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A FOLDED STRUCTURE FOR THE LAC-REPRESSOR HEADPIECE

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SUMMARY: A combination of selective ^1H nuclear Overhauser effects and other evidence indicates that the headpiece of the lac-repressor protein folds back on itself "head-to-tail" with residues in the N-terminal and C-terminal portions near to each other.

The isolated N-terminal 51 amino acid fragment (headpiece, HP) of the lac-repressor protein (1) retains ability to bind both to operator DNA and to non-operator DNA (2). Consequently there is considerable interest in determining the three-dimensional structure of this fragment and the nature of its interaction with DNA (3,4). ^1H NMR spectroscopy has revealed that the aromatic and aliphatic side chains as well as exchangeable protons in HP are chemical shift nonequivalent, suggesting extensive secondary and perhaps tertiary structure (5,6,7). We now report selective ^1H-^1H nuclear Overhauser enhancements (NOE) which are known to indicate proximity (distances <6 Å) between the groups whose resonances are affected. The NOE measurements combined with other evidence suggest that the HP folds "head-to-tail" with residues in the N-terminal and C-terminal portions near each other.

MATERIALS AND METHODS: Preparations of HP have been previously described (5,6). Fourier transform ^1H NMR spectra were obtained on the 360 MHz spectrometer at the Stanford Magnetic Resonance Laboratory. Nuclear Overhauser effects were measured in the following ways: (i) A selective low-power presaturation pulse of frequency f_2 and varying length (0.1 - 3 s) was applied before the accumulation of each free induction decay. After a few scans (typically 32), the free induction decay was stored. The presaturation pulse was then stepped to an off resonance frequency f_2' and a second free induction decay collected for the same number of scans and stored. Then the frequency was reset to f_2, the first free induction decay recalled into computer memory and the cycle repeated. In order to equilibrate the spin systems at each irradiation frequency f_2 or f_2', two scans were also collected and discarded before each stored set of scans. The two free induction decays obtained were Fourier transformed, then subtracted and phase corrected. (ii) Free induction

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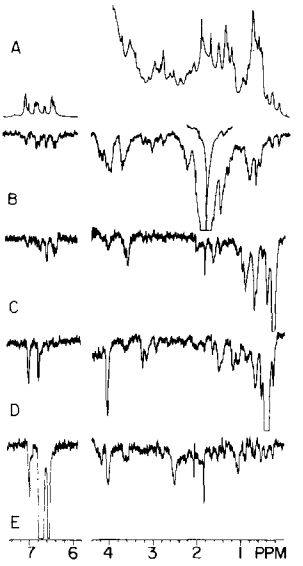

Fig. 1 360 MHz ^1H NMR spectra of the lac-repressor headpiece at 3 mg/ml in phosphate buffer in D_2O, pD 7.8, 23°C, sweep width ±2000 Hz, 16K points (A) reference Fourier transform spectrum with decoupling frequency off resonance (B-E) NOE difference spectra obtained with presaturation for a time τ = 2.0 s at: (B) the Met 42 SCH_3 singlet at ~1.8 ppm. Negative NOE are visible for tyrosine δ and ε CH doublet pairs at (a) 6.39, 6.33 ppm, (b) 6.70, 6.54 ppm and (c) 6.99, 6.76 ppm corresponding to Tyr 17, Tyr 12 and Tyr 47. In addition small NOE are visible at the 0.23, 0.63 ppm and the 0.39, 0.42 ppm leucyl doublets. (C) the 0.23 ppm Leu doublet. Negative NOE are visible at the Tyr 12 and Tyr 17 doublets and the Met 42 SCH_3 singlet. In addition, a weaker NOE appears at the 6.39, 6.98 ppm doublets corresponding to Tyr 7. (D) the 0.39 ppm leucyl doublet. "Large" negative NOE are visible for the Tyr 47 doublets. A weaker NOE appears at the Met 42 SCH_3 singlet. (E) the Tyr 12 2,6 protons (δ CH) at 6.70 ppm. Visible inter-residue NOE are seen at the Met 42 SCH_3 singlet, the 0.23, 0.63 ppm Leu and several alanyl doublets. The NOE at the 0.39, 0.42 ppm Leu arises from partial saturation of Tyr 47 at 6.76 ppm.

decays with the decoupler on and off resonance were alternately accumulated into the computer memory with the odd scans on resonance and even scans off resonance. The resulting FID's were then stored and processed as in (i). Similar methods have been described by Poulsen et al. (8), Campbell et al. (9) and Wagner and Wüthrich (10). The observations discussed in this paper were made in neutral ^2H_2O solutions containing 2.5 x 10^{-4} M HP protein.

