THE DELTA HELIX – A POSSIBLE LEFT-HANDED STABLE POLYPEPTIDE STRUCTURE IN THE N-TERMINAL SEGMENT OF THE *lac* REPRESSOR

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1. Introduction

Nuclear magnetic resonance and circular dichroism data on the N-terminal peptide (the headpiece) of the *lac* repressor indicate that the structure of the isolated peptide (51–59 amino acids) has a relatively high helical content of 30–50% [1]. Yet certain aspects of the NMR data are not readily compatible with the existence of a simple α-helix, or, alternatively, of a β-turn. Thus, the ^{1}H NMR spectrum suggests a stacking of tyrosines 7, 12 and 17 [1,2]. Such stacking is not possible in the α- and β-structures involving this segment of the polypeptide chain.

In the search for alternative structures which could account for this NMR result, we have found that if one constructs a 4.3_{14} helix for the 22 N-terminal residues of the *lac* repressor, tyrosines 7, 12 and 17 are arranged in a regular stacked array, with one of the possible ring spacings being 6.8 Å. The helix can be simply constructed by rotating all peptide bonds in the 3.6_{13} α-helix by 180°, and thus reversing the direction of the hydrogen bonds. A helix of this type was described in 1953 by Donohue [3], who suggested that it might be less stable than the α-helix by only ~4 kcal. Given that the *lac* repressor headpiece is known to contain the DNA-binding site, and that a regular stacking of tyrosines 6.8 Å apart is ideal for intercalation, the existence of a helix permitting such an arrangement would be of great interest. Here, we wish to present the result of theoretical calculations showing that a left-handed 4.3_{14} helix, to which we refer as the δ-helix, is indeed only slightly less stable than the right-handed α-helix. We wish to caution that the structure does not follow from NMR data, but is merely compatible with the following findings:

(i) A ~4 ppm upfield shift of ⩾30% of the α-CH resonances, indicating helix formation;
(ii) stacking of tyrosines 7, 12 and 17;
(iii) the appearance of 4 methyl groups shielded by tyrosine rings in the high field region of the ^{1}H NMR spectrum.

Nevertheless, the δ-helix should be considered as an interesting possibility for the backbone structure of residues 6–22 of the *lac* repressor.

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2. Results and discussion

Initial model building using CPK models gave a satisfactory structure for either the left- or the right-handed form. In a discussion of the model by O. J. with G. N. Ramachandran and R. E. Dickerson, the latter pointed out that the bad contacts for a right-handed 1→4 helix (O_1–R in the top right-hand quadrant of the Ramachandran diagram) would be much more severe than for a left-handed 1→4 helix (involving hydrogen atoms in only the bottom left hand quadrant of the diagram (fig.10 in [4])). A careful re-examination of the nature of helices having 'forward' hydrogen bonds $j\rightarrow 1$ (as contrasted with the 'backward' $1\rightarrow j$ hydrogen-bonded helices, α, 3_{10} and π) was therefore undertaken by R. C. A computer search for possible helical structures involving variations in only φ, ψ and ω, the latter over a range of

