Studies of the structure of bacteriophage λ *cro* protein in solution

Analysis of the aromatic region of the ^{1}H NMR spectrum

M.P. Kirpichnikov*, A.V. Kurochkin and K.G. Skryabin

Institute of Molecular Biology, USSR Academy of Sciences, Vavilov 32, 117984 Moscow, USSR

Received 1 November 1982

cro *Protein* *Repressor* *^{1}H NMR* *Chemical shift* *Resonance assignment*

1. INTRODUCTION

cro Protein encoded by bacteriophage λ is a repressor required for lytic growth of the phage. It competes for the binding to the operators o_L and o_R of the phage DNA with the λ repressor responsible for lysogeny [1]. *cro* Repressor is a basic protein consisting of 66 amino acid residues with the known sequence [2]. Its crystal structure has been analyzed [3]. We have predicted the secondary structure for a number of *cro* and λ repressors in lambda phages [4] and reported certain characteristics of ^{1}H NMR spectra for the *cro* repressor of phage λ [5].

Here we present a partial analysis of the ^{1}H NMR spectrum of *cro* protein in aqueous solution. Applying double resonance and NOE difference spectroscopy technique, resonance assignment of the aromatic protons was accomplished to the type of amino acid residues. Such an assignment is a necessary prerequisite for studying the interaction of *cro* protein with the DNA operator sites; moreover, it provides information about the structure of *cro* protein in solution.

* To whom correspondence should be addressed

Abbreviations: ^{1}H NMR, proton nuclear magnetic resonance; FID, free induction decay; NOE, nuclear Overhauser enhancement; EDTA, ethylendiaminetetraacetate

2. MATERIALS AND METHODS

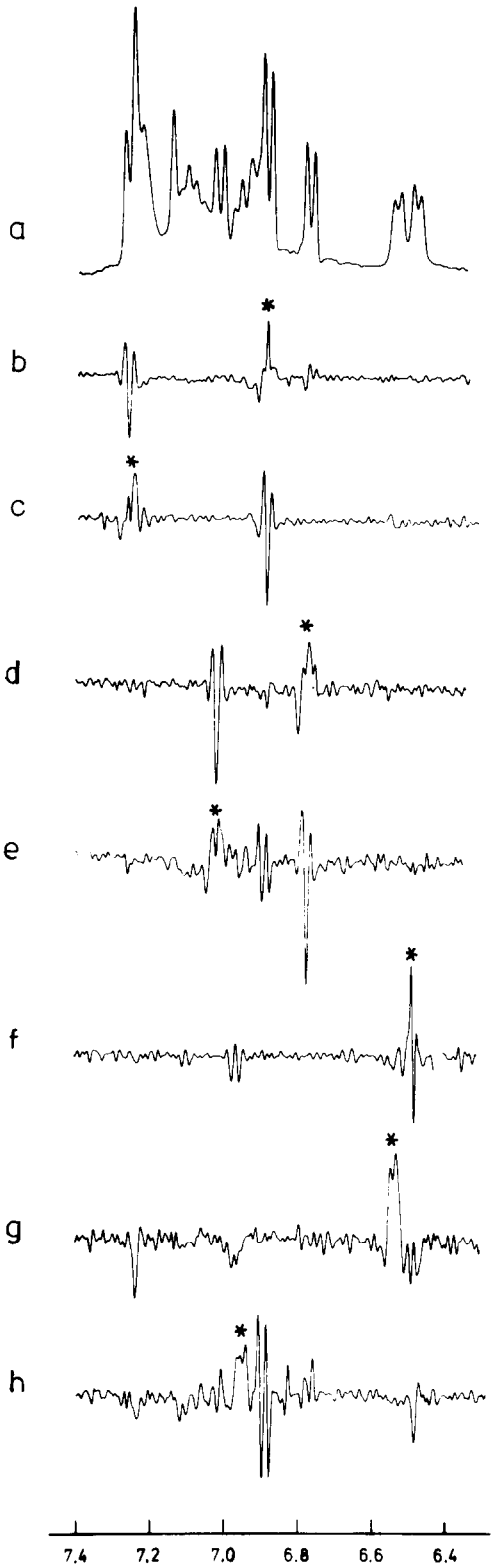
cro Protein was isolated from the overproducing *E.coli* strain pBR 294/214 [6] using the procedure [7] by our modification. After ion-exchange chromatography on a CM-Sephadex (C-50) column, the protein was loaded on a column packed with SP-Sephadex (G-25), eluted by buffer C with 0.35 M KCl, and the fraction corresponding to the second absorbance peak at 280 nm was subjected to gel-chromatography on a Sephadex G-75 (Superfine) column. The resultant, homogeneous *cro* protein was dialyzed against deionized distilled water and freeze-dried. The protein was dissolved in a buffer containing 10 mM Tris–HCl (pH 7.0), 2 mM EDTA in 99.8% ^2H_2O. Protein was ~1 mM.

