Interactions between cro repressor and the model specific binding site

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Binding of λ phage cro repressor to the synthetic half of O_R3, the most conservative half of the specific binding sites, was investigated by proton nuclear magnetic resonance spectroscopy. It was found that the α-helical segment (27–36) of the protein was involved in specific interactions with the model binding site. The 3-dimensional structure of cro repressor does not change noticeably upon complex formation. Intercalation can be excluded as a possible means of interaction.

cro repressor *Protein structure* *Operator* *Specific binding* *^{1}H NMR*

1. INTRODUCTION

The regulatory system of λ bacteriophage is composed of two repressors, cro and cI, whose interaction with operator sites in the genome determines whether phage growth will be lytic or lysogenic [1–4]. A study of this system will help us gain a better insight into both the regulation of gene activity and the molecular mechanisms of specific interactions between nucleic acids and proteins.

The 3-dimensional structures of cro repressor [5] and the N-terminal half of cI repressor [6] determined by X-ray diffraction analysis show a high degree of homology in the DNA-binding regions of these proteins [7]. This has made it possible to construct a model of their interaction with the operator sites.

NMR spectroscopy is a technique which provides direct information about the structure in solution. ^{1}H NMR was recently used to study cro repressor [8–10] and its interactions with DNA [10,11].

Here, we report on some structural characteristics of the complex of cro repressor protein with the synthetic half of O_R3: d(TATCACCGC)·d(ATAGTGGCG).

In the λ phage genome this sequence occurs in 4 out of 6 operator sites, for which cro repressor has a different affinity [3].

Abbreviations: NMR, nuclear magnetic resonance; HPLC, high pressure liquid chromatography; DSS, sodium 2,2-dimethyl-2-silapentane-5-sulphonate; CD, circular dichroism; NOE, nuclear Overhauser enhancement

2. MATERIALS AND METHODS

The oligodeoxynucleotides d(TATCACCGC) and d(ATAGTGGCG) were synthesized by the triether method in solution [12]. The protective groups in the synthesized compounds were removed by successive treatment with *p*-nitrobenzaldoxime anion, ammonia and 80% acetic acid. The compounds were isolated using high performance liquid chromatography (HPLC) on a Zorbax-C8 column (9.4 × 250 mm) in a linear methanol gradient (10–25%) in 0.1 M NH_4OAc, pH 7.0. The desalted samples were passed through a column packed with Dowex-Na^+.

The homogeneity of the compounds was controlled using HPLC on a Zorbax-C8 column (4.6 × 250 mm) in a linear acetonitrile gradient

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(0–25%) in 0.1 M NH_4OAc, pH 7.0. The primary structure was proved by the technique of nucleotide maps.

An equimolar mixture of the oligodeoxynucleotides was prepared by titrating a solution of one of the compounds with a solution of other and by using the hyperchromism of 9 bp duplex, i.e., double-stranded DNA, at 254–260 nm.

Cro repressor was isolated from the overproducing *Escherichia coli* K 802 strain containing plasmid pTR 214 [13], in accordance with the procedure described elsewhere [8].

^1H NMR spectra were recorded with a Bruker WH-360 spectrometer (360 MHz) at 20°C.

3. RESULTS

The interaction of the cro repressor with the duplex was studied at pH 7.0 and ionic strength 0.1 M NaCl. This ionic strength was required to stabilize the 9 bp duplex. Its melting curve obtained from the CD data showed that no less than 90% of the DNA was in the B-form of double helix under the experimental conditions (L. Minchenkova, unpublished). On the other hand, at such a salt concentration the complex remained stable.

