# $^1$H NMR STUDY OF THE LACTOSE REPRESSOR FROM *ESCHERICHIA COLI*

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## 1. Introduction

Although many investigations have been carried out on the functional properties of the *lac* repressor of *Escherichia coli* using biochemical or genetic methods (reviewed [1,2]) only few attempts have been made to elucidate the structure of this protein at the molecular level with physical methods. X-ray analysis of the tertiary structure has not yet been successful. $^{19}$F NMR studies have been performed with a protein derivative containing only [3-$^{19}$F]tyrosine residues instead of normal tyrosines [3]. A similar $^1$H NMR investigation of the tyrosine residues has been described [4]. The $^1$H NMR spectra were simplified by growing a special *E. coli* strain on a mixture of deuterated amino acids and [2,6-$^2$H]tyrosine. Hence all the ring proton resonances except the tyrosine 3,5-proton resonances have disappeared from the spectrum.

In order to analyse the interaction of the operator–DNA binding site of the *lac* repressor which is constituted of the amino-terminal 60 residues (reviewed [1,5]) with the aggregating and inducer binding part of the *lac* repressor [6] we have studied the tryptic digestion products of native *lac* repressor [7] with $^1$H NMR and compared the corresponding spectra with those of the native protein. We present evidence in favour of an identical and defined tertiary structure for both the amino-terminal region of the *lac* repressor and the corresponding dissected monomeric amino-terminal tryptic fragments [8] accounting for residues 1–51 and 1–59 of the *lac* repressor [7]. It is also suggested that this amino-terminal domain is linked to a rigid repressor core by a region of surprisingly high flexibility [8].

## 2. Experimental procedure

### 2.1. *Preparations*

*Lac* repressor from *E. coli* was isolated from strain BMH 593 as in [9,10]. The purity of the preparations before and after each NMR experiment was controlled by SDS–gel electrophoresis. Tryptic digestion was performed as in [7] using fresh preparations of TPCK-treated bovine trypsin (Serva, Heidelberg). After gel chromatography of the products on Sephadex G-150 (2 × 130 cm, 0.1 M $NH_4CO_3$) the core-containing and the 'headpiece'-containing fractions were concentrated by ultracentrifugation (Diaflo membranes UM02 and XM50, Amicon Corp., Lexington). N-terminal amino acids of the 'headpiece' preparations were identified as dansyl derivatives on polyamide sheets [11]. End group analyses and amino acid analyses of the 'headpiece' fraction showed the presence of the amino-terminal repressor fragments accounting for residues 1–51 and 1–59 as well as that of the tryptic peptide accounting for residues 52–59. The core preparation were analysed on SDS–polyacrylamide gels using native *lac* repressor and chymotrypsinogen as markers. Freshly prepared core samples run as a single band corresponding to mol. wt ~31 000. A further slow digestion of the core protein was observed. The samples were discarded as soon as more than one band was detected by SDS–gel electrophoresis.

### 2.2. *Measurements*

Protein solutions used for NMR measurements were prepared using 99.75% $^2H_2O$ containing 0.4 M NaCl, 0.1 mM dithiothreitol and 0.02% $NaN_3$. The samples contained ~10–15 mg/ml *lac* repressor or

core protein and about 2 mg/ml 'headpiece', respectively. pH measurements were performed with a Radiometer pH meter (PHM 26) in connection with a combined glass electrode (Ingold, Frankfurt). No correction of the pH meter readings were made for the $^2H$ effect of the electrode.

NMR measurements at 270 MHz were carried out with a Bruker WH 270 spectrometer in FT-mode employing a deuterium lock system. Probe diameter was 10 mm because of solubility difficulties. About 50 000 transients were recorded for each spectrum, using a block averaging technique. All chemical shifts were determined relative to the 2,2,3,3-tetradeuterotrimethylsilylpropionic acid (TSP) signal.

## 3. Results

Figure 1 shows the absorption region of the ring proton resonances of the aromatic amino acids of native *lac* repressor at two pH values. Only two signals exhibit a pH-dependent change in position. Obviously these two resonances can be assigned to the C2 and C5 protons of one or more histidine residues. From the pH dependence of the corresponding chemical shift values a p$K$ value has been determined using the Henderson-Hasselbalch equation and a least square curve fitting procedure (table 1).

