Recognition of base pairs by polar peptides in double stranded DNA

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ABSTRACT

The resonance Raman spectrum of native DNA has been obtained using excitation at 257 nm. In a first part, the spectral lines are assigned to the different nucleotide bases which provide the resonance effect. In a second part, the interactions of DNA with basic peptides (Arginine Methylester, Lysine Methylester, Arginyl-arginine) are investigated using excitation at 300 nm and 257 nm, which give complementary information about the DNA. Both Arginine Methylester and Arginyl-arginine recognize the A-T base pairs, the first one in the large groove, the second one in the narrow groove of DNA. The DNA-Lysine Methylester interaction is very likely not specific but can take place in the large groove of DNA.

INTRODUCTION

It is now well established that the different fractions of histones show some specificity for given DNA sequences (1). The H3 and H4 fractions seem to interact preferentially with the G-C rich regions (2) while the H1 (3) and H5 fractions (4) seem to bind to the A-T rich region of DNA.

The specificity of interaction still exists for the tetramer (H3)₂ (H4)₂ (1), but it was thought until recently that this specificity was disappeared for the histone octamer in chromatin. However, some recent work (1,7) suggest the possibility of specific interaction of histones and DNA in chromatin.

Several hypotheses have been proposed to explain the process of selective recognition of a nucleotide sequence by a protein. The simplest of these consists of a recognition of the DNA sequence by the Tyr or Trp (5) or by His (6) residues. However, the histones do not contain Trp and only a few Tyr or His ; in addition, the latter two residues are not located near or within the N-terminal part of histones, which appears to be mainly involved in the interaction with DNA in the case of the H2A-H2B-H3-H4 fractions (7). On the other hand, histones contain a large number of basic lysyl and

arginyl residues (from 20 % for H2A to 30 % for H1) often grouped together on high density segments (6). Numerous basic polypeptides have been used to simulate the action of these segments. Thermal denaturation and fractional precipitation studies have shown that poly-Lys interacts preferentially with A-T rich DNAs (8-10) while poly-Arg interacts preferentially with G-C rich DNAs (8-10). Unfortunately the same studies undertaken with oligopeptides (2 to 5 residues) similar to the segments found in the histones do not provide evidence for any selectivity (11,12). However, this lack of selectivity might be due to two sources :

i) The fractional precipitation used to investigate the DNA-basic polypeptide complexes might be inefficient for studying the DNA-oligopeptide complexes, because the fixation is not cooperative in that case : thus, the mixtures cannot precipitate.

ii) The side chains of arginyl and lysyl residues, which are positively charged, interact preferentially with the negatively charged phosphates of DNA, thus concealing a possible selectivity. This could be avoided by working at an ionic strength high enough to minimize electrostatic forces.

Thus, another investigation procedure had to be chosen to determine whether the nucleotide base environment might be selectively modified by the presence of a given basic peptide. In this paper it is shown that a possible approach is provided by resonance Raman spectroscopy, which yields information on local chemical groups of the DNA and their modification as a function of the changes in their environment.

Classical Raman spectra of various DNAs have been obtained using excitation in the visible range and are now satisfactorily interpreted in terms of contributions of the different nucleic bases (13-18). Recently, the spectra of the nucleic bases and of some derivatives were obtained under pre-resonance conditions (300 nm excitation), thus allowing to refine line assignments to local chemical groups of the bases (19) and to specify their contribution to the DNA spectrum obtained upon excitation at 300 nm (20). When excited at 257 nm, i.e. under pure resonance conditions, the same compounds yield still different spectra (21,22). These results and some complimentary experiments reported in this paper show that the contributions of the nucleic bases to the DNA spectrum recorded at 257 nm is different from that obtained with 300 nm or visible excitation. This is due to the fact that the Raman excitation profiles of the individual bases are quite different.

Thus, complimentary information is obtained from 300 nm and 257 nm

excited spectra of DNA-polar peptide complexes. This allows to propose a mechanism for recognition of nucleotide bases by the polar subunits of the proteins.

MATERIALS AND METHODS

Calf thymus DNA was prepared using the Schwander and Signer method (23). Lysine Methylester was purchased from Sigma, Arginine Methylester and $(\text{Arg})_2$ were purchased from Bachem Feinchemikalien A.G. (Switzerland) and used without further purification.