RESULTS AND DISCUSSION: The spectrum shown in Fig. 1B unequivocally demonstrates proximity between the Met 42 SCH_3 group and the Tyr 12, Tyr 17 and Tyr 47 side chains, all of which have been previously assigned (5,6). Figs. 1C and 1D show proximity between the two leucines in the headpiece (Leu 6 and Leu 45) and residues near the C-terminal end. Although the two pairs of Leu

doublets (0.23, 0.63 ppm and 0.39, 0.42 ppm) have not been individually assigned, it is apparent from Figs. 1C and 1D that the 0.23 ppm Leu is near Met 42, while the 0.39 ppm Leu is near Tyr 47. Thus, regardless of the assignment Leu 6 must be near one of the residues in the C-terminal segment. All NOE effects were found to be reciprocal; saturation of the tyrosine doublets gave corresponding effects in the aliphatic region. For example, in Fig. 1E, saturation of Tyr 12 gives effects at the Met 42 SCH_3 singlet and the 0.23 ppm leucyl doublet. The finding that the side chains of the four tyrosines at positions 7, 12, 17 and 47, the two leucines at positions 6 and 45 and the methionine at position 42 must lie near enough to each other to manifest these selective NOE's clearly indicates that the polypeptide chain of the headpiece is folded back on itself.

The near chemical shift equivalence of the tyrosine doublets gives NOE effects arising from the simultaneous irradiation of <u>two</u> tyrosine residues in a few cases; however these effects could be distinguished by stepping the decoupling frequency f_2 through all eight tyrosine doublets. For example, irradiation of the Tyr 12 2,6 protons (δ CH) at 6.70 ppm also partially saturates the Tyr 47 3,5 protons (ε CH) at 6.76 ppm. This effectively gives an apparent NOE at both the 0.23, 0.63 ppm and the 0.39, 0.42 ppm leucyl doublets (Fig. 1E). However, upon saturation of the clearly resolved Tyr 12 ε CH doublet at 6.54 ppm no NOE is visible at the 0.39, 0.42 ppm leucyl residue; subsequent saturation of the Tyr 47 δ CH doublet at 6.98 ppm gave NOE only at the 0.39, 0.42 ppm leucyl doublets with no significant effects at the 0.23, 0.63 ppm doublets.

Intra-residue negative NOE's of ca. -40% are seen between the ε and δ CH of each of the four tyrosine residues (7, 12, 17 and 47). No significant inter-residue NOE's between the four tyrosines in HP have been observed. Negative NOE's are seen at about 2.7-3.0 ppm, i.e. at the positions of the β-methylene protons of the tyrosines, and at other resonances in the methyl region. For example, irradiation of the Tyr 47 doublets reveals significant NOE peaks not

Table I Inter-Residue Nuclear Overhauser Effects Observed Between Aromatic and Aliphatic Side Chains in the Lac-Repressor Headpiece 1-51.[a]

Irradiated	Observed Peaks	
	Identified	Additional
Tyr 7	0.23, 0.63 ppm Leu	
Tyr 12	0.23, 0.63 ppm Leu Met 42 SCH_3	0.52 ppm, 0.60 ppm, 0.90 ppm 1.03, 1.08 ppm Val
Tyr 17	0.23, 0.63 ppm Leu Met 42 SCH_3	1.03, 1.08 ppm Val 1.48 ppm Ala
Tyr 47	0.39, 0.42 ppm Leu	1.43 ppm, 1.51 ppm, 1.63 ppm Ala
His 29	None	None
0.23 ppm Leu	Tyr 12, Tyr 17, Tyr 7 Met 42 SCH_3	1.00 ppm Thr
0.39 ppm Leu	Tyr 47	1.51 ppm Ala
Met 42	Tyr 12, Tyr 17, Tyr 47 0.23 ppm Leu	0.85 ppm Val

[a]The inter-residue NOE are of the order of a few percent, typically 2-8%. Larger intra-residue NOE have been omitted.

only at the 0.39, 0.42 ppm leucyl groups but also for several alanine doublets and valine doublets. Table I summarizes the effects seen with irradiation in both the aromatic and aliphatic regions of the spectrum. Since the individual assignments for the seven Ala and eight Val residues in HP have not been made, it is not possible at this time to give a highly detailed interpretation of all the NOE's observed. However, our conclusion rests on NOE's observed on the assigned residues and the two unassigned leucyl residues, which leaves two possibilities:

(1) If the 0.23, 0.63 ppm doublets are assigned to Leu 6 (Scheme A), the NOE's can be interpreted as follows: a) Leu 6 to Tyr 7, Tyr 12 and Tyr 17 within the N-terminal segment, b) Tyr 47 to Leu 45 and Met 42 within the C-terminal segment, and c) Tyr 12 and Tyr 17 to Met 42, and Leu 6 to Met 42 between the N- and C-terminal segments.