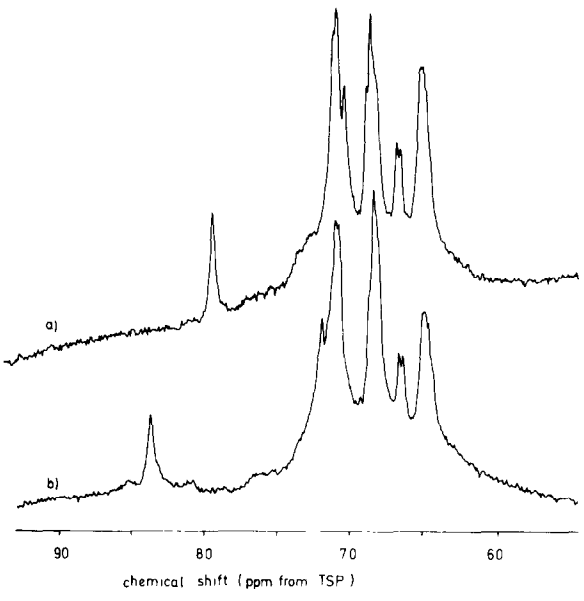


Fig.1. Aromatic region of the $^1H$ NMR spectra of the *lac* repressor in 0.4 M NaCl at (a) pH 6.87, (b) pH 5.99.

Table 1
p$K$ values as derived from the pH dependence of the histidine C2 proton resonances

| | p$K$ value |
|---|---|
| Native *lac* repressor | 6.40 ± 0.10 |
| 'Headpiece' | 6.50 ± 0.10 |
| Core protein | 6.65 ± 0.15 |

Apparently the few relatively narrow resonances of the spectra in fig.1 represent only a part of the 21 aromatic amino acids of the *lac* repressor subunit. The rest of the resonances is obviously not visible because of line width broadening. The observed resonances are surprisingly narrow indicating residues of high mobility. Assuming a rigid globular structure of the *lac* repressor a much larger line width would have been expected from the tumbling rate of the protein. Hence the *lac* repressor should contain regions of high flexibility. In order to get further evidence of flexible regions or domains within the *lac* repressor protein a tryptic digestion has been performed. The products of selective tryptic cleavage have been described [7]. A monomeric 'headpiece'-fraction accounting for residues 1–51 and 1–59 of the *lac* repressor and a tetrameric repressor core have been isolated and described [7,12]. Following these isolation procedures 'headpiece' and core protein were purified and looked upon by $^1H$ NMR under the same conditions as the whole *lac* repressor. Figures 2 and 3 show the ring proton resonance absorption region of the aromatic amino acids of the 'headpiece' and the core protein for different pH values. The comparison of the spectra reveals that the spectra of the 'headpiece' are almost identical with those of the whole *lac* repressor. However, the spectrum of the core protein is distinct and shows mainly broad overlapping resonances. The 'headpiece' contains 4 tyrosine residues [10,13], and indeed the analysis of the spectra allows the identification of seven out of eight 3,5- and 2,6-doublet resonances. These tyrosine resonances maintain essentially the same position in the intact protein spectra and in the 'headpiece' spectra. The linewidths are essen-

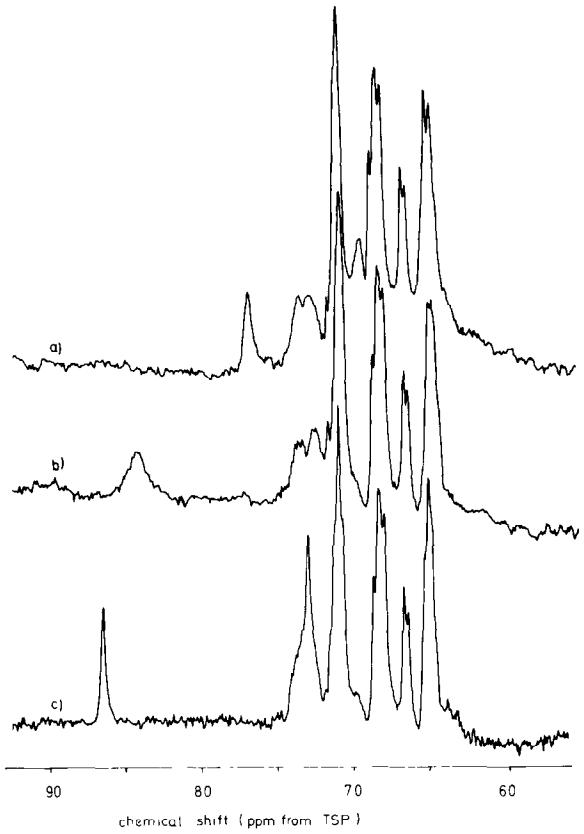


Fig.2. Aromatic region of the $^1$H NMR spectra of the *lac* repressor 'headpiece' in 0.4 M NaCl at (a) pH 9.12, (b) pH 6.05, (c) pH 4.60.

