Raman Spectroscopic Studies of the DNA Cro Binding Site Conformation, Free and Bound to Cro Protein†

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ABSTRACT: Raman spectra of the DNA binding site for cro repressor protein were obtained in the presence and absence of bound cro protein. The 17 base pair fragment is a consensus sequence of the six cro binding sites in phage λ, except that the second base to the right of the center of pseudosymmetry is altered. Analysis of the spectrum of the free DNA indicates that the molecule exists in a B-like conformation with deviations from the usual B form occurring mainly in the bands assigned to A-T vibrations. The spectrum of the bound DNA was obtained by subtracting the spectrum of free cro from the spectrum of the complex which was estimated to be 90% bound. The DNA undergoes significant structural changes upon binding to the protein; most notable of these changes is a destacking of the G-C bases reflected by increases in the 1240, 1262, and 1320 cm^{-1} bands. A decrease in the 1361 cm^{-1} band that occurs has also been assigned to a destacking in guanine bases. The appearance of a 705 cm^{-1} band and the decrease and downshift of the 670 cm^{-1} band are consistent with the appearance of A-like character in the A-T region of the binding site when the protein binds; however, the spectra indicate that the entire binding site remains in a distorted B-like conformation. We use the 705 cm^{-1} band to estimate A-like character because the 800–850 cm^{-1} region is obscured by interference from strong protein bands. Other shifts in both intensity and position cannot be assigned to characteristic changes in conformation and therefore must be attributed to the protein influencing the structure in a novel way.

Structural variation of DNA within the B family has been shown to occur in both solution and crystal studies (McCall & Calladine, 1988). This local variation can have a strong effect on DNA/protein interactions. Examples of secondary structure playing an important role in selectivity include the trp repressor (Otwinowski, 1988) and the histone octamer (Drew & Travers, 1985). The deformability of a sequence of DNA has also been postulated to contribute to binding specificity. These types of criteria for binding have become known as indirect readout (Otwinowski, 1988). These considerations are of course secondary to the main criterion for specificity, namely, the pattern of hydrogen bond donor and acceptor groups presented by the DNA of a certain sequence. The significance of DNA secondary structure and deformability is becoming more apparent as crystallography (Yoon et al., 1988; Anderson et al., 1987) and Raman studies (Wang et al., 1987; Patapoff et al., 1988; Taillandier et al., 1987) show the enormous amount of sequence-dependent variability in conformation that DNA is capable of undergoing. Raman studies in particular show that there is an enormous amount of structural variability within the B family of DNA in solution (Thomas et al., 1989; Wartell & Harrell, 1986).

We present here a Raman spectroscopic study of the cro operator site in solution both free and bound to cro protein. Cro protein is a repressor protein of λ phage and binds to the oligonucleotide

d(TATCACCGCGGGTGATA)

d(ATAGTGGCGCCCACTAT)

with a binding constant on the order of 10^{12} M^{-1} (Kim et al., 1986). The oligonucleotide is a consensus sequence of the six cro binding sites in λ, with the exception of a single guanine (G)[1] residue to the right of the center. This residue was changed from a cytosine to prevent hairpin formation. This change has no effect on the binding constant (Kim et al., 1987). The sequence of the binding site is quite amenable to a Raman study because the oligonucleotide can be roughly divided into thirds, with the first and last third being made up primarily of adenine and thymine residues and the center third being comprised entirely of guanine and cytosine residues. In this way, changes in the Raman spectra can be correlated to different regions of the binding site because bands due to the different base pairs are usually spectroscopically resolvable.

The solution studies presented here demonstrate not only that the complexed DNA has a structure that varies from average B-form DNA (Wartell & Harrell, 1986) but also that the free DNA conformation shows subtle but very real differences as well. Bands in the Raman spectra are assigned based on a number of previous Raman studies of DNA with specific conformational features (Peticolas et al., 1987). Results of previous normal mode calculations (Letellier et al., 1986, 1987) are also used in assigning bands and interpreting the changes in the DNA spectrum upon protein binding. Comparisons to native B DNA were made by using data from Wartell and Harell (1986) as a standard for canonical B conformation as well as data from the Peticolas lab on the oligonucleotide GGTATACC (Wang et al., 1987) in B and A form. The analysis of the cro binding site spectrum shows that the free DNA structure is slightly different than native B-form DNA in sugar pucker and degree of stacking in the A-T region of the DNA. These structural differences may aid cro recognition of the operator site as it slides along the DNA. Once the cro binds, the differences in the Raman

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[1] Abbreviations: CAP, catabolite activator protein; bp, base pair(s); A, adenine; T, thymine; C, cytosine; G, guanine.

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spectrum from average B-form Raman spectra become even more apparent. In the spectrum of the bound DNA that has the protein spectrum subtracted, we see the appearance of a more A-like character within the general B spectrum. These A marker changes include the appearance of a 705 cm^{-1} band due to a sugar-coupled purine stretch and a large decrease in intensity and 4 wavenumber downshift in the 672 cm^{-1} thymine stretching band. Other changes in the spectrum occur in bands that are sensitive to stacking interactions. These bands generally behave in a way that suggests an overall destacking of base pairs, particularly in bands assigned to G-C base pairs. The destacking of A-T pairs seems to be the result of an increase in A-like conformation based on positional shifts of bands, while the destacking of G-C base pairs is not accompanied by shifts in position or the appearance of A marker bands. The destacking then may be the result of some other conformational change such as bending upon complexation with the cro protein. In general, the binding site is in a B-family conformation while bound and free, with some interesting structural variations in these two states.

