Interactions of the 26–39 fragment of the *cro* protein from λ bacteriophage with nucleic acids

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A tetradecapeptide with a sequence identical to residues 26–39 of the *cro* protein from bacteriophage λ has been synthesized. This peptide has no secondary structure in an aqueous buffer but adopts an α-helical conformation in the presence of 20% hexafluoroisopropanol. The fluorescence of the single tyrosyl residue of the *cro* protein fragment is quenched upon binding to nucleic acids. Proton magnetic resonance has been used to investigate complex formation of the *cro* protein fragment with a self-complementary decadeoxynucleotide d(AATTGCAATT). Changes in resonance positions and linewidths have been observed for both partners in the 4 complexes which are obtained when either the single-stranded or double-stranded oligonucleotide is mixed with either the random coil or the α-helical peptide. These studies are presently extended to the specific complex formed by the *cro* protein fragment with the OR3 operator sequence.

cro *protein* *Protein–DNA interaction* *NMR* *α-Helix* *Oligodeoxynucleotide*

1. INTRODUCTION

Structural complementarity between proteins and nucleic acids is a prerequisite to the formation of specific complexes. Interactions between functional groups of both partners are then required to permit the selective recognition of nucleic acid base sequences [1]. The crystal structure of the *cro* protein from λ bacteriophage has been solved [2,3]. This protein recognizes the λ operator OR sequences and switches λ repressor synthesis off. On the basis of the crystal structure a model has been proposed whereby a *cro* protein dimer recognizes the partially symmetrical DNA operator sequences [2,3]. The specificity of the interaction would be due to the binding of an α-helical region (26–37) of each *cro* protomer into the major groove of the B-DNA double helix where most of the specific contacts are made.

To test this hypothesis, we have synthesized the fragment of the *cro* protein extending from residue 26–39. Conditions have been found where this oligopeptide adopts an α-helical structure in solution. Preliminary studies have been made of its interactions with a self-complementary decadeoxynucleotide d(AATTGCAATT) synthesized in our laboratory [4] and whose structure in solution has been already determined by NMR [5].

2. MATERIALS AND METHODS

The tetradecapeptide H-Tyr–Gln–Ser–Ala–Ile–Asn–Lys–Ala–Ile–His–Ala–Gly–Arg–Lys-amide was synthesized by classical solid-phase methods and purified by chromatography on carboxymethylcellulose. Details of the synthesis will be published elsewhere.

The decanucleotide d(AATTGCAATT) has been synthesized as previously described [4] and used after dialysis against water at pH 7.

NMR spectra were recorded with Bruker WH90 or WM400 Fourier-transform spectrometers. The spectrometer magnetic field was locked on an internal deuterium reference (D_2O). The temperature

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was regulated to ± 0.5°C. Chemical shifts were measured with respect to 2,2-dimethyl-2-silane pentane-5-sulfonate (DSS). Oligonucleotide was usually at 2.5 mM for NMR measurements. EDTA (2 mM) was added to remove metal cations. d^7-HFIP from Merck was used for NMR experiments in D_2O–HFIP (4:1, v/v) mixtures.

Circular dichroism spectra were recorded with a Jobin-Yvon mark III dichrograph. Fluorescence measurements were carried out with a Jobin-Yvon JY3C spectrofluorimeter. A pH 5–8 buffer containing 1 mM Na cacodylate, 1 mM NaCl and 0.2 mM EDTA (referred to as standard buffer) was used in most spectroscopic experiments.

3. RESULTS

3.1. *Conformation of the 26–39 fragment of* cro *protein in solution*

The circular dichroism spectrum of the 26–39 *cro* fragment in the standard pH 6 buffer is shown in fig.1. The strong negative band below 200 nm is indicative of a random-coil structure. Addition of hexafluoroisopropanol (HFIP) leads to the appearance of the characteristic spectrum of an α-helix (minima at 222 and 206 nm). The transition from random coil to α-helix is cooperative and is completed when the percentage of HFIP reaches 20%.

