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^{1}H NMR study of the interaction of bacteriophage λ Cro protein with the O_R3 operator. Evidence for a change of the conformation of the O_R3 operator on binding

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ABSTRACT

The specific complex between the λ phage O_R3 operator and the Cro protein has been studied by proton NMR spectroscopy at 500 MHz. The DNA imino proton resonances of this complex have been assigned to specific base pairs using the known assignments of these resonances for the free operator. Increase of the protein/DNA ratio to complete saturation of the O_R3 operator with the Cro protein made it possible to follow the shift changes of the resonances. Ambiguities were resolved by nuclear Overhauser effect measurements on the complex. The shifts of the imino proton resonance positions provide information on the changes induced in the conformation of the operator upon complex formation with a dimer of the Cro protein. The most striking shift occurs for the central (GC 9) base pair, which is known to have no direct contacts with the Cro protein. This shift may be induced by a bend in the O_R3 operator DNA at the GC 9 base pair to accomodate the operator for the binding of the Cro protein dimer. The imino proton resonances of two additional base pairs can be observed in the complex, demonstrating an overall stabilization of the DNA structure by the binding of the Cro protein.

INTRODUCTION

Temperate bacteriophage λ has an intricated system regulating the choice between lytic and lysogenic life-mode (1,2). Either of the modes is characterized by the expression of its own gene set. The key components of this genetic switch are encoded by the genes cI and Cro (called herein cI and Cro). The regulation takes place on binding of the cI and Cro repressors to sequence-specific sites of the operator regions of the λ phage genome (3-8).

Investigations of this system are of great interest since they may lead towards the understanding of the molecular basis of sequence-specific DNA-protein interactions and throw new light on many of the important questions of gene regulation.

The three known X-ray structures of sequence-specific DNA-binding proteins, Cro (9), catabolite gene activator protein (10), DNA-binding domain of cI repressor (11), and the predicted secondery structures (12) and comparisons of amino-acid sequences of many other DNA-binding proteins (13,14) have

revealed a high degree of structural similarity in the DNA-binding domains of these proteins.

NMR techniques are among the most informative methods for the investigation of structure and dynamics of proteins, DNA and DNA-protein complexes in solution. Recently NMR techniques have been used for studying the molecular basis of the genetic switch of bacteriophage λ. NMR studies of Cro (15,16), its nonspecic binding to DNA (17), and its binding to the 9 base pair duplex d(TATCACCGC)•d(ATAGTGGCG) (18) which comprises half of the O_R3 operator, the most preferential binding site for Cro, have been published. Recently the assignments of the imino protons of the λ phage O_R3 operator have been made (19,20).

In the present paper we report a ^1H NMR study of the imino proton absorption region of the specific complex between the λ phage O_R3 operator and Cro. By increasing the protein/DNA ratio in small steps up to complete saturation of the O_R3 operator with Cro we were able to follow the resonances of most of the DNA imino protons from their positions in the free operator to their respective positions in the complex with Cro. Ambiguities in this procedure were solved by nuclear Overhauser effect (NOE) measurements. The shifts in the resonance positions of the imino protons provide information on the changes induced in the DNA structure of the operator upon complex formation with Cro. Implications of these data for the general model of Cro protein binding to DNA are discussed.

MATERIALS AND METHODS

The 17 base pair oligodeoxynucleotide dTATCACCGCAAGGGATA and its complementary strand were prepared by a modified phosphotriester method in solution (18). The yield of each strand was determined UV-spectrophotometrically after digestion of a DNA sample with snake venom phosphodiesterase (21) using the known values of the molar extinction coefficients of mononucleotides at 260 nm. Cro was isolated and purified according to procedures described previously (15). Cro concentration was determined using the molar extinction coefficient at 276 nm of 4330 (22).

Prior to NMR measurements the single-stranded oligonucleotides were dissolved in 0.2 M KCl, 1 mM EDTA, 10% ^2H_2O, 10 mM Tris pH 7.4 at a concentration of 8 mg/ml. Equimolar amounts of the complementary strands were combined, heated to 80°C and slowly cooled down to room temperature. To avoid extensive dilution in the course of an NMR titration experiment, Cro protein was dissolved in the same buffer and concentrated by ultrafiltration (Nucle-

pore membrane type 1C, molecular weight cut-off 1000 D) to 18 mg/ml.