Materials and Methods

The 17 base pair oligonucleotide used in this study is the consensus sequence of the six cro binding sites in λ, except that the second base to the right of the center is altered. This was done to prevent hairpin formation. Cro binds to this oligo as well or even better than the highest affinity OR3 site (Kim et al., 1987). The DNA was synthesized by the University of Oregon Biotechnology Laboratory utilizing an Applied Biosystem Model 380 DNA synthesizer. Purification was done by reverse-phase HPLC equipped with a 10 × 250 mm Vydac C4 column. The collected sample eluted with a single symmetric band which occupied about 90% of the total absorption area. Each strand was then run on an Amicon Centricon-3 filter (Danvers, MA) with two washes of 500 mM NaCl and three washes of water to first remove any HPLC buffer and then to remove as much salt as possible. Absorbance readings were taken, and equimolar amounts of each strand were annealed, aliquoted, and dried by rotary evaporation. Purified cro was the gift of Dr. Richard Brennan. The cro contained high glycerol and sulfate concentrations so that washing 3 times with binding buffer (100 mM KCl/1 mM EDTA, pH 7) using Centricon-3 microconcentrators was necessary. By this method, the concentration of protein was raised from 2 to 11.4 mg/mL.

Samples of free DNA were prepared by dissolving the DNA in 10 mL of binding buffer so that the final DNA concentration was about 909 mM ends or about 1% (w/v) phosphate. DNA/cro complex was made by dissolving the DNA in 10 mL of concentrated cro solution. The concentration of dimeric cro was about 815 mM. This corresponds to a DNA solution that was about 90% bound if we use the accepted binding constant for this oligo of about 1×10^{12} M^{-1} (Kim et al., 1987). Spectra of the free protein were also obtained. All spectra were taken at a temperature of 2 °C. The 514.5-nm light with 200-mW output power from an argon laser was used as the excitation beam to obtain Raman spectra for the solution samples. The experimental apparatus has been described previously (Thomas & Peticolas, 1983). About 40 scans were taken for each spectrum: free DNA, free protein, and DNA/protein complex. The Raman spectrum of water was also obtained. The spectral resolution of all spectra was about 2 cm^{-1}. The spectrum of the bound DNA was resolved from the complex by subtracting the spectrum of the cro which had previously had the water spectrum subtracted from it. Two bands in the protein spectrum were used to monitor the subtraction at 1004 and 1463 cm^{-1}, where the signal from the DNA is very weak. A subtraction coefficient of 0.9 was eventually used. The Raman spectrum of the DNA undergoes marked changes upon protein binding. These changes are quantitated by comparing heights of peaks relative to the 1095 cm^{-1} band which has been assigned to a phosphate backbone vibration. The height of this band is assumed to be conformationally insensitive. The height of each band is determined by fitting the base-lined spectrum to a sum of Lorentzian curves by a nonlinear least-squares fit program. This program has been described elsewhere (Patapoff et al., 1988).

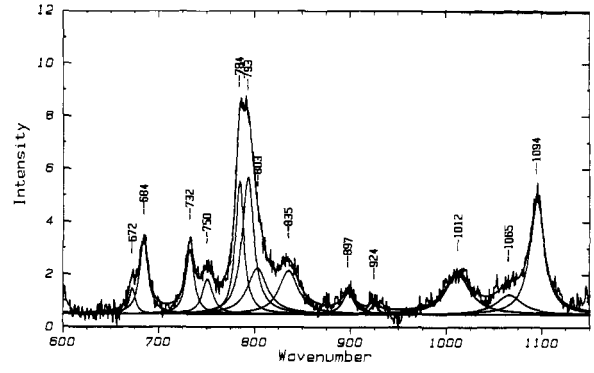

FIGURE 1: Raman spectrum (600–1150 cm^{-1}) of the consensus sequence of the six λ cro binding sites, taken with 200 mW of 514.5 nm argon laser light at pH 7.0, 2 °C. The concentration of DNA is 0.9 mM ends. The smooth curve is the sum of Lorentzians used to fit the data.

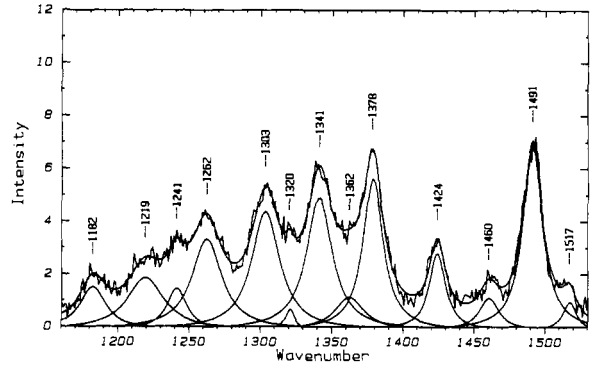

FIGURE 2: Raman spectrum (1150–1530 cm^{-1}) of the consensus sequence of the six λ cro binding sites. Same conditions as in Figure 1.

Results

The DNA sequence examined can be roughly divided into thirds, with the ends being made up predominantly of A,T base pairs and the middle region consisting entirely of G,C base pairs. This allows one to use changes in bands assigned to specific bases to say something about structural changes in different regions of the binding site. Spectra of both free and complexed DNA were normalized to the 1095 cm^{-1} PO_2^- stretch. The intensity of this band is known to be conformationally insensitive (Wartell & Harrell, 1986; Peticolas et al., 1987). The spectra of both free and bound DNA show an overall B-form conformation with some subtle and striking variations.