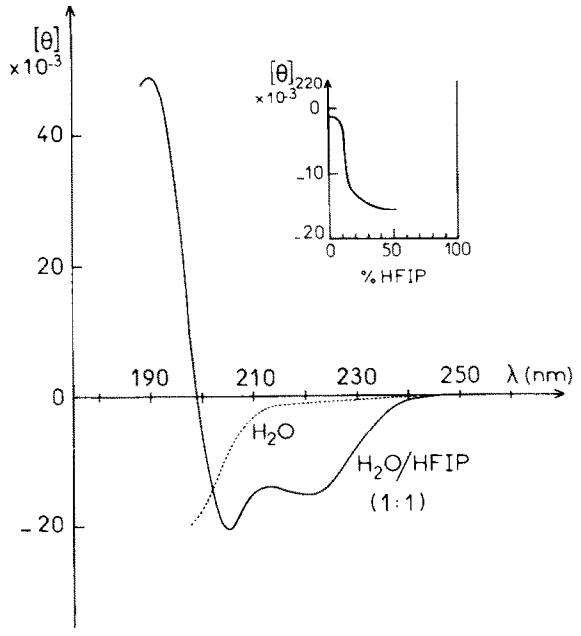


Fig.1. Circular dichroism spectra of the *cro* protein fragment (26–39) in the standard pH 6 buffer (– – –) and in a mixture buffer/HFIP (1:1, v/v) (——). Insert: Intensity of the circular dichroism at 220 nm *vs* [HFIP].

The helical content of the tetradecapeptide depends upon solvent, pH, ionic strength and temperature (not shown). In the mixture H_2O–HFIP (4:1, v/v) the ellipticity value at 222 nm per peptide bond (-20000 deg.cm^2.dmol^{-1}) should be compared to that (-28000) for the α-helix spectrum computed on the basis of the crystal structures and CD spectra of 15 proteins. In this compilation the average helix length contained 10 residues [6]. In the crystal structure of the *cro* protein, the α-helix extends from residue 27–36. If the α-helix induced by HFIP in the tetradecapeptide 26–39 involves only 10 out of the 14 residues, then the experimental value determined above is identical to the computed value. (It was assumed that the CD spectrum in the peptididic region was not perturbed by the tyrosyl contribution.)

The 400 MHz PMR spectra of the 26–39 *cro* fragment in D_2O and in the mixture D_2O–HFIP (4:1, v/v) also reveal the conformational change induced by HFIP (fig.2,3). The different resonance lines were assigned on the basis of reported spectra of free amino acids [7] and on the basis of double irradiation experiments. Many changes in chemical shifts and coupling constants can be seen when HFIP is added to the peptide solution. The δ and γ Ile methyl resonances and the Ala methyl resonance are notably affected as well as the β-CH_2 Tyr resonance. Self-association of the peptide in the D_2O–HFIP mixture might be responsible for a slight broadening of the resonance lines.

3.2. *Structure of the self-complementary decadeoxynucleotide d(AATTGCAATT)*

Self-association of the decamer d(AATTGCAATT) can be followed by absorption, circular dichroism and NMR spectroscopies. At 0.1 mM, the duplex $\rightleftharpoons$ single strand transition in an aqueous buffer at pH 6 is characterized by a T_m of ~20°C. Upon addition of HFIP, this value is decreased. Under the conditions where the 26–39 *cro* protein fragment is completely α-helical (20% HFIP), the T_m of the decanucleotide is decreased by ~20°C (not shown).

The PMR spectra of d(AATTGCAATT) have been measured at 90 and 250 MHz [4,5]. It was concluded that a duplex structure was formed at low temperature and that a B-type conformation was obtained. The 400 MHz spectra recorded in D_2O and D_2O–HFIP (4:1, v/v) are shown in fig.2,3. The coupling constants between the 1′ and the 2′ and 2′′ protons, $J_{1'2''} + J_{1'2''}$, are 13.3–15.0 Hz; this value is characteristic of a C2′-endo structure of the deoxyribose as expected for a B-DNA structure.

