTAINING PHENYLALANINE AND TYROSINE RESIDUES WITH DNA BY FOURIER ^{1}H NMR SPECTROSCOPY

V. I. Permogorov, V. S. Gasparov,
Yu. A. Semiletov, Yu. P. Shvachkin,
and B. V. Tyaglov

UDC 577.125.126+547.92

Complexes of native and denatured DNA with model tripeptides containing phenylalanine or tyrosine residues flanked by lysine or arginine residues, respectively have been investigated by pulsed Fourier ^{1}H NMR spectroscopy. The existence of shifts into the strong-field region of the signals of aromatic protons of the model tripeptides in the complexes both with native and with denatured DNA has been shown. Results have been obtained that indicate the possibility of the intercalation of the side chains of aromatic amino acid residues into the DNA double helix.

At the present time, among the model systems used for studying various types of interactions by means of which the binding of proteins and nucleic acids is effected, an important place is occupied by peptides including aromatic amino acid residues [1-6]. The importance of the investigation of the interaction of the side chains of aromatic amino acids with nucleic acids is due to the fact that these planar systems are capable of becoming inserted (being wedged) between the planes of the nitrogen bases — of intercalating. This phenomenon may bear within itself many important biological functions in the vital activity of the cell.

However, in spite of the numerous investigations of complexes of various polynucleotides with model peptides containing aromatic acid residues, hitherto there has been no complete clarity in relation to the question of whether, in the process of complex-formation, the intercalation of the side chains of tyrosine or phenylalanine into the double helix of the polynucleotide takes place.

In view of the importance of the problem and the absence of a single opinion relative to the capacity of the side chains of tyrosine and phenylalanine for intercalating in polynucleotide double helices [1, 3, 4, 7-10], we have investigated complexes of DNA with model peptides having the general formulas H-L-Arg-L-Tyr(Phe)-L-Arg-OH (I),(II) and H-L-Lys-L-Tyr(Phe)-L-Lys-OH (III), (IV) by high-resolution pulsed Fourier ^{1}H NMR spectroscopy.

An analysis has been made of the magnitudes of the different chemical shifts $\Delta\delta$* and of the structure of the absorption lines of the protons of the aromatic amino acids present in the model peptides (I-IV) (Table 1). The measurements were performed under conditions excluding aggregation effects.

The assignment of the absorption lines of the aromatic protons of tyrosine and phenylalanine residues of the model tripeptides in the presence of DNA was made by comparison with the signals of the corresponding protons of the tyrosine and phenylalanine residues of the free peptides. The signals of the aromatic protons of the phenylalanine residues (ABC spin system [12]) had the form of a multiplet; for the tyrosine derivatives, two doublets (AA'BB' spin

*By the difference in chemical shifts of the signals of the protons of the individual groups of the complex ($\Delta\delta$) in the literature is understood a magnitude consisting of the difference between the values, δ_1, of the signals of the protons of individual groups and of individual protons, and the values, δ_2, of the signals of the corresponding protons in the complex [11]. The chemical shifts were measured to the center of gravity of the resonance signal.

All-Union Scientific-Research Institute of the Genetics and Breeding of Industrial Microorganisms, Moscow. Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 751-755, November-December, 1986. Original article submitted March 11, 1986; revision submitted June 2, 1986.

0009-3130/86/2206-0699$12.50

system [12]) were observed, the signal from the meta protons in the weak field and those from the ortho protons in the strong field.

As can be seen from Table 1, in the spectra of the peptides in the presence of DNA the signals of the aromatic protons of peptides (I-IV) undergo an upfield shift as compared with the resonance peaks of the corresponding protons in the spectra of the free peptides. Such a phenomenon most probably indicates the influence of the magnetic anisotropy of the nitrogen bases of the DNA on the protons of the aromatic system of the ligands when these systems are close and are bound through stacking interaction [11, 13-15]. It must be mentioned that the signals of the ortho protons of the aromatic systems of the tyrosine residues undergo a somewhat greater upfield shift, which indicates a large interaction of the magnetic anisotropy of the nucleic bases with just these protons. This, in its turn, is realized only when, in the complex-forming process, intercalation into the polynucleotide helix of the phenol ring of the tyrosine residue takes place. Unfortunately in the case of the complexes formed by the peptides (II) and (IV) with DNA no such analysis is possible, since the signals of the protons of the benzene ring of the phenylalanine residue have a complex profile. However, in this case, as well, together with an upfield shift a change in the structure of these resonance signals is observed which is due to the dissimilar actions of the magnetic anisotropy of the nucleic bases on the protons of the benzene ring, this apparently being a reflection of its intercalation into the DNA double helix.