The preparation of the DNA solutions has been described elsewhere (24) and their concentration determined by 260 nm absorbance measurements ($\epsilon_{260 \text{ nm}}=6500$). The starting concentrations were 1 mM (phosphate) for the studies at 300 nm and 6 mM for excitation at 257 nm, in Tris buffer 1 mM, pH 7.2, NaCl 2mM. The peptides were then added in (phosphate) equimolar concentration and the solutions immediately homogenized on Vortex. The formation of the complexes was checked by circular dichroism as in our previous studies (25). For 257 nm excitation, the complex was studied directly ; in the case of excitation at 300 nm, the complex was centrifuged overnight at 75000 g, and the pellet was then used in our experiments.

The Raman spectra were obtained in the range of 1000 to 1800 cm^{-1} using a Jobin-Yvon Ramanor HG2S double monochromator (concave holographic gratings) equipped for UV transmission and working in the second order. The 300 nm excitation wavelength was provided by a Chromatix CMX4 pulsed dye laser. The data acquisition system has been described elsewhere (19).

The 257 nm emission is the first harmonic of the 514,5 nm line of a 95-4 Lexel Ar^+ continuous laser. The doubling system is a temperature matched ADP crystal (Inrad Corp.), and the fundamental and the first harmonic of the 514,5 nm line are split by a quartz prism. Quartz spinning cells have been used to avoid the photodegradation of the samples at this wavelength corresponding nearly to the maximum of the absorption spectrum. The Raman signal is measured in a photon counting system (Ortec) equipped with a parallel BDC digital output, and the spectra are recorded step by step in a multichannel analyser. It is then possible to do a numerical treatment of the data, such as smoothing by the Savitsky-Golay method (26), and normalization of the Raman line intensities, taking the 3500 cm^{-1} band of water as an internal standard.

RESULTS AND DISCUSSIONA. UV Raman spectra of DNA

For excitation at 300 nm, the preresonance Raman spectra of the nucleotide bases was presented in a previous paper (19) and a refinement of the assignment of the Raman lines to the vibrations of local chemical groups was proposed, supported on the one hand by the study of the 8- and 5-bromosubstituted species of purines and pyrimidines respectively, on the other hand by comparison with the theoretical results obtained by Tsuboi and coworkers (27). This preliminary work allowed us to give (20) an interpretation of the DNA preresonance Raman spectrum obtained under the same excitation (300 nm). Only the main results, which are of some interest for this paper, are recalled here (Table I).

For excitation at 257 nm, i.e. under pure resonance conditions, the relative intensities of the Raman lines observed for the four nucleotides are quite different from those obtained for excitation at 300 nm. Figure 1 presents the spectra of AMP, GMP, CMP and TMP obtained in neutral aqueous solutions with a concentration of 0.1 mg ml^{-1} . Figure 2 shows the calf thymus DNA spectrum obtained under the same excitation conditions (and in Tris buffer 1 mM, pH 7.2, $5 \times 10^{-3} \text{ M NaCl}$). The various contributions of the bases to the DNA spectrum are shown also: the intensity of the 1340 cm^{-1} line of AMP has been arbitrarily taken as unity, the other scales being determined to obtain the best fit between the reconstructed spectrum (combination of the four bases) and the original spectrum of native DNA. Table II recapitulates the results of the figures 1 and 2, and gives a proposed assignment of the DNA lines to the different bases, as we discuss below.

For excitation at 257 nm, the DNA spectrum exhibits five principal lines in the $1000\text{--}1800\text{-cm}^{-1}$ range, i.e. 1256, 1340, 1490, 1584 and 1660 cm^{-1} . The prominent 1340-cm^{-1} line is the easiest to assign: it is mainly due to an adenine mode of vibration. However, this line shows some broadening on both

TABLE I - The main Raman frequencies of DNA, in the $1000\text{--}1800 \text{ cm}^{-1}$ range, and their assignments to the different bases, under preresonance conditions: $\lambda_{\text{exc}} = 300 \text{ nm}$. (From Ref. 20).