(2) If the 0.23, 0.63 ppm doublets are assigned to Leu 45 (Scheme B), the NOE's can be interpreted as follows: a') Leu 6 to Tyr 47, b') Leu 45 to

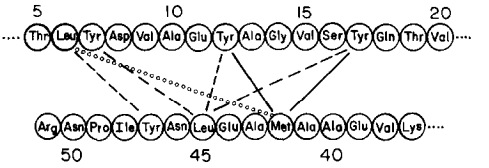

Fig. 2 Schematic of NOE between side chains in the N- and C-terminal segments of the lac-repressor headpiece: (——) NOE between assigned resonances Tyr 12, Tyr 17 with Met 42. (∘∘∘) NOE between Met 42 and Leu 6 with assignments of the 0.23 ppm doublet to Leu 6 and the 0.39 ppm doublet to Leu 45. (---) NOE between Tyr 47 and Leu 6 and NOE between Tyr 7, Tyr 12 and Tyr 17 and Leu 45 upon assignment of the 0.23 ppm doublet to Leu 45 and the 0.39 ppm doublet to Leu 6.

Tyr 7, Tyr 12 and Tyr 17 and c') Tyr 12 and Tyr 17 to Met 42. N-terminal to C-terminal interactions are evident in either scheme (Fig. 2).

The "head-to-tail" folding inferred from NOE measurements is consistent with several other pieces of evidence. No inter-residue NOE effects are observed between the four tyrosines and His 29, or between Met 42 and His 29, suggesting that side chains located in the middle of the primary sequence are not folded close to the N- or C-terminal portions. The Tyr 47 and Met 42 side chains are particularly insensitive to chemical modification, suggesting these side chains are not readily accessible to the surface (5,6). Chemical or enzymatic cleavage of the peptide chain at positions between Pro 3 and Pro 49 leads to a rapid loss of the entire native structure. For example, cyanogen bromide treatment cleaving at Met 42 (and Met 1) leads to peptide fragments (2-42, 43-51) that are random coils. Cleavage of the C-terminal portion with carboxypeptidase Y proceeds with a particularly rapid loss of structure upon removal of Tyr 47 (5). Enzymatic removal of residues 1-11 and 36-51 with Staphylococcal protease destroys the folded structure. The removal of amino acids exterior to the Pro residues, e.g. N-terminal Met 1 and C-terminal Arg 51 however does not result in a detectable alteration of the folded HP structure (6).

The folded headpiece structure, as monitored by the behavior of the tyrosine and leucine residues in the N- and C-terminal portions, is known to be

stabilized at high ionic strength (11), i.e. at conditions where repulsive interactions between charged side chains are reduced and the "hydrophobic" clustering of non-polar groups enhanced (12).

The headpiece is known from both CD and NMR measurements to have an extensive secondary structure (7). Approximately 35 ± 3 of the 51 residues in HP 51 are hydrogen-bonded. The α-CH groups of all four tyrosines, at least one of the leucines and isoleucine 48 are shifted to higher fields, indicating that the secondary structure encompasses both the C-terminal and the N-terminal regions. The CD and NMR evidence to date (7), though not completely unequivocal, is more consistent with a helical, rather than a β-structure. The exact location of the hydrogen-bonded domains in the peptide chain can not yet be specified. However, the extent of hydrogen bonding and the involvement of both the N-terminal and the C-terminal residues in it strongly suggests the existence of two such domains, one each in the N-terminal and the C-terminal segment with a less regular structure in between. Provisionally we visualize the headpiece as containing two hydrogen-bonded domains, possibly helices, in close apposition in the regions of Leu 6-Tyr 17 and Met 42-Leu 45. A "head-to-tail" folding allows the clustering of a number of hydrophobic side chains which dominate the primary sequence from Pro 3 to Val 20 and Val 38 to Pro 49, leaving the segment from Ser 21 to Glu 39, which has a substantially larger population of polar side chains, as a major part of the surface.

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