^1H NMR spectra were recorded at 500 MHz on a Bruker AM 500 spectrometer. Chemical shifts were measured relative to the TSP (sodium 3-trimethylsilyl-(2,2,3,3-$^2H_4$)propionate) peak. Chemical shifts values were determined with an accuracy of ±0.01 ppm for the titration experiments and of ±0.03 ppm for the NOE measurements. The H_2O solvent resonance was suppressed by means of the Redfield 2-1-4 pulse sequence (23) with a total pulse length of 241 µsec and a carrier offset of 4872 Hz downfield from the water resonance (acquisition time 0.27 sec, relaxation delay 0.3 sec). Before Fourier transformation and phase correction spectra were resolution enhanced by a Lorentzian to Gaussian transformation as provided by the Bruker DISNMR/P program, except when needed for integration of resonances, and a one time zero-filling was applied. NOE experiments were carried out at 20°C either in the direct subtraction mode or in the interleaved accumulation mode where the accumulated FID's from the on and off resonance irradiation were stored seperately (24,25). In either case 32 scans were recorded in which a selective low power preirradiation pulse (35-41 dB below 0.1 W, duration between 0.4 and 0.6 sec) was applied at the chosen frequency. This was followed by an exactly equal cycle of 32 scans where the presaturation pulse was applied at an off resonance position. The irradiation power was set to a level that just saturated the irradiated resonance completely. NOE difference spectra were given a linebroadening of 4-10 Hz for a better signal to noise ratio.

RESULTS

The imino proton resonances of the 17 base pair O_R3 operator and the changes induced in their spectral positions upon complex formation with Cro are shown in Fig. 1 A-D. The spectrum of the free DNA was found to be identical to the spectra reported by others (19,20). Since the exchange between the free operator and the operator-Cro complex is fast on the NMR time scale, the changes induced by the formation of the protein- DNA complex can be followed by carefully titrating the O_R3 with Cro. The formation of the complex results in only moderate line broadening of the DNA imino proton resonances (Fig. 2). Saturation of the operator with Cro is reached at a ratio of two molecules Cro per 17 base pair O_R3 operator (Fig. 3). Most of the imino proton resonances in the complex can unambigiously be correlated to the respective resonances of the free DNA (Fig. 3), for which the assignment has been established (19,20).

Some of the GC base pair imino proton resonances remain unchanged or exhi-

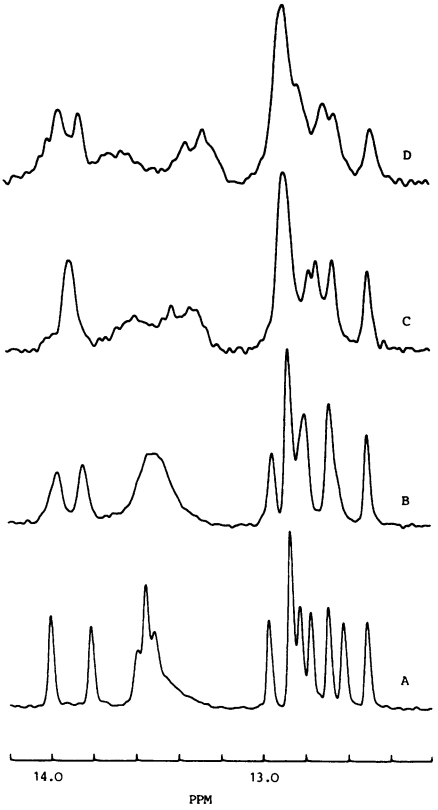

Fig. 1. Absorption region of imino proton resonances of the O_R3 operator at 500 MHz: (A) free operator, (B) in the presence of Cro at a Cro dimer/O_R3 ratio of 0.2, (C) in the presence of Cro at a Cro dimer/O_R3 ratio of 0.5, (D) in the presence of Cro at a Cro dimer/O_R3 ratio of 1.0. (Conditions see text).