Unbound DNA. The Raman spectrum of free DNA in solution (Figures 1 and 2) is essentially that of B DNA (Wartell & Harrell, 1986; Peticolas et al., 1989). Models for average B-DNA conformation include a series of native DNAs and polynucleotides with varying GC content analyzed by Wartell and Harrell (1986) as well as the Dickerson dodecamer analyzed by Peticolas and co-workers (1989). The

presence of this large body of Raman data on B-form DNA now allows comparison with sequences of biological interest to determine if deviations from standard B form exist. The B conformation of the cro binding site is indicated by the position and height of the 673 and 684 cm^{-1} bands. The 673 cm^{-1} height is 0.229, and the 684 cm^{-1} height is 0.650 relative to 1095 cm^{-1} which is consistent with B-form DNA (Peticolas et al., 1987). This differs from Raman studies of the 21 bp CAP binding site where these bands' heights are in slightly different ratios to the 1095 cm^{-1} band (De Grazis et al., 1988). Further evidence for a generally B-like structure is indicated by the 786 cm^{-1} peak which is comprised of two bands, a 784 cm^{-1} cytosine vibration and a 793 cm^{-1} phosphate mode. A broad 835 cm^{-1} peak is assigned to the C2′ endo conformation of the furanose ring and exists at a relative height which is consistent with B-form DNA (Wartell & Harell, 1986; Thomas et al., 1989). A few bands show a slight deviation from native B DNA. These include an 803 cm^{-1} band which is assigned to the C3′ endo sugar pucker (Erfurth & Peticolas, 1975) and is significantly more intense than in native B-form DNA as well as appearing at a lower than average frequency. Wartell and Harrell have shown that the 809 ± 6 cm^{-1} band has an intensity of about 0.25 (for B DNA with a 53% G-C content) relative to the 1095 cm^{-1} band. Here, it has an intensity of 0.40, indicating an above-average population of C3′ endo ring pucker. A spectrum of the free binding site was also obtained at 25 °C, and the ratio of the 803 cm^{-1} band to the 1095 cm^{-1} band remained about 0.40 (data not shown). If we assume a C3′ endo population of between 10% and 20% (G. A. Thomas, unpublished results; Wartell & Harrell, 1986) in average B DNA, then the height of the 803 cm^{-1} band in the cro binding site corresponds to a 25% C3′ endo sugar pucker population.

The 733 cm^{-1} band which normally appears at 729 cm^{-1} is assigned to sugar-coupled adenine base vibrations (Letellier et al., 1987) and also deviates in intensity from native DNA. This band has a height relative to the 1095 cm^{-1} band of 0.47 in heterogeneous B DNA of similar GC content (Wartell & Harrell, 1986). In the cro binding site, the height is about 0.57 relative to the 1095 cm^{-1} band, which, along with the 4 wavenumber downshift in frequency, cannot be explained in terms of a more A-like character but may reflect a novel change in sugar conformation in the AT region of the binding site.

The spectrum of the 1150–1550 wavenumber region of free DNA is shown in Figure 2. The spectrum in this frequency region shows only mild differences from native B-DNA spectra, and the differences cannot be assigned to any known structural characteristics. The non-B-like bands are the 1423 cm^{-1} band normally seen at 1420 cm^{-1} and the 1460 cm^{-1} band which is characteristically seen at 1463 cm^{-1} in heterogeneous DNA (Small & Peticolas, 1971). Both bands are assigned to primarily adenine base vibrations. Normal mode calculations have shown that the 1420 cm^{-1} band is coupled to sugar stretching modes (Letellier et al., 1987). These shifts may reflect a sequence-specific difference in glycosidic bond angle or sugar conformation. In general, only mild deviations from canonical B form exist in the free DNA spectrum, and all of these differences appear to be in bands assigned to adenine or sugar modes. These differences do however reflect deviations from standard B conformation and may somehow affect the ability of the protein to recognize the specific binding site as it slides along the DNA.

Bound DNA. To facilitate analysis of the DNA structure while bound to cro (Figure 3), the protein spectrum was

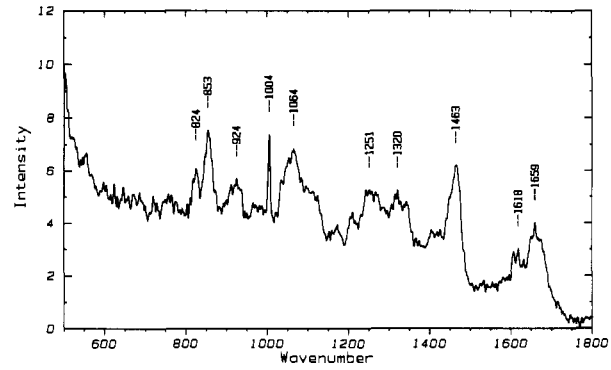

FIGURE 3: Raman spectrum (500–1800 cm^{-1}) of cro protein taken with the same conditions as for Figure 1. Protein concentration is 11.4 mg/mL. The water spectrum is subtracted so that only protein bands remain.