Binding with the duplex has a noticeable effect on the protein spectrum (fig.1 and 2). The complex formation is accompanied with a considerable broadening of the lines. For example, TyrIII

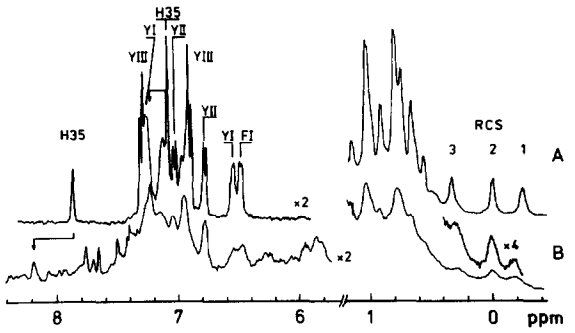

Fig.1. The downfield and upfield regions of the ^1H NMR spectrum at 360 MHz for cro repressor (A) and for its complex with the operator fragment (B). The protein concentration is 0.8 mM in a buffer containing 10 mM Tris–DCl (pH 7.0), 0.1 M NaCl, 0.1 mM EDTA in D_2O. Chemical shifts are given with respect to the DSS signal.

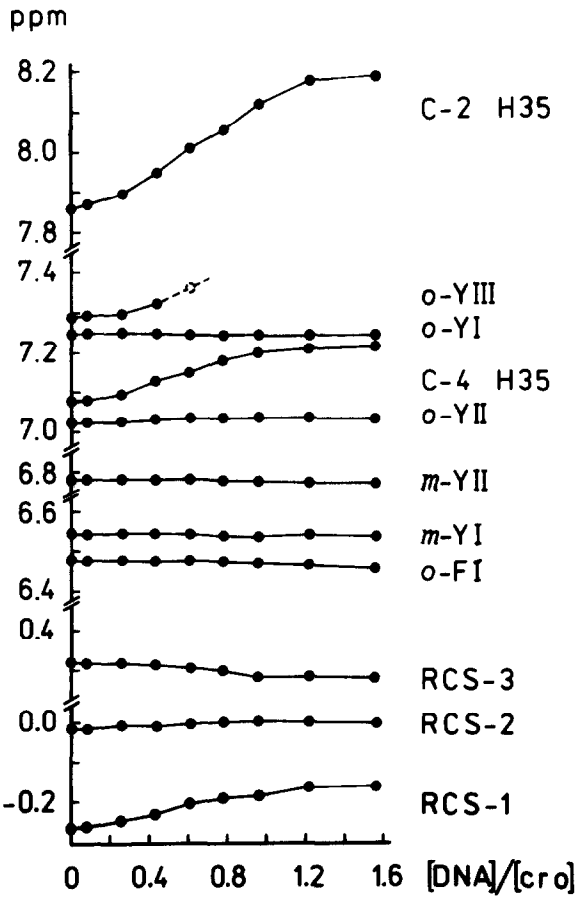

Fig.2. The chemical shifts of the aromatic proton resonances and ring current shifted methyl resonances of cro repressor as a function of the relative concentration of the operator fragment [DNA]/[cro].

signals become so broad that they cannot be identified at a relative concentration of the 9 bp duplex greater than 0.4. It is noteworthy that the proton signals of DNA bases broaden in a different manner in the complex, although these signals have almost identical intensities and widths in a spectrum of the free DNA duplex (not shown).

The binding causes a shift of certain signals including those of histidine-35, TyrIII and some ring current shifted (RCS) methyl resonances. However, the chemical shifts of TyrI, TyrII, PheI and RCS-2 remain unchanged.

The complex remains stable within a pH range from 5.5 to 8.2. Only histidine signals are titrated in this region (fig.3). As was shown by comparing