^{1}H NMR spectra were recorded using a Bruker WH-360 spectrometer with a working frequency of 360 MHz at 26°C. The spectrum width was 4 kHz with the same filter band width collecting 8 K data points. The interval between pulses was 1.024 s, without an additional relaxation delay. Prior to Fourier transformation, the FID was multiplied by the Gaussian function [8] with the parameters $LB = -6.0$ and $GB = 0.15$.

Double resonance and NOE were recorded as difference spectra [9,10].

The values of chemical shifts are given relative to the resonance of sodium 2,2,3,3-tetradeutero-3-trimethylsilylpropionate (TSP) with the necessary correction [11].

Published by Elsevier Biomedical Press
00145793/82/0000—0000/$2.75 © Federation of European Biochemical Societies



3. RESULTS AND DISCUSSION

The downfield region of the *cro* protein spectrum in fig. 1a is a superposition of resonances of the aromatic protons from 3 tyrosines (Tyr-10, −26 and −51), 3 phenylalanines (Phe-14, −41 and −58) and one histidine (His-35) in the *cro* protein molecule.

The two singlets titratable at neutral pH with the chemical shifts of 7.15 and 7.88 ppm (not shown) can be assigned unambiguously to the protons of the C-2 and C-4 in His-35, respectively [12].

As can be seen from the double resonance difference spectra (fig. 1), the doublets with the chemical shifts of 6.88 and 7.26 ppm represent one closed spin system and should be assigned to the 3.5 and 2.6 protons of tyrosine, respectively, designated as Tyr III in fig. 2a. The two doublets at 6.77 and 7.02 ppm correspond to the 3.5 and 2.6 protons of another tyrosine (fig. 1d,e) designated as Tyr II in fig. 2a. The wiggles at 6.88 ppm are caused by the Ziegert-Bloch effect as a result of dispersion of spin decoupler power.

Irradiation of the doublet at 6.54 ppm gives a signal at 7.24 ppm (fig. 1g), and the shape of its line in the difference spectrum indicates that it is a doublet [9]. Obviously, the signals at 6.54 and 7.24 ppm belong to the 3.5 and 2.6 protons of Tyr I in fig. 2a. A minor signal at 6.96 ppm in the spectrum shown in fig. 1g is caused by leakage of spin decoupler power to the doublet with a chemical shift of 6.48 ppm whose irradiation (fig. 1f) produces a signal at 6.96 ppm with the shape typical of a triplet [9]. When this triplet located in the crowded region is irradiated (fig. 1h), many signals appear, including those at 6.48 and 7.1 ppm. This

Fig. 1. 360 MHz ^1H NMR spectra (aromatic region) of 1 mM *cro* protein solution in ^2H_2O (pH 7.0): (a) resolution-enhanced reference spectrum; (b) a difference spectrum obtained by subtracting the FID with irradiation of the signal at 6.88 ppm from the FID with an off-resonance spin decoupler frequency; (c) as (b), but with irradiation of the signal at 7.26 ppm; (d) as (b), but with irradiation of the signal at 6.77 ppm; (e) as (b), but with irradiation of the signal at 7.02 ppm; (f) as (b), but with irradiation of the signal at 6.48 ppm; (g) as (b), but with irradiation of the signal at 6.54 ppm; (h) as (b), but with irradiation of the signal at 6.96 ppm. The irradiated resonances are indicated with an asterisk.