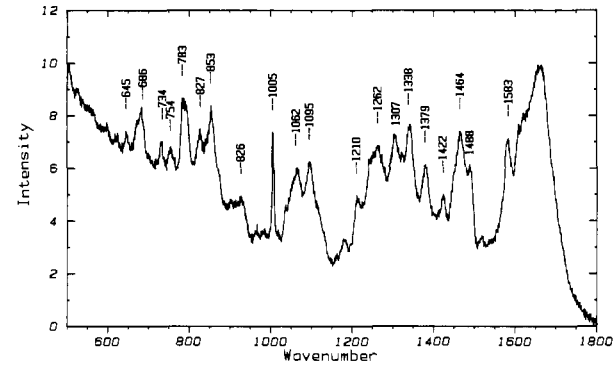

FIGURE 4: Raman spectrum (500–1800 cm^{-1}) of cro protein and consensus sequence binding site. Cro protein concentration is 11.4 mg/mL; DNA concentration is 0.9 mM ends. Data collection conditions are the same as for Figure 1.

computer-subtracted from the complex spectrum. Two bands in the protein spectrum (Figure 4) were used to monitor the subtraction, a 1004 cm^{-1} phenylalanine stretch and a CH_2 backbone vibration at 1463 cm^{-1} where the signal from DNA is very weak. When the 1463 cm^{-1} band was completely subtracted, the spectrum looked most like a pure DNA spectrum. The resulting spectrum contains bands almost exclusively attributed to the oligonucleotide. Even so, the spectrum may contain potential contributions arising from the protein that are due to structural differences between the bound and free protein. A case in point in the 815–860 cm^{-1} region is the two tyrosine stretching vibrations at 824 and 853 cm^{-1}. These bands are known to change in relative height to one another with changes in the hydrogen bond interactions of the tyrosine moiety (Siamwiza et al., 1975). Cro protein has six tyrosines in the dimer; four of the six change local environment when the protein binds DNA. Two are directly involved in H bonds with the oligonucleotide, and two change environment as the protein changes conformation to the specific binding mode in the crystal structure (Brennan et al., 1990). Therefore, this region of the complex spectrum cannot be adequately subtracted nor can it be used to draw conclusions about the state of the sugar puckers in the bound DNA. The subtracted spectrum (Figure 5) also shows residual intensity of the 1004 cm^{-1} band at 1006 cm^{-1}. This is believed to be the result of a decreased phenylalanine signal due to Raman hypochromism in the bound cro, caused by small conformational changes upon binding. This is the only region of the protein spectrum where small conformational changes have such an impact on the spectrum. The amide III region from 1100 to 1400 cm^{-1} is sensitive only to changes in the amount of β sheet and α helix. Such changes do not occur when cro

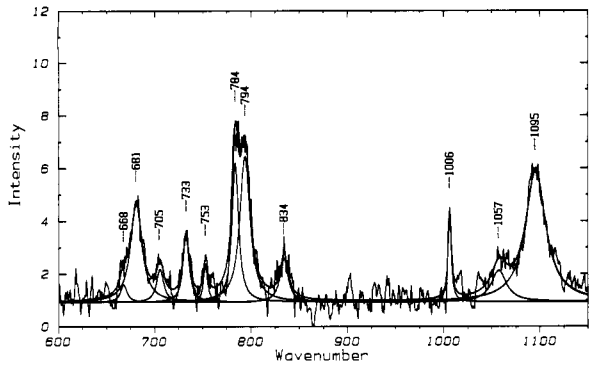

FIGURE 5: Raman spectrum (600–1150 cm^{-1}) of oligonucleotide bound to cro protein. The cro spectrum (Figure 3) has been subtracted out from the cro/DNA complex spectrum (Figure 4). The smooth curve is the sum of Lorentzians used to fit the DNA spectrum and can be compared to Figure 1.

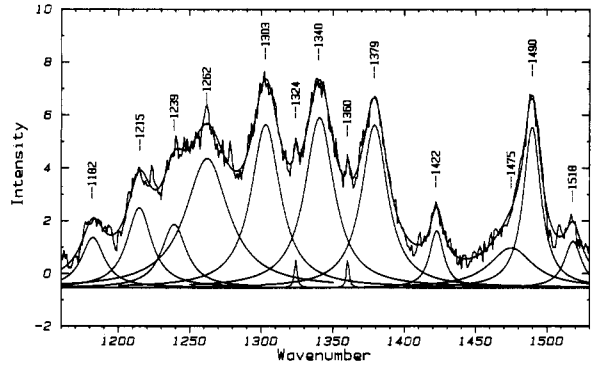

FIGURE 6: Raman spectrum (1150–1530 cm^{-1}) of the oligonucleotide bound to cro protein. The cro spectrum (Figure 3) has been subtracted out from the cro/DNA complex spectrum (Figure 4). The smooth curve is the sum of Lorentzians used to fit the DNA spectrum and can be compared to Figure 2.

protein binds DNA in the crystal and are not expected to occur in solution (Brennan et al., 1990). Complete subtraction of this band causes the appearance of oversubtraction in other regions of the spectrum. The intensity of this band is considerably weaker in the spectrum of the protein-subtracted complex than in that of the free protein. Although the subtraction criteria are subjective, the degree of subtraction used here gives the most reasonable DNA spectrum; furthermore, only changes between the free and bound DNA spectrum greater that 10% are considered as significant. The rest of the spectrum (Figures 5 and 6), however, shows an excellent subtraction and seems to give a pure DNA Raman spectrum.

The 600–1150 cm^{-1} region of the bound oligonucleotide spectrum (Figure 5) contains some striking differences from the free DNA spectrum (see Table I). Some of the strongest changes include a 40% decrease in height and a 4 wavenumber downshift of the 672 cm^{-1} bands, as well as the appearance of a peak at 705 cm^{-1}. The 672 cm^{-1} band is assigned to a thymine base vibration, and although the band is small, a 40% decrease is certainly significant and can easily be seen without using the curve-fitting program. There are no protein contributions in this region, so it is impossible to obtain this result from oversubtraction of protein bands. The decrease in the 672 cm^{-1} band is consistent with either an increase in A-like character or a destacking in the thymine-rich region of the binding site. This band has been shown to split into two much smaller bands at 642 and 662 cm^{-1} upon B to A transition (Wang et al., 1987). No 642 cm^{-1} band is present, so it does not appear that the AT region of the binding site adopts an entirely A-like conformation; however, some conformational