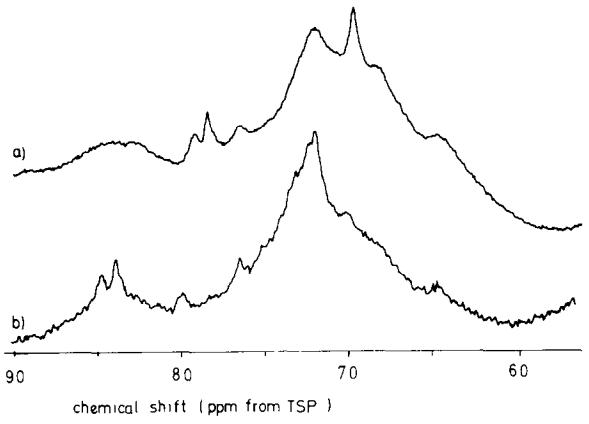


Fig.3. Aromatic region of the $^1$H NMR spectra of the *lac* repressor core protein in 0.4 M NaCl at (a) pH 7.17, (b) pH 6.11.

tially the same in both species. In addition, the chemical shift values are, with the exception of minute changes, invariant with pH between pH 4.6 and pH 9.1.

All three proteins investigated, the intact repressor, the 'headpiece' and the repressor core, reveal relatively sharp histidine C2 and C5 proton resonances. Their chemical shifts are changing with pH in the well known characteristic way. The p$K$ values derived from the pH dependence of the chemical shifts are listed in table 1. Owing to a slow exchange process, possibly with a concomitant interaction with the surroundings, the observed proton resonances of the single 'headpiece' histidine 27 is line broadened and almost disappearing around the imidazole p$K$. The chemical shift values of the C2 and C5 proton resonances of both the intact *lac* repressor and the 'headpiece' are similar. The same holds also for their p$K$ values. However, the observed histidine resonances of the intact *lac* repressor cannot be assigned with certainty to His 27 since we have also observed relatively narrow C2 and C5 proton resonances of at least one histidine residue in the repressor core protein (fig.3 and table 1).

Although the structure of the N-terminal part of the *lac* repressor is not markedly changed after dissection a perturbation of the structure of the core part is observed. The flexibility of some groups of the core protein seems to be increased as a consequence of the removal of the 'headpiece' as indicated by the appearence of a broad intensive resonance at ~7.2 ppm and of several small resonances at lower field, which cannot yet be assigned.

## 4. Discussion

Studies of the limited proteolysis of liganded and unliganded wild-type *lac* repressor and of mutationally altered *lac* repressors devoid of operator–DNA binding have led to the conclusion that the region accounting for residues 52–59 of the *lac* repressor subunit is the inducible part and therefore a highly flexible part of the operator–DNA binding site of *lac* repressor [5,14]. A weak secondary structure for the amino acids between residues 50 and 60 which may form a flexible hinge region was also suggested [15]. The results of our $^1$H NMR

studies are in very good agreement with these assumptions. The linewidths of the ring proton resonances of the aromatic amino acids of the N-terminal region are essentially the same in the whole *lac* repressor and the isolated 'headpiece'. The flexibility of the 'hinge region' must be so pronounced that there is no or only little interaction of the 'headpiece' with the larger core protein. If there were some interaction of the 'headpiece' with the core protein at least small differences in the chemical shifts of the observed resonances should be found. Only the linewidths of the His 27 imidazole proton resonances may be different in the 'headpiece' and the whole *lac* repressor. However, this linewidth broadening is observed in the 'headpiece' due to a slow exchange process. In the whole *lac* repressor the corresponding histidine C2 and C5 proton resonances are relatively narrow. Although we cannot definitely assign these resonances we would rather assume that these narrow resonances correspond to the 'headpiece' His 27 resonances.

Despite of the fact that the 'headpiece' of the whole *lac* repressor apparently may tumble in a similar way as the cleaved isolated 'headpiece' the structure of the N-terminal region does not seem to be random. All of the tyrosine ring proton resonances are different indicating a tertiary structure of the 'headpiece'. As a conclusion of our $^1$H NMR investigation we suggest that the 'headpiece' of the *lac* repressor forms a domain which is covalently linked to a rigid core protein by a region of surprisingly high flexibility.

Preliminary results of $^1$H NMR measurements of the *lac* repressor in the presence of IPTG show that there is little or no change of this N-terminal domain due to complex formation with an inducer. This is in agreement with the observation that the inducer alters the proteolytic susceptibility of the highly flexible region of the residues 52–59 [5,14] but has no effect upon non-specific binding to double-stranded DNA [16,17] which resides in the region covered by the small 'headpiece' accounting for residues 1–51 [5,12,18].

In order to obtain detailed information on the structure of the *lac* repressor–DNA complex intensive investigations of this interaction are presently being carried out in our laboratory.

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