bit only minor shifts, namely the resonances assigned to the GC base pairs 4 and 14. The situation is more complicated for the resonances of the GC base pairs 8 and 13 (at 12.86 ppm in the free DNA) since the resonances of the GC base pair 6 and 9 (12.76 and 12.62 ppm in the free DNA, respectively) undergo downfield shifts and the resonance of the GC base pair 7 (12.97 ppm in the free DNA) undergoes an upfield shift. Therefore, in the spectrum of the complex the resonances of five protons are detected between 12.8 and 12.9 ppm, only one of which can be resolved (at 12.80 ppm), whereas the other four resonances form one large peak at 12.88 ppm. However, from the spectra at a low protein/DNA ratio it can be concluded that the resonances of the GC base

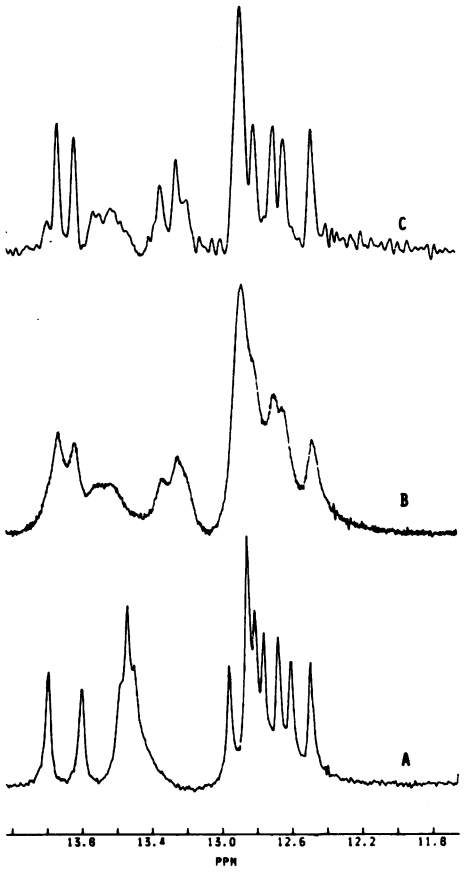

Fig. 2. Effect of complex formation on the line widths of the imino proton resonances of Fig. 1. (A) spectrum of the free operator without resolution enhancement. (B) spectrum of Fig. 1,D without resolution enhancement. (C) spectrum of Fig. 1,D with resolution enhancement by Lorentzian to Gaussian transformation of the FID (LB= -40; GB= 0.16).

pairs 8 and 13 at most undergo changes of less than 0.03 ppm to lower field. The resonance of the base pair 6 obviously coalesces with the resonances of the base pairs 8 and 13 in the complex. Whether the resonance at 12.80 ppm in the spectrum of the complex has to be assigned to base pair 7 or 9 could not be decided unambigiously from the titration measurements of the operator with Cro. The resonance of the GC base pair 12 is shifted from 12.82 ppm in the free DNA to 12.70 ppm in the complex.

Of the nine AT imino protons of the 17 base pair O_R3 operator only five

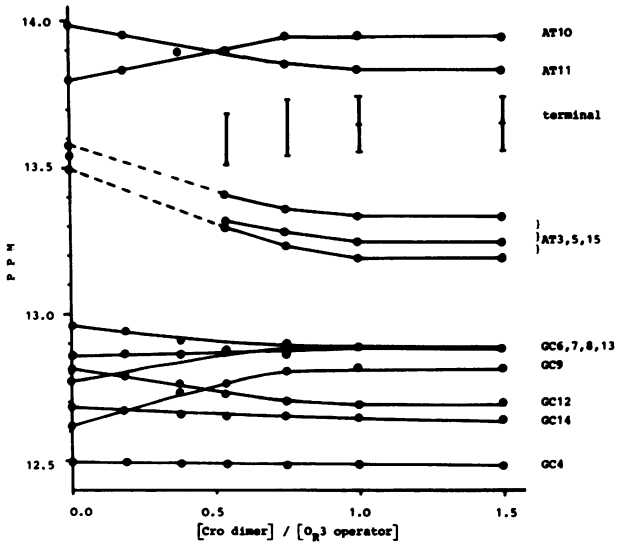

Fig. 3. Chemical shifts of the imino proton resonances of O_R3 in dependence on the Cro dimer/O_R3 ratio.