Table I: Position, Height,[a] and Assignment of Raman Bands of the Consensus Sequence Binding Site Unbound and Bound to Cro Protein, 600–1100-Wavenumber Region

unbound		bound			
position	height	position	height	% change	assn
672.2	0.229	667.8	0.133	−41.9	Thy[c]
684.4	0.650	680.9	0.749	15.2	Gua[c]
		705.6	0.255		bk[d]
732.6	0.569	732.8	0.507	−10.9	Adn[c]
751.7	0.308	753.1	0.293	−4.9	Thy[c]
784.2	1.169	784.0	1.063	−9.1	Cyt[c]
792.8	1.201	794.0	1.115	−7.2	bk[b]
802.7	0.403				bk[d]
835.3	0.382	834.2	0.337	−11.8	bk[b]
897.2	0.194				bk[e]
924.3	0.088				bk[b]
		1006.1	0.695		
Adn/Thy[b]					
1011.8	0.352				
1065.2	0.171	1057.4	0.242	41.5	bk[b]

[a] Height relative to 1095 cm^{-1}. [b] Erfurth & Peticolas (1975). [c] Letellier et al. (1986, 1987). [d] Thomas & Benevides (1985). [e] Nishmura et al. (1985).

changes are clearly taking place. The 705 cm^{-1} band is also a marker band for A-form DNA. This band cannot be correlated to a specific region of the binding site because this band appears when both A-T and G-C polymers adopt the A conformation (Thomas & Benevides, 1985; Nishmura et al., 1985). Normal mode calculations have assigned it to purine stretching vibrations that are also coupled to sugar modes (Letellier et al., 1987). This band appears only in A-form DNA, so it was used as a measure of the C3′ endo population in the bound DNA. This was done because the traditional A-form marker band and 808 cm^{-1} band are not resolvable due to interference from strong tyrosine bands at 824 and 853 cm^{-1} which change in intensity upon binding. The height of the 705 cm^{-1} band relative to the 1095 cm^{-1} band is 0.255. An estimate of the C3′ endo population in the bound DNA is based on a comparison of the relative height of the 808 cm^{-1} band of GGTATACC in low salt, in high salt, and in the A-form crystalline state where the amount of C3′ endo can be estimated for each case. Using a conversion factor of 1.9 that was determined from the slope of a line "% C3′ versus 808 cm^{-1} height" in GGTATACC, we can calibrate the height of the 705 cm^{-1} band relative to the C3′ population for that oligonucleotide. By using the GGTATACC oligo as a standard, we can estimate the C3′ population in the cro binding site using the 705 cm^{-1} bandwidth to be about 34%. This is about 10% higher than in the unbound state and about 15% higher than in average B DNA.

Other indications of subtle structural changes upon binding which are more difficult to interpret include a 15% increase in intensity and 4 wavenumber downshift in the 685 cm^{-1} band and a slight decrease in the 732 cm^{-1} band. The 685 cm^{-1} band is assigned to guanine base vibrations (Letellier et al., 1986) and has been shown to decrease in intensity when G-C polymers are melted and stacking is disrupted (Erfurth & Peticolas, 1975). Normal mode calculations indicate that this band undergoes a slight downshift when the sugar goes from C2′ endo anti to C2′ endo syn (Dohy, 1989). Studies on A-form poly(dG)-poly(dC) report an 18 cm^{-1} downshift of the 685 cm^{-1} band during the B to A transition (Nishimura et al., 1985). The downshift in position of the 685 cm^{-1} band is not nearly this dramatic, however, in the bound DNA spectrum. The peak is about 30% broader in the bound than in the free oligonucleotide spectrum. This could indicate an increase in the variation of the sugar pucker about the C2′ endo anti

conformation coupled to guanine moieties. The 732 cm^{-1} band is assigned to a sugar-coupled adenine vibration. It undergoes a very small decrease in height, which is difficult to interpret in light of the fact that this band normally increases slightly when the stacking is disrupted during melting and is relatively insensitive to the B to A transition in A-T polymers. Overall, it can be seen from this region of the spectrum alone that the cro has a profound influence on the local structure of the DNA. This becomes more apparent when we analyze the higher wavenumber region of the spectrum.

The 1100–1530 cm^{-1} region of the spectrum (Figure 6) also undergoes some dramatic changes upon complex formation. This region contains many base vibrations which are sensitive to stacking interactions. Spectral changes here include many larger increases and decreases in height as well as small changes in the position and half-width of several peaks. Interpretation in this region is difficult because of the large number of bands and the fact that many peaks contain contributions from both purine or both pyrimidine residues. This makes it harder to localize the structural changes to specific parts of the binding site. The overall spectrum indicates that while the protein seems to influence DNA structure, the binding site retains an overall B-like conformation.

A number of peaks can be analyzed with confidence however (Table II). These include the 1241, 1262, 1320, and 1361 cm^{-1} bands. The first three bands increase in height by about 40%, and the 1361 cm^{-1} band decreases by about 8%. Positional shifts also occur in these bands when cro binds: 1241 cm^{-1} shifts to 1239 cm^{-1}, and 1320 cm^{-1} shifts to 1323 cm^{-1}. The bands are all assigned to guanine or cytosine base vibrations, except for the 1241 cm^{-1} band, which contains strong thymine contributions (Peticolas et al., 1987). The first three have been shown to increase in height when stacking is disrupted during melting, and the last decreases under the same conditions (Erfurth et al., 1975). There are also charcteristic changes when poly(dG)-poly(dC) goes into A form (Benevides et al., 1986). Overall, in bands that can clearly be assigned to guanine or cytosine, stacking seems to decrease in a manner that is more like simple destacking rather than an B to A transition. This conclusion is drawn from the types of positional shifts observed in these bands upon binding since both the B to A transition and melting involve destacking of the base pair by two different means and the overall height effect in the bands is the same. Shifts in position, however, are slightly different for the two transitions. The 1320 cm^{-1} band upshifts about 4 wavenumbers upon binding, which is consistent with a melting transition and not a B to A transition.