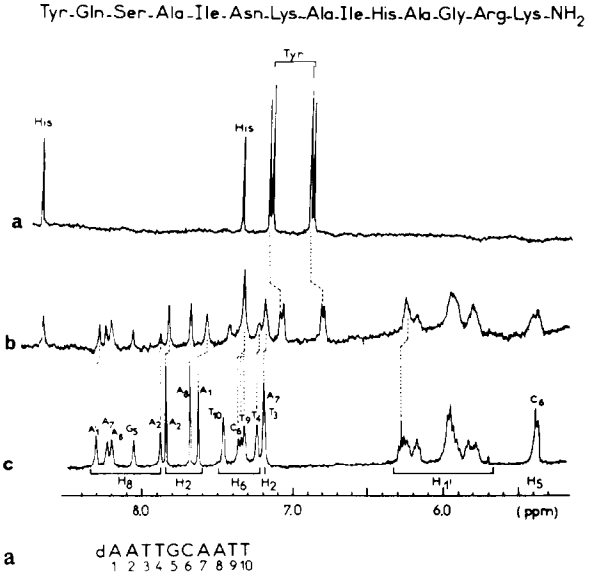
Addition of HFIP leads to a decrease of the transition temperature between the duplex and the single-stranded structures as observed by absorption (see above). The melting temperature in the mixture D_2O–HFIP (4:1, v/v) is 28°C at 2.5 mM.

3.3. *Interaction of the 26–39* cro *fragment with the decanucleotide d(AATTGCAATT) in aqueous solution*

The 26–39 *cro* peptide in its random coil conformation interacts with the duplex form of the decanucleotide d(AATTGCAATT) at low temperature. An increase in the melting temperature of double-stranded DNA is observed at low ionic strength indicating that the *cro* protein fragment prefers duplex to single-stranded structure. A quenching of the single tyrosine fluorescence is observed upon binding. A small decrease of the first positive band of the circular dichroism spectrum of the decanucleotide is induced upon *cro* peptide binding while the negative band is not affected (not shown).

The 400 MHz PMR spectrum of the complex formed by the duplex structure of the decanucleotide with the *cro* fragment at pH 5.5 and 17°C is shown in fig.2. Several changes are observed when this spectrum is compared to those of the separated molecules:

- Several proton resonances of the decanucleotide are shifted upfield (see fig.2 for the numbering of bases): H_8 proton of A_1, H_2 protons of A_1, A_2 and A_7, H_6 protons of C_6, T_4, T_9 and T_{10} and methyl groups of T_3, T_4, T_9 and T_{10};
- The following resonances of the base protons are not affected: H_8 of A_7, A_8, G_5 and A_2. It



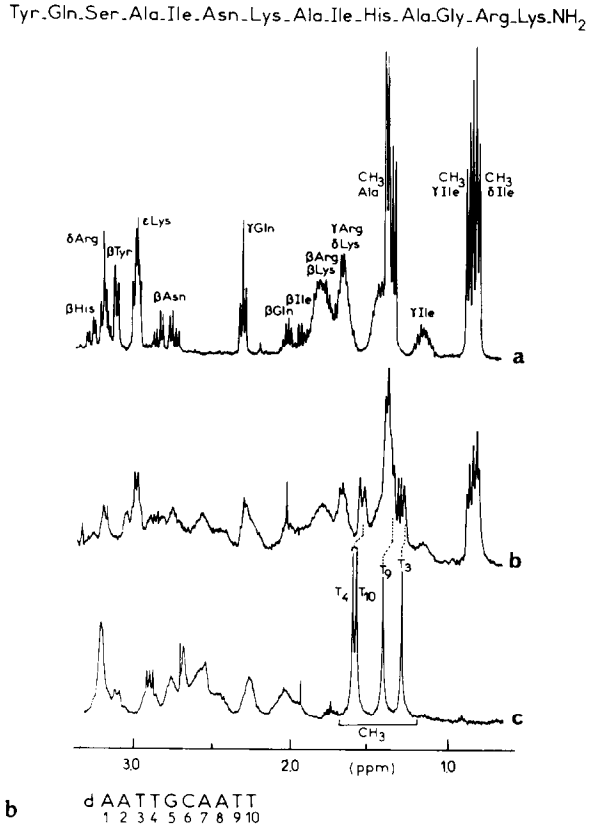


Fig.2. 400 MHz PMR spectra of the *cro* fragment (26–39) (2.5 mM) (a), of d(AATTGCAATT) in duplex form 2.5 mM (c), and of the 1:1 complex (b) (1 peptide/1 single strand) at 17°C and pH 5.5 in the presence of 1 mM EDTA.

should be noted that H_8 proton resonances are usually less sensitive to oligonucleotide conformation than H_2 resonances;

- Some of the $H_{1'}$ resonances of the deoxyribose are altered (fig.2);
- All resonance lines are broadened in the complex. The linewidth of the thymine methyl resonances increases from ~2.7–4.3 Hz.