can be detected in the free DNA at 20°, the resonances of the two terminal base pairs at each end being lost due to fraying. Upon the binding of Cro the resonances of the AT base pairs 10 and 11 (at 13.81 and 13.99 ppm, respectively, in the free DNA) cross over each other and appear at 13.93 and 13.83 ppm in the spectrum of the complex. In addition to the signals of the base pairs 10 and 11 five more resonances can be detected in the complex between 13.0 and 14.0 ppm. Even though the signals of the AT base pairs 3, 5 and 15, which are poorly resolved in the spectrum of the free DNA (Fig. 1A), are partially obscured during the course of the titration experiment, it is obvious from the spectra at low protein/DNA ratio (Fig. 1B) that all of these resonances shift to higher field. Therefore, the peaks at 13.34, 13.25 and 13.19 ppm (Fig.1D and 2 C) should be assigned to the AT base pairs 3, 5 and 15. The extremely broad resonances at 13.7 and 13.6 ppm do not correspond to any signal found in the free DNA at 20°C and most likely represent the resonances of two of the terminal AT base pairs which are lost in the spectrum of the free DNA due to fraying, but can be detected in the complex with Cro. This interpretation is confirmed by comparison of the total signal intensities in the GC and AT base pair resonance regions. Integration over all signals (without resolution enhancement) gives a ratio of 8 GC to 5 AT pro-

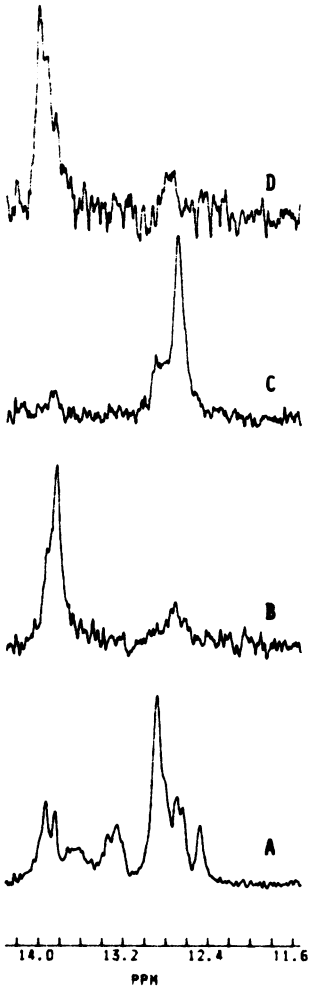

Fig. 4. NOE difference spectra of the Cro-O_R3 complex. (A) normal spectrum of the Cro-O_R3 complex. (B-C) NOE connectivities between the base pairs AT 11, GC 12 and AT 10 (see text). (B) irradiation of AT 11, NOEs at GC 12 and AT 10. (C) irradiation of GC 12, NOEs at AT 11 and GC 13. (D) irradiation of AT 10, NOEs at GC 9 and AT 11.

tons for the free DNA and a ratio of 8 GC to 7 AT protons for the complex. When the O_R3-Cro complex was dissociated by increasing the ionic strength up to 2 M KCl, the additional resonances in the imino proton absorption region disappeared and the spectrum of the free operator was recovered. These additional resonances might be assigned to the AT base pairs 2 and 16.

Some of the assignments above are not quite unambigious. In particular the exact assignment of the peak at 12.80 ppm in the GC region and of the peaks around 13.25 ppm in the AT region cannot be derived unambigiously from the titration experiment. Therefore, a series of inter-imino proton NOE experiments were done (Fig. 4). The saturation of a single resonance without "spill

over" of decoupler power to a neighboring peak is only possible for a few signals, i.e. at 13.93, 13.83 and 12.48 ppm, because of overlapping of resonances. On saturating the signal of the AT base pair 10 at 13.93 ppm, a NOE was detected at 12.80 ppm, allowing the unambigious assignment of this resonance to the GC base pair 9. Saturation of the signal of the AT base pair 11 at 13.83 ppm led to an NOE at 12.70 ppm, confirming the assignment of this peak to the GC base pair 12. A very broad difference peak between 13.4 and 13.2 ppm was observed on saturation of the GC base pair 4 at 12.48 ppm indicating that the resonance at 13.34 ppm and one of the resonances around 13.20 ppm have to be assigned to the base pairs 3 and 5.