Of the many bands which contain adenine or thymine contributions, only three have very slight or no contributions from guanine or cytosine vibrations. These are the 1181, 1378, and 1460 cm^{-1} bands. The 1181 cm^{-1} band is assigned to a sugar-coupled thymine base vibration. This band normally increases in both the melting (Small & Peticolas, 1971) and B to A transition (Thomas et al., 1984; Erfurth et al., 1975), although it is a much smaller increase in the B to A transition. Here it shows a 16% increase, which makes it difficult to assign to a particular transition because we can imagine cro protein disrupting the stacking by a small amount without changing the base tilt angle to that of A-form DNA. The 1459 cm^{-1} band broadens and shifts considerably from its position in the free DNA. This leads one to believe that this is probably a subtraction artifact of the very strong 1459 cm^{-1} band which is assigned to protein CH_3 asymmetrical deformation (Siamwiza, 1975). This protein band is believed to be conformationally insensitive, and so the artifact is due to the subtraction of a very strong band from a relatively weak band. The 1378 cm^{-1} band which does have small contributions from guanine base vibrations is mainly due to adenine sugar-coupled C'1 N9 stretches as well as C6 N6 base stretches. This band is relatively insensitive to melting phenomena and shows only a slight decrease in B to A transitions. Here we note that the band does not change significantly in height or width or position upon cro binding.

The rest of the DNA bands in this region are the result of contributions from both guanine and adenine; therefore, changes in structure associated with changes in band height or position cannot be correlated to specific regions of the binding site. Bands that contain vibrational contributions from both purines or both pyrimidines include the 1281, 1303, and 1340 cm^{-1} bands, which all increase in height, and the 1423 cm^{-1} band which decreases. The 1218 cm^{-1} band contains contributions from thymine around 1210 cm^{-1} and cytosine at 1220 cm^{-1}. Both of these peaks decrease when stacking is disrupted either by a B to A transition or by melting. When cro binds, there is a significant increase in peak height in the spectrum of the bound DNA, which is inconsistent with both G-C and A-T bands in other regions of the spectrum. Bands that support the notion of an overall decrease in the stacking interaction include the 1303, 1340, and 1423 cm^{-1} bands, all of which are the result of contributions from adenine and guanine base vibrations. The 1303 cm^{-1} band is a sugar-coupled purine stretch that is due mainly to adenine vibrations. It is relatively insensitive to the B to A transition and increases during the melting of poly(A-T). When cro binds, this band increases approximately 38%. The 1340 cm^{-1} band is also a sugar-coupled purine stretching mode that is made up of the unresolved 1334 and 1344 cm^{-1} bands. This band is relatively insensitive to melting phenomena while displaying a slight increase and downshift during the B to A transition. In the spectrum of the bound binding site, the height of the 1340 cm^{-1} band increases but is not accompanied by any shift in position, indicating a novel change in the stacking interaction caused by cro binding. The 1423 cm^{-1} band is a small band with relative contributions from every region of the DNA including the sugar–phosphate backbone. This band downshifts position when changing to A form and increases very slightly when melting. Here it decreases about 25%, but since this band contains contributions from all parts of the DNA, it is impossible to assign a conformational change to this decrease in height except to say that it may be a novel structural change associated with protein binding.

Discussion

Raman studies of cro operator in solution, bound to cro protein and free, indicate that the DNA exists in a B-family conformation with mild structural variations that become more pronounced when the protein binds. The deviations from B-type structure in free DNA fall into two categories: differences that can be attributed to an A-like character and differences that have not been correlated to a particular conformational transition. Indications of a slightly increased A-like sugar conformation include the intense and broad 803 cm^{-1} band characteristic of a C3′ endo sugar conformation, as well as a downshifted and significantly smaller than B form 733 cm^{-1}1 band. Other features in the spectrum of the free DNA which are not consistent with B DNA are found in the higher wavenumber region where stacking interactions have a large influence. Those bands which are shifted include the 1423 cm^{-1} bands, normally seen at 1420 cm^{-1}, and the 1460 cm^{-1} band, normally seen at 1463 cm^{-1}. Both bands are assigned to adenine vibrations, and neither shift can be correlated

Table II: Position, Height,[a] and Assignment of Raman Bands on the Consensus Sequence Binding Site Unbound and Bound to Cro Protein, 1150–1530-Wavenumber Region

unbound		bound			
position	height	position	height	% change	assn
1181.8	0.380	1182.2	0.386	16.5	Thy/Gua/Cyt[b]
1218.8	0.458	1214.6	0.614	53.5	Thy/Cyt/bk[b]
1240.9	0.366	1238.9	0.489	53.2	Cyt/Thy/bk[b]
1262.0	0.796	1262.0	0.990	42.4	Cyt/bk[c]
1303.1	1.039	1302.9	1.251	37.9	Adn[c]
1320.3	0.178	1323.9	0.216	39.0	Gua/bk[c]
1340.7	1.162	1340.5	1.303	28.5	Adn[c]
1361.6	0.289	1360.4	0.232	−8.0	Gua/Cyt[c]
1378.4	1.326	1378.9	1.246	7.7	Adn/Gua[c]
1423.6	0.674	1422.5	0.438	−25.4	Adn/Gua/bk[c]
1459.9	0.280	1474.8	0.307	25.6	Adn/bk[c]
1490.9	1.602	1489.6	1.230	−12.0	Gua/bk[c]
1516.8	0.248	1518.2	0.358	65.4	Adn[c]

[a] Height relative to 1095 cm^{-1}. [b] Peticolas et al. (1987). [c] Letellier et al. (1986, 1987).

to any known change in conformation. In general, it seems that deviation from canonical B form in free DNA are not large and occur either in backbone or in adenine residues, thus localizing the changes to the end regions of the binding site. It is interesting to speculate about how these structural variations influence recognition by the cro protein.