In the case of the tripeptides (I-IV), in the presence of denatured DNA the values of $\Delta\delta$ are substantially greater than the corresponding difference chemical shifts found for the tripeptides in the presence of the native DNA, and this is explained by the ready accessibility of the nitrogen bases of the denatured DNA for the formation of stacking interactions with the side chains of the aromatic acid residues present in peptides (I-IV).

It must be mentioned that the half-widths of the spectral lines of the aromatic protons of tripeptides (I-IV) are increased only to a certain extent in the case of the formation of complexes both with the native and with the denatured DNA. This probably indicates the short lifetime of the complexes under consideration on the NMR time scale.

The process of the formation of complexes by the model tripeptides (I-IV) with DNA can be represented schematically in the following way. In the solution of a peptide there are three forms: 1) free peptide; 2) the peptide bound through electrostatic interactions of the guanidine and amino groups of the arginine and lysine residues with the phosphate groups of the DNA; and 3) the peptide bound through electrostatic interactions and through the intercalation of the side chains of the aromatic amino acid residues. A rapid exchange probably takes place between the three states of the peptide in which the contribution (proportion) of the third form is insignificant at room temperature.

It is well known that a rise in the temperature causes a high lability of the double-stranded structure of DNA and, in the first place, of the A-T pairs [16]. The contribution of the intercalation complex should probably increase with a rise in the temperature, which should lead to an increase in the corresponding difference in chemical shifts. Table 1 gives the values of $\Delta\delta$ for the protons of the tripeptides (I-IV) in the presence of DNA at two temperatures (28 and 43°C). It must be mentioned that at 43°C, as we have established spectrophotometrically, the DNA in a complex is present in the native state. As can be seen from Table 1, the $\Delta\delta$ values of the protons of the peptides (I-IV) obtained at 43°C exceed the $\Delta\delta$ values of the corresponding protons at 28°C, which apparently witnesses an increase in the proportion of the form intercalated into the DNA double helix.

The results of the present work and those on the thermal stability of the complexes of model peptides (I-IV) with polynucleotides that we obtained previously [6, 17] indicate that the side chains of the aromatic amino acid residues are probably capable of intercalating into the double helix.

To a certain extent, the results can remove the contradictions arising between proponents of the possibility of the intercalation of side chains in complexes of model peptides of polynucleotides [3-5, 10] and Wang [18], who has shown the absence of an untwisting of circular closed DNAs in the presence of tyramine for which the existence of an intercalation form in the complex with DNA was shown by the methods of ^{1}H NMR spectroscopy [1, 19-21]. It is quite understandable that under the conditions of the experiment the proportion of intercalation complex should, according to our results, be insignificant and, apparently, the author was unable to detect it.

TABLE 1. Values of the Difference in Chemical Shifts, $+\Delta\delta$, of the Signals of the Aromatic Protons of Peptides (I-IV) in the Presence of Native and Denatured DNA (D_2O, 10^{-3} M, Tris-DCl, pD 7.4, containing 10^{-3} M NaCl and $5 \cdot 10^{-5}$ M EDTA)

Type of DNA	Temperature, °C	$+\Delta\delta$, ppm*					
		I		II	III		IV
		ortho-	meta-		ortho-	meta-	
Native	28	0,03	0,02	0,07	0,02	0,01	0.06
Native	43	0,04	0,02	0,10	0,03	0,01	0.08
Denatured	28	0,22	0,19	0,21	0,20	0,17	0,19

*Concentration of peptides in the sample $5 \cdot 10^{-4}$ M and of DNA $1.5 \cdot 10^{-3}$ M.