Frequencies	1246 w	1305 w	1340 sh	1380 m	1493 vs	1530 sh	1584 w	1665 s
Assignment	T	C	A	T	G(A)	C	A	T

Fig. 1. Resonance Raman spectra in the range of 1000 to 1800 cm^{-1} of the four mononucleotides in neutral aqueous solution, 0.1 mg ml^{-1} . $\lambda_{\text{exc}} = 257 \text{ nm}$. A. Adenine monophosphate ; B. Guanine monophosphate ; C. Thymine monophosphate ; D. Cytidine monophosphate. The height of the main line has been chosen the same over four spectra but the intensity scale is different from one spectrum to another.

Fig. 2. A. Upper part. Resonance Raman spectrum (range 1000–1800 cm⁻¹) of native calf thymus DNA in Tris buffer 1 mM, pH 7.2, 5×10⁻³ M NaCl and reconstructed spectrum.

B. Lower part. The relative contribution of the four mononucleotides, which give the best fit between the native DNA spectrum and the reconstructed spectrum.

TABLE II - The Raman frequencies of the four mononucleotides and DNA, in neutral aqueous solution, under pure resonance conditions : $\lambda_{\text{exc}} = 257 \text{ nm}$.

AMP	TMP	GMP	CMP	DNA
1249 w	1190 w 1245 s		1245 m	1256 w, T, C
		1334 m	1298 m	1334 sh, G
1340 vs		1370 sh	1370 w	1340 s, A
	1378 s			1378 sh, T
1420 w	1460 sh			
1486 s		1490 vs		1490 s, A-G
		1584 s	1531 s	1584 m, A-G
1583 m		1630 w		
			1652 broad m	
	1662 vs			1660 w, T

sides compared to the 1340-cm^{-1} line of free AMP. It might be due to a guanine contribution around 1335-cm^{-1} (1334-cm^{-1} of medium intensity for GMP) and on the higher energy side to a thymine contribution near 1375-cm^{-1} (1378-cm^{-1} of strong intensity in the case of TMP). The very strong 1490-cm^{-1} line is a mixture of those observed at 1486-cm^{-1} for AMP and of the prominent 1490-cm^{-1} frequency shown in the spectrum of GMP. It corresponds to a wave of condensation and rarefaction along the long axis of the purine molecules. In the same way the 1584-cm^{-1} line can be attributed to a mixture of purine contributions corresponding to stretching vibrations of $\text{N}_3\text{-C}_4 = \text{C}_5\text{-C}_6$ bonds in both purines. The last principal high frequency line observed at 1660-cm^{-1} for DNA excited at 257 nm can be at least partially assigned to the thymine carbonyl modes of vibration : TMP shows its prominent line at 1662-cm^{-1} when excited under the same conditions.

We note that the thymine and cytosine contributions to the spectrum of DNA excited at 257 nm remain weak. They are situated in the region around

1250 cm^{-1} of the DNA spectrum (1245 cm^{-1} for both CMP and TMP) and in the high frequency part near 1660 cm^{-1} (1652 cm^{-1} for CMP, and 1662 cm^{-1} for TMP). For CMP, this is due to the fact that the resonance conditions are much less favourable for this base than for the other three : at 257 nm, the molar absorption coefficient of CMP is the weakest, compared to the other nucleotides, and the gap between the excitation and the maximum absorption wavelength the largest. This has been confirmed by recording at 257 nm the spectrum of poly dG-poly dC (28) : the contribution of cytosine is again very weak, compared to the guanine contribution. For TMP, we have shown elsewhere (29) that the thymine lines show a very important hypochromism when the base is included in a stacked structure, which is the case of the DNA used here in our experiments.

B. DNA-basic peptide interactions

Now we want to focus our attention on the variations observed for the DNA spectrum, when DNA is complexed with basic peptides, i.e. Arginine-Methyl ester (Arg ME), Lysine-Methyl ester (Lys ME) and Arginyl-arginine (Arg)₂. The nucleotide bases providing the resonance phenomenon, these spectral variations concern the vibrational modes of the bases only.

It is to be noted that if one of the ligands induces a local destacking or distortion of the double helix through electrostatic interactions, but without any interaction with the bases, one would expect an increasing of all the thymine lines of the spectrum (Raman hypochromism of the thymine lines when thymine is included in a stacked structure). This has been shown with a 300 nm excitation in the case of DNA by fractional melting of the double helix (30). This was also the case for poly (d(A-T))-poly (d(A-T)) complexed with an unwinding protein, i.e. pancreatic ribonuclease (29).