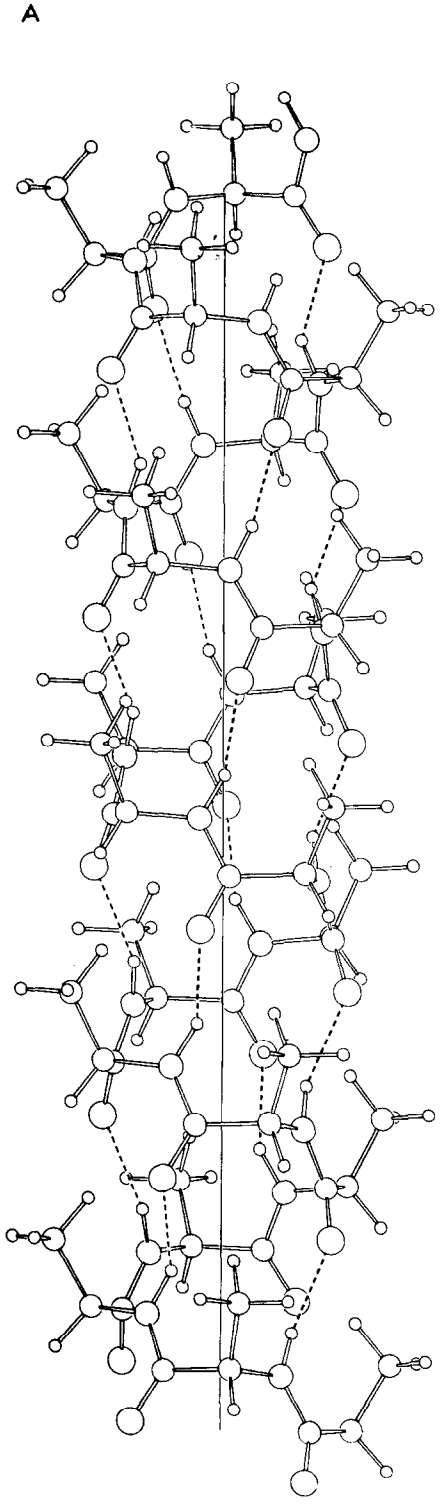
±20° about the planar value of 180°, was negative. This indicated the need to vary more parameters in the peptide backbone, and the one chosen for this purpose was the bond angle τ(N–C^{α}–C) at the C^{α}-atoms. When τ was decreased to 105° and $\Delta\omega$ was made −10°, good $N_1H_1 \ldots O_4$ hydrogen bonds could be obtained, and at the same time, all the contacts in the left-handed helix were also satisfactory near about $\phi = -90°$ to −100° and $\psi = -80°$ to −90°. From energy calculations (following the procedure adopted for the α-helix [5] and using the latest torsional potential functions [6]) it was found that the new helix has a good energy minimum of −11.1 kcal/mol.residue^{-1} for a poly(L-Ala) chain for the conformation listed in table 1. It is interesting to note that, with the same theory, the left-handed α-helix (α_M) had a minimum energy of only −9.2 kcal/mol.residue^{-1}, although the standard right-handed α-helix (α) had a minimum energy of −14.6 kcal/mol.residue^{-1}. (The right-handed 3_{10}-helix had a stable value of −12.6 kcal/mol.residue^{-1}.) Thus, among possible left-handed helices, the most stable is the new left-handed helix, which has been named the δ-helix. Its right-handed counterpart is extremely unsatisfactory, having a minimum energy of only −3.8 kcal/mol.residue^{-1}. A detailed analysis of the relative stabilities of the various types of possible

Table 1
Dihedral angles and coordinates of the left-handed δ-helix

Atom	Cartesian			Cylindrical	
	x	y	z	r	ϕ
C^{α}	2.647	0.000	0.000	2.647	0.0
C	1.811	−1.280	−0.046	2.217	−35.3
O	1.718	−1.946	−1.089	2.596	−48.6
N	1.225	−1.581	1.098	2.001	−52.2
H	1.433	−1.110	1.955	1.812	−37.8
C^{β}	3.422	0.104	1.327	3.424	1.7
H^{β_1}	2.717	0.050	2.169	2.717	1.1
H^{β_2}	4.142	−0.725	1.397	4.205	−9.9
H^{β_3}	3.962	1.062	1.362	4.102	15.0
H^{α}	3.377	0.023	−0.823	3.377	0.4

Coordinates (x, y, z and r in Å and ϕ in degrees)

Minimum energy conformation of poly-(L-Ala): $\phi = -98°$; $\psi = -80°$; $\omega = 170°$; $\tau = 105°$; unit height $h = 1.23$ Å; unit twist $t = -85.4°$; no. units/turn $n = -4.2$; energy = −11.1 kcal/mol.residue^{-1}



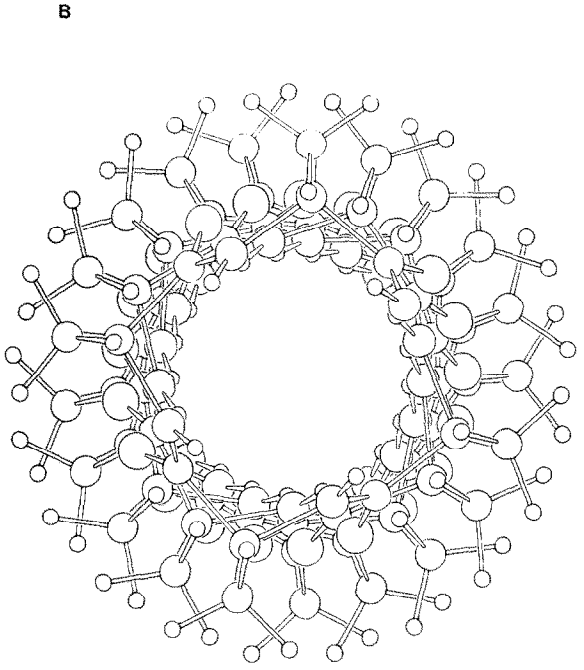


Fig.1. The left-handed δ-helix of poly(L-Ala). (A) The polypeptide chain is progressing upwards from the amino to the carboxyl end. The backbone NH. . .O hydrogen bonds (- - -) are slightly inclined with respect to the vertical helix-axis (——). (B) A view down the helix-axis (the dot at the center) shows that the side chains are pointing away from the helix, a feature which would favor the tyrosine ring intercalation with DNA in the *lac* repressor.