DISCUSSION

The site-specific binding of the Cro dimer to the O_R3 operator leads to an overall stabilization of the double-stranded structure of the DNA. This is demonstrated by the relatively moderate changes of the imino proton resonances of most of the base pairs, which have been proposed as the sites of the direct contacts of the operator with Cro (26), and by the appearance of two additional AT base pair imino proton resonances. These resonances are absent in the spectra of the free DNA due to terminal fraying, but can be observed in the complex.

The chemical shifts of the imino protons within the double-stranded DNA are highly sensitive to the base pair ring currents and strongly influenced by the local geometry and chemical environment (27). In the present study we found no changes in the resonance postions of the DNA imino protons larger than 0.3 ppm. Recently the effects of short nonspecific DNA fragments (17) and of the 9 base pair duplex d(TATCACCGC)•d(ATAGTGGCG) (18) on the Cro ^1H NMR spectrum have been studed. In both cases most of the aromatic amino acid resonances shift slightly downfield and no upfield shifts are observed. Since intercalation of aromatic groups into the helical structure of DNA results in large upfield shifts of the DNA imino proton resonances (28) and upfield shifts of the protein aromatic amino acid resonances (29,30,31), intercalation can be excluded as a possible way of the interaction of Cro with the O_R3 operator.

The most striking result of our studies on the specific Cro dimer-O_R3 complex is the considerable change that takes place in the chemical shift value of the imino proton resonance of the central (GC 9) base pair (Fig. 1 and 3). In Figure 5 the results of the chemical protection studies on the Cro-O_R3 complex (4,32) and the known relative affinities of Cro for mutant

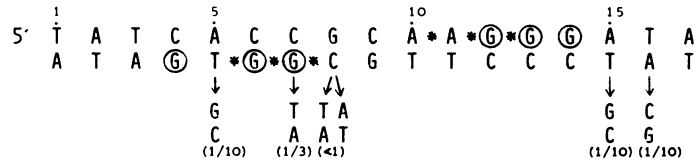

Fig. 5. Sequence of the O_R3 operator. The mutants and their relative affinities (in brackets) to Cro are indicated below (one arrow corresponds to one mutant) (7,32,33). GC base pairs for which the N7 positions are protected from methylation in the Cro-operator complex are circled (34). Phosphate groups which prevent or hinder complex formation with Cro, when being ethylated are marked with * (4,32).

O_R3 operators (7,32,33) are summarized. From these data three lines of evidence follow. First, Cro binding to the O_R3 operator is hindered by the ethylation of those phosphates that are apart from the center of the operator (4,32).Second, binding of Cro protects the N7 positions of the guanines, which are located far from the center,from methylation (34). Third, the known mutations of the O_R3 operator which strongly reduce the Cro affinities relative to the operator are also located apart from the center (7,32,33). These lines of evidence suggest that Cro does not touch the central part of the operator. Therefore, the change of the chemical shift of the GC 9 base pair imino proton cannot be explained by any direct contacts between the central part of the operator and Cro.

We believe that, as proposed in (26), a dimer of Cro binds to the major groove of the right-handed B-DNA O_R3 operator and each monomer of Cro interacts mainly with one half of the operator. Since Cro binds to O_R3 as a dimer which touches the operator further away from the center, a bend of the DNA axis at the central (GC 9) base pair may be required in order to optimize the interaction surface for the attachment of Cro. In crystallized short right-handed B-DNA pieces similar axial bends of different angles, which compress the major groove, are known (35). The bend may induce changes in the ring-current-shielding effects, thereby producing shift in the resonance position of the GC 9 imino proton.

A comparison of our data with a related system, the complex of the lac operator with the intact lac repressor and the lac repressor headpeace suggests similar assumptions. There is experimental evidence for a significant change of the operator structure upon the binding of the intact lac repressor (36), whereas only minor changes occur upon the binding of the isolated lac repressor headpeaces (37,38). In the last case two separate lac repressor

headpeace molecules interact with the operator DNA independently. In the former case additional changes of the operator conformation are required to optimize the interaction between the protein and the operator DNA.

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