A comparison of the $\Delta\delta$ values of the complexes formed by peptides (I) and (III) and by (II) and (IV) showed that the signals of the protons of the phenol rings in the complexes of the tyrosine-containing peptides with native DNA undergo a smaller shift than the absorption lines of the protons of the phenyl nuclei in the case of the corresponding complexes formed by the phenylalanine-containing peptides. The fact that in the spectra of tyrosine-containing peptides the shifts of the signals of the aromatic protons were smaller than those of their phenylalanine analogs may be due either to a smaller tendency of the more hydrophilic rings of the tyrosine residues to undergo intercalation or to an interaction of the hydroxy groups of the phenol rings of tyrosine residues in the intercalated state with one of the acceptor groups of the nitrogen bases of the DNA.

An analysis of the $\Delta\delta$ values of the peptides (I) and (II) and the peptides (III) and (IV) in the presence of DNA showed that somewhat greater values of $\Delta\delta$ are characteristic for arginine-containing peptides than for the corresponding lysine-containing peptides. This indicates that the aromatic residues of phenylalanine and tyrosine probably intercalate in both cases. We have shown previously [17, 22] on the basis of experiments on complexes of these peptides with glucosylated (phage T4) and nonglucosylated (amber mutant with respect to α,β-glucosyl transferase of phage T4) DNAs (binding was recorded from the parameters of the melting curves of the DNAs in the presence of the tripeptides) that lysine-containing tripeptides are localized in the small groove and arginine-containing tripeptides in the large groove of the DNA double helix.

Summarizing what has been said, it may be assumed that the intercalation of aromatic amino acid residues apparently takes place in both the large and small grooves of the double helix but the intercalation process is more probable in the first case (arginine-containing peptides).

The authors express their gratitude to N. F. Sepetov, A. M. Lysenko, and N.B. Petrov for assistance in the investigation.

EXPERIMENTAL

Model Tripeptide. The synthetic stereoregular tripeptides (I) and (II) and also commercial preparations of peptides (III) and (IV) (FRG) were used.

The concentrations of tripeptides (I) and (III) were determined from their UV absorption, using the value $E_{Tyr}^{275} = 1600$ [23]. The concentrations of the tripeptide (II) and (IV) were determined after the hydrolysis of aliquot samples under standard conditions (5.7 N HCl, 105°C, 24 h). A Durrum model 500 automatic amino acid analyzer was used for the quantitative analysis of the hydrolysates.

The DNA Preparations. Calf thymus DNA (USA) was used. To eliminate polysaccharides from the DNA preparations we used centrifugation at 50,000g (22,000 rpm) in a MSE machine at +5°C for 2 h. The DNA was subjected to disintegration in a Virtis-60 apparatus (8000 rpm, 30 min) to obtain fragments with a molecular weight of $2 \cdot 10^6$ daltons [24].

The preparations were dialyzed three times against 10^{-3} M Tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl buffer, pH 7.0) containing $5 \cdot 10^{-5}$ M disodium ethylenediaminetetraacetate (EDTA) and 10^{-3} M NaCl and were lyophilized. After lyophilization, the preparations were

dissolved in D_2O (99.9%), and this soution was kept for 12 h and was lyophilized, this procedure being repeated three times, after which the residue was dissolved in D_2O containing 10^{-3} M Tris-DCl, pD 7.4, and 10^{-5} M EDTA.

Preparation of the Complexes. The complexes of DNA with the tripeptides were prepared directly before an experiment by mixing solutions of DNA and of the model tripeptides in a ratio by weight of 3:1. The solutions of the model tripeptides were subjected to lyophilization three times and the residues were then dissolved in D_2O containing 10^{-3} M Tris-DCl, pD 7.4, 10^{-3} M NaCl, and $5 \cdot 10^{-5}$ M EDTA. The concentration of tripeptide in a sample was $5 \cdot 10^{-4}$ M and the concentration of the DNA solution $1.5 \cdot 10^{-3}$ M.