One can observe here spectral variations concerning the Raman lines of the four nucleotide bases and not the thymine only ; on the other hand, when thymine is involved, a few lines, but not all, of this base show some variations of their intensities. Thus, it is assumed that these spectral variations can be interpreted in terms of interaction with local chemical groups of the bases, and they should be useful for looking for a mechanism for the recognition of the bases by the peptides.

Figures 3 and 4 present the spectra of native DNA and of the different complexes, observed by excitation at 300 nm and 257 nm respectively.

a) In the case of the DNA-Arg ME complex (Figures 3C and 4C), the comparison with the native DNA (Fig. 3A and 4A) shows variations concerning

Fig. 3. Raman spectra of native DNA and of its complexes with basic peptides, $\lambda_{\text{exc}} = 300 \text{ nm}$. A. Native DNA ; B. DNA-Lys ME complex ; C. DNA Arg ME complex ; D. DNA-(Arg)₂ complex.

essentially the adenine lines (1340 and 1584 cm^{-1} at 300 nm , 1340 , 1490 and 1584 cm^{-1} at 257 nm) and to a lesser extent the thymine 1662-cm^{-1} line for both excitations. The G-C line intensities are essentially un-

Fig. 4. Same as Fig. 3, but $\lambda_{\text{exc}} = 257 \text{ nm}$.

changed, as one can see from the spectra recorded at 300 nm (Fig. 3C). For this excitation, the 1490-cm^{-1} line of DNA is almost exclusively due to a guanine mode of vibration, the adenine contribution at 1485 cm^{-1} re-

maintaining very weak (as has been confirmed on the poly(d(A-T)) spectrum (29)). Thus we conclude that the variations observed for the 1490-cm^{-1} line on the spectrum recorded at 257 nm (Fig. 4C) is due to a change in the intensity of the adenine mode of vibration, whose contribution is important for this excitation.

Once the assignment of Raman lines to local chemical groups of the bases is known, an attempt can be made to correlate the observed spectral variations of the complexes to some changes in the environment of these chemical groups. We have been helped by a Corey-Pauling model of peptides and double stranded DNA, to check if our assumptions were compatible with the possibilities of steric hindrance.

In the spectrum of adenine, the 1340-cm^{-1} and 1585-cm^{-1} lines mainly involve the $C_4 = C_5 - C_6$ bonds (coupled with a scissoring effect of the 6-amino group) : they are very sensitive to changes in the $C_6 - NH_2$ group (29). On the other hand, the 1490 cm^{-1} involves accordion motions along the long axis of the molecule (27), and is greatly affected by alterations at the N7 position (31). As we have previously described, these three lines show important variations in the DNA-Arg ME complex. Consequently, we assume that the DNA-Arg ME interaction involves the atoms of adenine located in the large groove of DNA, and might satisfy the following model :

- As demonstrated earlier (5,32), two electrostatic interactions can occur between negatively charged phosphates of one DNA strand and the two positively charged C_α and C_ϵ amino groups of Arg-ME.

- Two hydrogen bonds might be present in the large groove between Arg-ME and adenine : one between a hydrogen of the adenine C_6 amino group and the peptide C_α carbonyl group, another one between the N_7 of adenine and the N-H part of the Arg side chain.

Additionally, the involvement of the adenine $6-NH_2$ group is also supported by the weak intensity variations of the 1660-cm^{-1} line of thymine. This line is assigned to $C = O$ vibrations ; the hydrogen bond of the $C_4=O$ of thymine with the adenine amino group can be slightly perturbed by the interaction of this group with the peptide, thus explaining the intensity variations of the 1660-cm^{-1} line.

b) In the case of the DNA-(Arg)₂ complex (Fig. 3D and 4D) the comparison with the native DNA (Fig. 3A and 4A) shows spectral variations very different from those observed in the preceding case, between DNA and its complex with Arg ME. The changes in the thymine lines are now predominant, particularly in the spectrum obtained at 300 nm (Fig. 3D). The intensity of the

1660-cm⁻¹ line, due to stretching motions of C₂ and C₄ carbonyls of thymine, and that of the 1247-cm⁻¹ line, involving the N₃-region (27), are greatly enhanced in the complex. On the other hand, some modifications of the adenine 1340 and 1584-cm⁻¹ line intensities are in the present case small but detectable. These observations are consistent with the variations observed with the 257 nm excitation between DNA and its complex with (Arg)₂ (Fig. 4A and 4D respectively). The intensity changes of the 1490 and 1584-cm⁻¹ lines correspond to variations of adenine contributions.