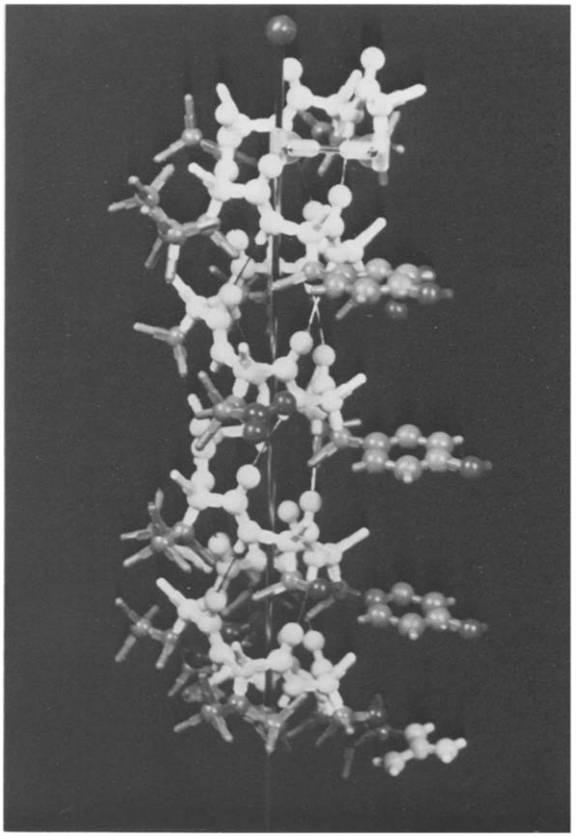


Fig.2. A Nicholson model of the δ-helix for residues 6–22 of the *lac* repressor, showing regular stacking of tyrosine side chains and the proximity of the methyls of Leu 6, Val 9 to Tyr 7 and Val 15 to Tyr 17.

single helices for polypeptides will be published elsewhere (R. C., in preparation).

Thus, purely from theory, it can be predicted that, under suitable conditions, the δ-helix would be observed, and further that it is energetically superior to α_M (which, incidentally, has never been observed in globular proteins). Two views of the δ-helix are shown in fig.1. The regular stacking of tyrosines on a δ-helix involving residues 6–22 of the *lac* repressor is shown in fig.2.

As mentioned above, the δ-helix is only slightly less stable than the standard α-helix or 3_{10}-helix and would therefore be expected to occur for a regular polypeptide sequence. However, if the polymer undergoes a transition from a right- to a left-handed helical structure, the δ-helix would be the best choice for two main reasons:

(1) The conformational changes from α to δ are minimum, $<30^\circ$ in ϕ and ψ values;
(2) The energy barrier involved is fairly low, since the two forms occur in the same lower left quadrant of the Ramachandran diagram.

Moreover, for certain side chains, the δ-helix has a conformation which favors side chain–backbone hydrogen bonds, and hence may be a preferred structure for such polypeptides. A good example is the side chain $-C^{\beta}H_2-N^{\gamma}H3^{+}$ in poly(L-α, β diaminopropionic acid) (PDPA). In this case, an $N^{\gamma}H^{\gamma}$. . .O bond, between the side chain and backbone, can also occur for each residue of the α- as well as the δ-helix, but this is not possible for the α_M-helix. When the side chain conformation corresponds to χ^1 around

$-60°$, the α- and δ-helices are stabilized by $N_5^{\gamma}H_5^{\gamma}\ldots O_1$ and $N_1^{\gamma}H_1^{\gamma}\ldots O_4$ hydrogen bonds, respectively. The calculated minimum energies for the α-, α_M and δ-helices are -22.5, -14.1 and -19.8 kcal/mol.residue^{-1}, respectively, showing again a definite preference for the δ-helix to the α_M-helix. It is remarkable that the energy gap between the α- and δ-helices has dropped from 3.5 in the case of poly(L-Ala) to 2.7 kcal/mol.residue^{-1} for PDPA. This clearly indicates that once the δ-helix is nucleated, the side chains can provide additional stability, through $N_1^{\gamma}H_1^{\gamma}\ldots O_4$ hydrogen bonds and thus lower its energy to be approximately in the vicinity of the α-helix. The detailed coordinates, and an analysis of other theoretically expected examples of the δ-helix will be presented elsewhere (R. C., in preparation).

It is interesting that the right-handed analogue of the δ-helix with 1→4 hydrogen bonds is not possible for L-residues, but is perfectly suitable for D-residues. Thus, if the fragment 6–22 of the *lac* repressor were to be synthesized with D-residues (instead of the natural L-residues), it may not bind by intercalation to DNA, unless the DNA has local regions with a left-handed twist, instead of the standard right-handed twist throughout, as in the Watson–Crick double helix of DNA. This may serve as an interesting experiment to check the alternating-helix structure proposed for DNA (Ramachandran, unpublished; [7,8]).

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