Table 1 gives the linewidths of the adenine proton resonances in the free and bound state:

- The aromatic proton resonances of Tyr are shifted upfield in the complex and are broadened from 0.2–2.5 Hz while those of protonated His are not affected;
- The spectra of the CH_2 (δ) protons of Arg and of the CH_2 (ϵ) protons of Lys are affected;
- The spectra of the CH_2 (γ) and CH_2 (β) protons of Gln, the CH_2 (β) of Tyr, the CH_3 (γ') and CH_3 (δ) of the 2 Ile residues and the CH_3 of the 3 Ala residues are also modified.

It is not possible to obtain the decanucleotide in a single-stranded form at 17°C due to the self-complementarity of its base sequence. This precluded any comparison between the single strand and duplex structures in the peptide complex under the same experimental conditions. Such a study can be carried out with two single strands which have complementary sequences but which are not themselves self-complementary. Work is now in progress along this line. At 50°C the decanucleotide d(AATTGCAATT) is in a single-stranded form. Addition of the *cro* fragment induced some upfield shifts and line broadening indicating that binding of the peptide to single strands does occur. However, the magnitude of the shifts and their distribution among the different base protons were different from those observed at 17°C indicating that the complex structure had changed.

3.4. *Interaction of the 26–39* cro *fragment with the decanucleotide d(AATTGCAATT) in the solvent mixture H_2O-HFIP (4:1, v/v)*

Addition of the *cro* fragment to the decanucleotide d(AATTGCAATT) in a mixture of H_2O and HFIP (4:1, v/v) leads to an important broadening of all resonance lines of the oligopeptide. When the peptide concentration reaches 1 peptide/decanucleotide duplex precipitation is observed. Therefore all PMR measurements were carried out with a 1:2 ratio (1 peptide:2 duplexes) and at 17°C to prevent too much broadening. Fig.3 shows the corresponding 400 MHz PMR spectra. The low peptide concentration and the broadening of the resonance lines did not allow us to follow the behavior of all resonance lines. Nevertheless several preliminary observations can be made:

- The tyrosine aromatic proton resonances are shifted upfield in the complex;
- Several important changes occur for the CH (α) protons and for the CH_2 (γ), CH_3 (γ') and CH_3 (δ) of Ile residues;
- The CH_2 (ϵ) resonance lines of Lys residues are shifted;
- Several H_2, H_8 or H_6 proton resonances of the decanucleotide are shifted upfield or downfield (see fig.3). Since the peptide:decanucleotide

Table 1

Linewidths of the H_2 and H_8 protons of adenine bases in the free and bound decanucleotide

	A_1		A_2		A_7		A_8	
	H_2	H_8	H_2	H_8	H_2	H_8	H_2	H_8
d(AATTGCAATT) 12 78	1.0	3.8	0.4	2.7	1.4	6.4	0.4	4.6
Decanucleotide + *cro* fragment (1 single strand:1 peptide)	3.1	7.3	1.2	5.3	4.0	8.6	1.6	7.0