Upon binding with the cro protein, the DNA seems to undergo some further structural changes, while still maintaining an overall B conformation. These changes are reflected in bands assigned to all the bases as well as backbone vibrations. The spectrum indicates an overall destacking of residues as well as the appearance of the 705 cm^{-1} A-marker band. The decrease in stacking interactions is consistent with NMR results of a similar system (Metzler & Lu, 1989). Changes in the height of bands assigned to guanine or cytosine residues correlate well with simple melting phenomena, while changes in the height of bands assigned to adenine or thymine residues are often accompanied by positional shifts associated with a B to A transition. The disruption of G-C stacking observed in the Raman spectrum of the cro/oligo complex in solution can be attributed in part of the appearance of an overall bend in the binding site when cro binds that has been reported in the crystallography data of the cocomplex (Brennan et al., 1990). In addition, phosphate–phosphate distances in the G-C portion of the crystalline cocomplex decrease to values close to A-form DNA. The Raman data suggest that formation of a band and the related decrease in the interphosphate distances in the center C,G section can occur by disruption of base stacking similar to melting and unwinding, without adoption of a formal A-form structure. The outer A,T sections in the crystalline cocomplex at the resolution currently available appear to adopt B-form structure. The Raman data concerning the cocomplex in solution suggest that the outer A,T sections are in a B form though the A,T sections contain more A-like character when the oligo is bound than when unbound. This information was not directly available from crystallographic studies because the resolution does not allow detailed analysis of the DNA sugar pucker which may in any case be altered by crystal packing forces.

The data indicate that the conformation of the unbound cro consensus sequence also differs slightly from the canonical B form of DNA observed in solution for DNA of heterogeneous sequence. The slight deviation may play a role in the recognition of cro protein for the specifc binding sites. Recognition of the overall difference between the binding site and other regions of the DNA could cause the cro protein to go into the specific binding mode and engage in all the specific hydrogen bonds that establish specificity.

Once the specific complex forms, further conformational changes occur in the entire binding site. These changes include a slight increase in A-like character in the backbone as well as a slight destacking of the bases. The ability of the DNA binding site to distort in the protein/DNA complex may add to the overall favorable specific binding energy and compensate for energy lost when stacking is disrupted. Stacking interactions are clearly very important to duplex stability. Maximum base interaction is achieved by a purine–purine pair, while the weakest interaction occurs with a pyrimidine–pyrimidine pair. Much more conformational variability is possible in the case of a purine–pyrimidine or pyrimidine–purine dinucleotide because stacking interactions are possible between opposite purines. It is interesting to note that out of 16 dinucleotide units in the cro binding site, 11 are purine–pyrimidine or pyrimidine–purine steps and 5 are purine–purine or pyrimidine–pyrimidine steps. We can imagine that this conformational flexibility contributes positive binding energy to the cro/DNA complex.

Registry No. d(TATCACCGCGGGTGATA)·d(ATAGTGGCGCCCACTAT), 104121-09-7.

References

Anderson, J. E., Ptashne, M., & Harrison, S. C. (1987) *Nature 326*, 846.

Benevides, J. M., Wang, A. H.-J., Rich, A., Kyogoku, Y., van der Marel, G. A., van Boom, J. H., & Thomas, G. J. (1986) *Biochemistry 25*, 41.

Brennan, R., & Matthews, B. W. (1990) *Proc. Natl. Acad. Sci. U.S.A. 87*, 8165.

De Grazia, H., Brown, K., Cheung, S., & Wartell, R. (1988) *Biochemistry 27*, 6359.

Dohy, D., Ghomi, M., & Taillandier, E. (1989) *J. Biomol. Struct. Dyn. 6*, 741.

Drew, H. R., & Travers, A. A. (1985) *J. Mol. Biol. 186*, 773.

Erfurth, S. C., & Peticolas, W. L. (1975) *Biopolymers 14*, 247.

Kim, J., Takeda, Y., Matthews, B. W., & Anderson, W. F. (1987) *J. Mol. Biol. 1*, 149.

Letellier, R., Ghomi, M., & Taillandier, E. (1986) *J. Biomol. Struct. Dyn. 3*, 671.

Letellier, R., Ghomi, M., & Taillandier, E. (1987) *J. Biomol. Struct. Dyn.* 4, 663.

McCall, M., & Calladine, C. R. (1988) *Annu. Rev. Cell Biol. 4*, 1.

Metzler, W. J., & Lu, P. (1989) *J. Mol. Biol. 205*, 149.

Nishimura, Y., Torigoe, C., & Tsuboi, M. (1985) *Biopolymers 24*, 1841.

Otwinowski, Z., Schevitz, R. W., Zhang, R. G., Lawson, C. L., & Jochiamiak, A. (1988) *Nature 355*, 321.

Patapoff, T. W., Thomas, G. A., Wang, Y., & Peticolas, W. L. (1988) *Biopolymers 27*, 493.