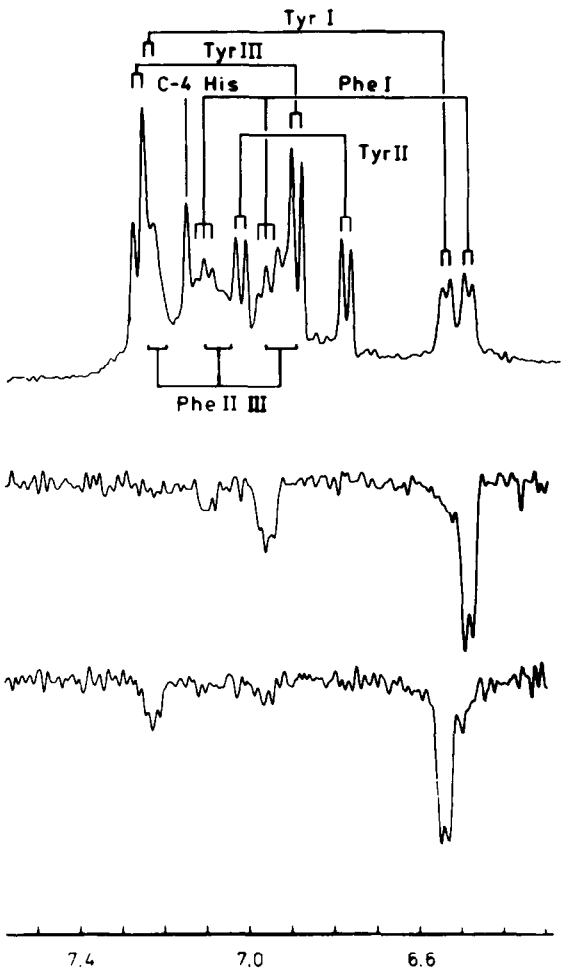


Fig. 2. 360 MHz NOE difference spectra for aromatic protons in 1 mM *cro* protein solution in ^2H_2O (pH 7.0): (a) resolution enhanced reference spectrum with resonance assignment; (b) a spectrum obtained by subtracting the FID with pre-irradiation of the resonance at 6.48 ppm for 1 s from the FID with an off-resonance spin decoupler frequency; (c) as (b) but with pre-irradiation of the resonance at 6.54 ppm.

makes it possible to assign the signals at 6.48, 6.96 and 7.1 ppm to the 2.6, 3.5 and 4 protons, respectively, in one of the phenylalanines designated as Phe I in fig. 2a. The assignment is supported by NOE experiments (fig. 2) with pre-irradiation of the doublets at 6.48 and 6.54 ppm.

We could not identify the resonances of the two other phenylalanine residues in three groups of signals centered at 6.93, 7.1 and 7.22 ppm; however, NOE experiments [12] are indicative of the fact that the signals of one of these (Phe II) are located in the two groups; viz., at 6.93 and 7.1 ppm.

Our assignment of the resonances differs in many respects from that reported in [13], although the aromatic spin system of one of the tyrosines (Tyr II in our nomenclature) was correctly identified by them.

A broad range of the chemical shifts of the aromatic protons point to the different surroundings of these residues in the *cro* protein molecule. The chemical shifts of signals for only one of the three tyrosines, Tyr II are close to those for free tyrosine or for a tyrosine residue in linear tetrapeptides [11]; this, however, implies neither its surface location nor its accessibility to a solvent [12].

The results reflect the complex three-dimensional structure of *cro* protein in solution. To study this structure as well as conformational changes and interactions with the operator DNA, one has to assign the identified signals to particular tyrosines and phenylalanines in the sequence. This work is in progress.

ACKNOWLEDGEMENTS

The authors express grateful thanks to Professor A.A. Bayev for his encouragement and stimulating discussions, and to V.N. Gagloyev from the Oncological Center of the USSR Acedemy of Medical Sciences for his technical assistance in obtaining the spectra.

REFERENCES

[1] Johnson, A.D., Poteete, A.R., Lauer, G., Sauer, R.T., Ackers, G.K. and Ptashne, M. (1981) Nature 294, 217–223.
[2] Hsiang, M.W., Cole, R.D., Takeda, Y. and Echols, H. (1977) Nature 270, 275–277.
[3] Anderson, W.F., Ohlendorf, D.M., Takeda, Y. and Mattews, B.W. (1981) Nature 290, 754–758.
[4] Ptitsyn, O.B., Finkelstein, A.V., Kirpichnikov, M.P. and Skryabin, K.G. (1982) FEBS Lett. 147, 11–15.
[5] Kirpichnikov, M.P., Kurochkin, A.V. and Bayev, A.A. (1982) Dokl. Akad. Nauk SSSR 266, 738–741.
[6] Roberts, T.M., Kacich, R. and Ptashne, M. (1979) Proc. Natl. Acad. Sci. USA 70, 760–764.

[7] Johnson, A.D., Pabo, C.A. and Sauer, R.T. (1980) Methods Enzymol. 65, 839–856.
[8] Ferrige, A.G. and Lindon, J.C. (1978) J. Mag. Res. 31, 337–341.
[9] DeMarco, A., Tschesche, H., Wagner, G. and Wüthrich, K. (1977) Biophys. Struct. Mech. 3, 303–315.
[10] Richarz, R. and Wüthrich, K. (1978) J. Mag. Res. 30, 147–150.
[11] Bundi, A. and Wüthrich, K. (1979) Biopolymers 18, 285–297.
[12] Kurochkin, A.V. and Kirpichnikov, M.P. (1982) FEBS Lett. 150, 411–415.
[13] Ivahashi, H., Akutsu, H., Kobayashi, Y., Kyogoku, Y., Ono, T., Koga, H. and Horiuchi, T. (1982) J. Biochem. 91, 1213–1221.