^{1}H NMR Spectra. High-resolution ^{1}H NMR spectra were recorded on a Bruker WM-500 pulsed NMR spectrometer at a frequency of 500 MHz. Recording conditions: length of a pulse 6 μsec; interval between pulses 1.8 sec; number of accumulations usually 300. The spectra were recorded in 5-mm tubes at temperatures of 28 and 43°C. The chemical shifts are given in the δ scale relative to tert-butyl alcohol as internal standard (1.29 ppm) with an accuracy of the determination of 0.001 ppm or, allowing for the multiplicity of the signals, 0.005 ppm.

SUMMARY

New information has been obtained in favor of the intercalation of the side chains of aromatic amino acid residues of model peptides with the general formulas H-L-Arg-L-Tyr(Phe)-L-ARg-OH and H-L-Lys-L-Tyr(Phe)-L-Lys-OH into the DNA double helix.

It has been shown that the side chains of the aromatic amino acid residues are capable of intercalating into the double-helical structure of DNA both in the small groove (in the case of the lysine tripeptides) and in the large groove (in the case of the arginine tripeptides).

LITERATURE CITED

1. C. Helene and J. C. Maurizot, C.R.C. Crit. Rev. Biochem., 2, 213 (1981).
2. C. Helene and C. Lancelot, Prog. Biophys. Mol. Biol., 39, 1 (1982).
3. E. J. Gabbey, K. Sanford, and C. S. Baxter, Biochemistry, 11, 3249 (1972).
4. E. J. Gabbey, K. Sanford, C. S. Baxter, and L. Kapicak, Biochemistry, 12, 4021 (1975).
5. D. E. Brown, Biochim. Biophys. Acta, 213, 282 (1970).
6. B. V. Tyaglov, V. E. Minaev, A. V. Trubnikov, and V. I. Permogorov, Mol. Biol., 15, 454 (1981).
7. J. L. Dimicoli and C. Helene, Biochemistry, 13, 714 (1974).
8. J. L. Dimicoli and C. Helene, Biochemistry, 13, 724 (1974).
9. J. L. Dimicoli and C. Helene, Biochimie, 53, 331 (1971).
10. K. G. Wagner and R. Lawaczek, J. Magn. Resonance, 8, 164 (1972).
11. P. O. P. Ts'o, N. S. Kondo, M. P. Schweizer, and D. P. Hollis, Biochemistry, 9, 997 (1970).
12. J. W. Emsley, J. Feeney, and L. H. Sutcliffe, High Resolution NMR Spectroscopy, Pergamon, Oxford (1965-1966).
13. B. W. Baugeter and S. I. Chan, J. Am. Chem. Soc., 91, 3910 (1969).
14. C. Giessner-Prettre and B. Pullman, J.Theor. Biol., 27, 81 (1970).
15. C. Giessner-Prettre and B. Pullman, J. Theor. Biol., 65, 189 (1977).
16. E. L. Ulrich, E. M. John, G. R. Gough, M. J. Brunden, P. T. Gilham, W. M. Westler, and J. L. Markley, Biochemistry, 22, 4362 (1983).
17. V. I. Permogorov, T. N. Fomenko, Yu. A. Semiletov, Yu. P. Shvachkin, and B. V. Tyaglov, Mol. Biol., 19, 1514 (1985).
18. J. Jacobsen and J. C. Wang, Biochim. Biophys. Acta, 335, 49 (1973).
19. C. Helene, J. L. Dimicoli, and F. Brun, Biochemistry, 10, 3802 (1971).
20. C. Helene, T. Montenay-Garestier, and J. L. Dimicoli, Biochim. Biophys. Acta, 254, 349 (1971).
21. C. Helen, Stud.Biophys., 57, 221 (1976).
22. B. V. Tyaglov, V. E. Minaev, and V. I. Permogorov, in: Symposium on Biophysics of Nucleic Acids and Nucleoproteins, Tallinn (1981), p. 130.
23. B. V. Tyaglov, S. V. Zenin, G. B. Sergeev, Z. A. Shabarova, and M. A. Prokof'ev, Mol. Biol., 9, 652 (1975).
24. N. B. Petrov, in: The Molecular Foundations of Genosystematics [in Russian], Moscow (1980), p. 51.