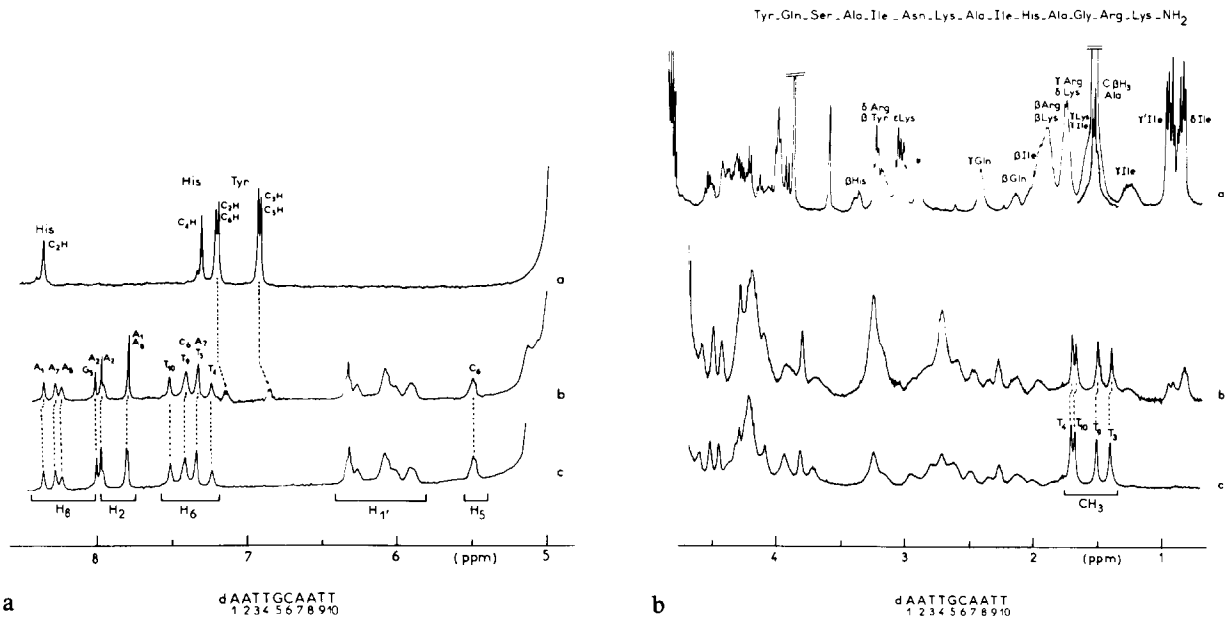


Fig.3. 400 MHz PMR spectra of the *cro* protein fragment (26–39) 2.5 mM (a), of d(AATTGCAATT) in duplex form 2.5 mM (c), and the 1:2 complex (b) (1 peptide/2 duplexes) at 17°C, pH 5.5 in a mixture D_2O/HFIP (4:1, v/v) in the presence of 1 mM EDTA.

ratio was small, part of the decanucleotide is certainly free in solution and therefore only limited shifts are expected for the decanucleotide protons.

At 50°C complex formation between the peptide and the single-stranded decanucleotide (T_m = 28°C at 2.5 mM) leads to a broadening of all resonance lines but only small shifts are observed for the resonances of the decanucleotide. The tyrosine protons are shifted upfield as observed at lower temperature (see above). Important changes occur for the CH_2 (δ) of Arg, CH_2 (ϵ) of Lys, CH_2 (β) of Tyr, CH_3 of Ala and CH_3 (δ and γ') of Ile residues.

4. CONCLUSION

Spectroscopic measurements using absorption, fluorescence, circular dichroism and PMR, have shown that the *cro* protein fragment 26–39 interacts with the decanucleotide d(AATTGCAATT) both in its double-stranded and in its single-stranded conformation. It has been possible to induce the formation of an α-helix in the *cro* fragment by addition of the helix-forming solvent HFIP. In a H_2O–HFIP mixture (4:1, v/v) the transition from random coil to α-helix is completed. Under these solvent conditions the decanucleotide d(AATTGCAATT) is still able to form a double-stranded structure even though the melting temperature is decreased by ~20°C. It is thus possible to study 4 types of complexes:

peptide (random coil) ⟷ oligonucleotide (duplex)

peptide (α-helix) ⟷ oligonucleotide (single strand)

The above PMR results indicate that the conformations of both partners are different in all 4 complexes. It should be noted that circular dichroism spectra demonstrate that the α-helical conformation of the peptide in H_2O–HFIP (4:1, v/v) is retained upon binding to the decanucleotide duplex in the same solvent mixture. NMR spectra reveal however that the peptide α-helical conformation is certainly affected upon complex formation.

The decanucleotide used in this work does not contain any of the base sequences which are recognized by the *cro* protein when it binds to λ operators. To investigate the specificity of this in-

teraction at the level of *cro* protein fragments, it is necessary to use one of these specific sequences. We have recently completed the synthesis of the left-part of the OR3 operator sequence (N. Thuong et al., unpublished) and its interactions with the *cro* 26–39 fragment are presently under investigation. Using a combination of different spectroscopic techniques and solvent conditions under which the peptide adopts an α-helical conformation, should allow us to get some insight into the role that α-helices could play in the recognition of base sequences in the large groove of DNA.

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