Peticolas, W. L., Kubasek, W. L., Thomas, G. A., & Tsuboi, M. (1987) *Biological Applications of Raman Spectroscopy* (Spiro, T., Ed.) Wiley, New York.

Peticolas, W. L., Thomas, G. A., & Weng, Y. (1989) *J. Mol. Liq. 41*, 367.

Siamwiza, M. N., Lord, R. C., Chen, M., Takamatsu, T., Harada, I., & Shimanouchi, T. (1975) *Biochemistry 14*, 4870.

Small, E., & Peticolas, W. L. (1971) *Biopolymers 10*, 1377.

Taillandier, E., Ridoux, J. P., Liquier, J., Leupin, W., Denny, W. A., Wang, Y., Thomas, G. A., & Peticolas, W. L. (1987) *Biochemistry 26*, 3361.
Thomas, G. A., & Peticolas, W. L. (1983) *J. Am. Chem. Soc. 105*, 993.
Thomas, G. J., & Benevides, J. (1985) *Biopolymers 24*, 1101.
Thomas, G. A., Kubasek, W. L., Peticolas, W. L., Greene, P., Grable, J., & Rosenberg, J. M. (1989) *Biochemistry, 28*, 2001.
Wang, Y., Thomas, G. A., & Peticolas, W. L. (1987) *J. Biomol. Struct. Dyn. 5*, 249.
Wartell, R. M., & Harrell, J. T. (1986) *Biochemistry 25*, 2664.
Yoon, C., Prive, G. G., Goodsell, D., & Dickerson, R. E. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*, 6332.

The Membrane Interaction of Amphiphilic Model Peptides Affects Phosphatidylserine Headgroup and Acyl Chain Order and Dynamics. Application of the "Phospholipid Headgroup Electrometer" Concept to Phosphatidylserine†

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ABSTRACT: Deuterium nuclear magnetic resonance (^{2}H NMR) was used to study the interaction of amphiphilic model peptides with model membranes consisting of 1,2-dioleoyl-*sn*-glycero-3-phospho-L-serine deuterated either at the β-position of the serine moiety ([2-^{2}H]DOPS) or at the 11-position of the acyl chains ([11,11-$^2H_2$]DOPS). The peptides are derived from the sequences H-Ala-Met-Leu-Trp-Ala-OH (AX, one-letter code with X = MLWA) and H-Arg-Met-Leu-Trp-Ala-OH (RX^+) and contain a positive charge of +1 ($AXme^+$) or +2 ($RXme^{2+}$) at the amino terminus or one positive charge at each end of the molecule ($AXetN^{2+}$). Upon titration of dispersions of DOPS with the peptides, the divalent peptides show a similar extent of binding to the DOPS bilayers, which is larger than that of the single charged peptide. Under these conditions the values of the quadrupolar splitting ($\Delta\nu_q$) of both [2-^{2}H]DOPS and [11,11-$^2H_2$]DOPS are decreased, indicating that the peptides reduce the order of both the DOPS headgroup and the acyl chains. The extent of the decrease depends on the amount of peptide bound and on the position of the charged moieties in the peptide molecule. The effects exerted by the peptides on the $\Delta\nu_q$ value of [2-^{2}H]DOPS are consistent with the PS headgroup responding as a molecular electrometer to the surface charge resulting from the presence of the peptides in the lipid–water interface. The effects on the acyl chain deuterons are in agreement with a localization of the peptides intercalated in between the lipid headgroups. Titrations of DOPS with poly(L-lysine)$_{100}$, which were included for reasons of comparison, reveal increased $\Delta\nu_q$ values. When the peptide–lipid titrations are carried out without applying a freeze–thaw procedure to achieve full equilibration, two-component ^{2}H NMR spectra occur. The apparently limited accessibility of the lipid to the peptides under these circumstances is discussed in relation to the ability of the peptides to exhibit transbilayer movement. ^{2}H spin–lattice relaxation time $T1$ measurements demonstrate a decrease of the rates of motion of both headgroup and acyl chains of DOPS in the presence of the peptides.

The interactions of peptides and proteins with the lipid phase of biological membranes are essential for many biological processes. Due to the complexity of biological membranes, model systems consisting of isolated compounds are often used to investigate at a molecular level how the mutual interaction of peptides and lipids may contribute to a particular biological function. A set of interrelated model peptides based on the sequence H-Ala-Met-Leu-Trp-Ala-OH has been designed for the purpose of studying molecular aspects of membrane insertion and translocation processes of peptides/proteins. In a previous study (de Kroon et al., 1990b) the lipid affinity and the membrane topology of several peptide derivatives varying in net charge and hydrophobicity have been assessed by using the fluorescence properties of the tryptophan residue. These peptides attain a localization in the membrane–water interface, the insertion depth depending on the peptides' charge and hydrophobicity. Particularly, the accessibility of the peptides' tryptophan residue to bromines attached at different positions along the phospholipids' acyl chains, reported in that study, raised questions concerning the ability of these peptides to perturb the acyl chains. Here the influence of these peptides on the order and dynamics of the lipid molecules at the level of the headgroup and the acyl chains is reported.

^{2}H NMR,[1] a nonperturbing biophysical technique, provides a suitable method to address these questions (Seelig & MacDonald, 1987). The main experimental parameters obtained from ^{2}H NMR encompass the deuterium quadrupole

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[1] Abbreviations: NMR, nuclear magnetic resonance; HPLC, high-performance liquid chromatography; DOPS, 1,2-dioleoyl-*sn*-glycero-3-phospho-L-serine; DMPS, 1,2-dimyristoyl-*sn*-glycero-3-phospho-L-serine; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; POPS, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-L-serine.

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