Finally, the G-C lines seem essentially unaffected by the (Arg)₂ interaction : in particular the 1490-cm⁻¹ line of guanine which is the prominent line of the DNA spectrum recorded with 300 nm excitation, and usually very sensitive to changes in the environment of the guanine, is strictly unchanged here.

These spectral variations involve on the one hand the N₃H and C₂ = O region of thymine, on the other hand the N₃ region of adenine (33). These groups are located in the narrow groove of DNA, and as in the preceding case, according to a Corey-Pauling model, we assume the following mode of interaction :

- Three electrostatic interactions can exist between the NH₃⁺ groups of arginyl-arginine and the negatively charged phosphates of the phosphate chain, two on one strand, the third on the opposite strand, along the narrow groove of DNA.

- The interaction might establish a hydrogen bond between the N-H group of the arginyl moiety and N₃ of adenine, another hydrogen bond occurring between the C₂ = O of thymine and the N-H of the peptidic bond.

c) Figures 3B and 4B show the spectra of the DNA-Lys ME complex obtained with excitation at 300 nm and 257 nm respectively. The comparison with the spectra of native DNA shows that almost all lines change their intensities in the interaction : 1305, 1340, 1380 and mainly 1492 cm⁻¹ for a 300 nm excitation, 1340, 1490, 1584 and 1660 cm⁻¹ for a 257 nm excitation.

The variations observed in the intensities of the 1340-1380-cm⁻¹ (300 nm) and 1340-1660-cm⁻¹ (257 nm) frequencies might be assigned to distortion in the hydrogen bonding between adenine and thymine, approximatively in the same way as for the DNA-Arg ME complex, thus involving the large groove of DNA.

On the other hand, large intensity variations of the 1490-cm⁻¹ frequency of guanine and of the 1305-cm⁻¹ frequency of cytosine (300 nm) are now detectable. Thus they involve again the large groove of DNA through the G-C bonding between the NH₂ group of cytosine and the C₆ = O of guanine. Thus, it can be assumed that the DNA-Lys ME interaction involves the large groove

of DNA, but seem to be not specific of a given nucleotide base.

CONCLUSION

The most important feature is that the different lines of the Raman spectra of the DNA can be selectively modified by different ligands. The intensity variations of a line reflects the modification, due to the ligand, of the environment of some given chemical groups. The charge and dimension of Lys ME and Arg ME being almost identical, the observed differences between the DNA-Arg ME and DNA-Lys ME complexes cannot be solely due to steric hindrance, or to electrostatic interaction ; they reflect a specificity of interaction of the Arg residues with the adenine bases in the large groove, a recognition of A-T region by (Arg)₂ in the narrow groove, and no specificity of the Lys residue for a DNA sequence.

Up to now, we have no knowledge of a method allowing the observation of some recognition process between the basic peptides and the DNA. However, we can compare our results to those obtained with oligo- or polypeptides, and basic proteins. It has been shown (34) by Raman spectroscopy that the polylysine interacts in a non-specific manner with the DNA. On the other hand, the results obtained in classical Raman spectroscopy for DNA-protamine complexes (34,35) (the protamine is a basic protein which contains more than 60 % of Arg residues and very few Lys residues) and for chromatin (36), show an interaction of protamine and histone in the narrow groove of DNA, as in the case of DNA-(Arg)₂ complexes. For DNA-protamine complexes (35,36), one cannot observe any noticeable modification of the guanine vibrations, but an important intensity change of the 1580 cm⁻¹ line of the adenine, thus characterizing the specific interaction of the arginyl residues. The histones of the chromatin give almost identical results (36), which confirm the important role of Arginyl residues for core formation (37,38).

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