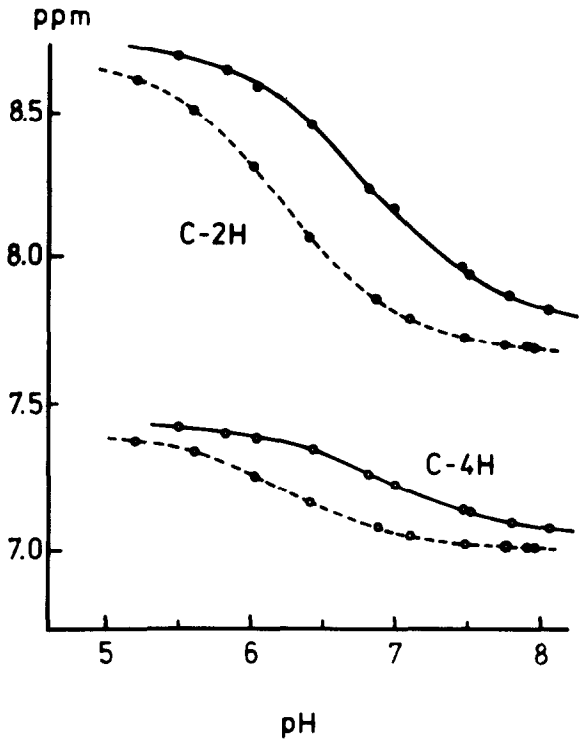

Fig.3. The titration curves of histidine-35 signals in free cro repressor (– – –) and its complex with the operator fragment (——).

titration curves of the protein in complex with the 9 bp duplex and those of the free repressor, taken under identical conditions, the complex formation makes the ionization constant change by approx. 0.7 pH unit and hardly affects the values of chemical shifts corresponding to the protonated and deprotonated states.

4. DISCUSSION

Overall broadening of the lines in the protein spectrum upon addition of DNA is indicative of an increase of the effective molecular mass, i.e., of complex formation. It was confirmed by complete dissociation of the complex and normal line widths when increasing an ionic strength up to 0.3 M NaCl. Selective broadening of the DNA signals in the spectrum of the complex suggests that the complex is the specific one.

At the same time, a non-specific interaction with DNA [10] led to broadening of all the lines and to downfield shifts of nearly all the aromatic proton resonances. Here, also, no upfield shifts were observed. Since intercalation of aromatic groups into the helical structure of DNA results in upfield shifts of the protein aromatic amino acid resonances [14,15], intercalation can be excluded as a possible means of interaction of cro repressor with this 9 bp duplex.

It is noteworthy that the signals of PheI, TyrI and TyrII, located inside the protein globule [9], do not shift upon complex formation (fig.2), indicating that there is no remarkable change in the environment of these residues and in 3-dimensional structure of cro repressor upon complex formation.

The most interesting results of our studies on the complex of cro repressor with 9 bp duplex are the changes which take place in positions of TyrIII and His-35 resonances (fig.1 and 2). TyrIII, which is the only tyrosine residue accessible to the solvent [9], is involved in interactions with the DNA duplex. The chemical shifts of *ortho-* and *meta-*proton resonances of TyrIII in the free protein are nearly identical (although measured in a mixture D_2O/hexafluoroisopropanol; 4:1, v/v) to those of tyrosine signals in the α-helical synthetic peptide corresponding to the fragment 26–39 of cro repressor [16]. On the other hand, the α-helical structure of the segment 26–37 in the protein polypeptide chain was predicted theoretically [17] and found in the crystal [5]. As was shown, the structure of cro repressor in solution is similar to that in the crystal [18]. Therefore, according to the data of X-ray structure analysis [5] and taking into account that TyrIII is the only exposed tyrosine residue in the protein [9], we can conclude that this tyrosine is Tyr-26.

It is evident from fig.1 and 2 that histidine-35 also is involved in interactions with the 9 bp duplex. An increase in the pK of His-35 imidazole in the complex shows that a negative charge is located near the imidazole. Such a charge appears to come from the internucleotide phosphate group in the duplex. As demonstrated in [19], the distance between the imidazole and the charge located near it can be represented as a function of ΔpK, the local dielectric constant of the medium and the remoteness of the cycle from the protein surface. Under our experimental conditions, this distance may roughly be estimated as 5 Å.

Tyr-26 and His-35 are located in the same α-helical region (α_3) which, as proposed from X-ray analysis of the free protein [5], is involved in specific interaction of cro repressor with O_R3. Our NMR data provide direct support for this model of cro repressor–DNA complex.

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