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Termination of right handed helices in proteins by residues in left handed helical conformations

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An analysis of 636 helical segments, ranging in length from 4 to 32 residues, from 123 independent protein crystal structures reveals that helix termination by residues in left handed (α_L) helical conformations is a common occurrence. Gly and Asn residues are the most frequent α_L helix terminators, with the former having a very high propensity to adopt such conformations. The α_R-α_R-α_R-α_L segment at the C termini of protein helices often possesses a $6 \rightarrow 1$ (π-type) hydrogen bond between the CO of residue i and the NH of residue i + 5 with residue i + 4 occurring in the α_L conformation. A stereochemical analysis of 216 examples shows that in 62 cases the $6 \rightarrow 1$ hydrogen bond is absent. The present analysis provides a quantitative measure of the propensity of the 20 amino acids to adopt α_L helix terminating conformations.

Helix termination; $6 \rightarrow 1$ Hydrogen bonds; Protein conformation; Protein data analysis

1. INTRODUCTION

$6 \rightarrow 1$ (C_{16} or π-type) hydrogen bonds involving the carbonyl oxygen of residue i and the amide hydrogen of residue i + 5 occur infrequently in proteins, as compared to the more widely observed $4 \rightarrow 1$ (C_{10}) and $5 \rightarrow 1$ (C_{13}) hydrogen bonds which are found in β-turns/3_{10}-helices and α-helices, respectively [1]. Helices in proteins often terminate with a reversal of helix sense at the C terminus, the helix breaking residue adopting a left-handed, α_L ($\phi \approx +50°$, $\psi \approx +50°$) conformation. This feature generally results in the formation of a $6 \rightarrow 1$ hydrogen bond, along with a $5 \rightarrow 2$ (C_{10}) hydrogen bond, with residue 5 occurring in the α_L conformation, as first reported by Schellman [2]. A subsequent analysis, using a data set of 40 protein structures, revealed the occurrence of $6 \rightarrow 1$ hydrogen bonded features, with concomitant $5 \rightarrow 2$ hydrogen bond formation in isolated hairpins, referred to as 'paper-clips' [3]. Residue 5 adopts the α_L conformation and is predominantly Gly and to a lesser extent Asn [3], an observation also highlighted in earlier studies [2]. A recent observation of the $6 \rightarrow 1/5 \rightarrow 2$ hydrogen bonded π-turn at the C terminus of the crystalline, helical peptides, with the achiral α-aminoisobutyryl (Aib) residue adopting the α_L conformation [4], stimulated a detailed re-examination of this helix terminating structural feature in proteins. This communication presents the results of an analysis based on a large data set of 636 helices, of length ≥ 4 residues, observed in 116 independent protein crystal structures. The overwhelming majority of helix terminating residues with α_L conformations in proteins are Gly residues. Asn residues also show an appreciable propensity for this conformation. This analysis provides a quantitative estimate of the propensity of the 20 amino acid residues to occur in this helix terminating structural feature.

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2. MATERIALS AND METHODS

A data set of 123 protein structures determined at resolution ≤ 2 Å were extracted from the Brookhaven Protein Data Bank [5]. The data set consists of largely non-homologous structures and will be detailed elsewhere. Helices were identified using the criterion that four successive backbone ϕ, ψ values [6] should lie within the region defined by the limits ($-120° \leq \phi \leq 0°$; $-60° \leq \psi \leq 0°$; a rectangular box in the (ϕ–ψ) map). The helix data set so formed consisted of 636 helical segments ranging in length from 4 to 32 residues. The residue immediately succeeding the helix at the C-terminal end was designated as the 'terminator' residue and ϕ, ψ values were computed If these ϕ, ψ values lie within the box as defined by the limits ($0° \leq \phi \leq 120°$; $-40° \leq \psi \leq 120°$), then the conformation of that corresponding residue was considered as left-handed helical (α_L) and such residues along with other 'terminator' residues were picked out for further study as outlined below.

Propensity ($p^i_{\alpha_L}$) of a residue i to occur at the C-terminal end of the helices (terminator position) having α_L conformation was computed as follows:

$$\text{Propensity, } p^i_{\alpha_L} = \frac{f^i_{\alpha_L}}{F^i_i}$$

where, $f^i_{\alpha_L}$ is the % occurrence of a 'terminator' residue i, in α_L conformation and F^i_i is the % occurrence of a residue i that is generally found

at the terminator position. These terms were in turn evaluated using the following,

$$f^{i}_{\alpha_L} = \frac{n^{i}_{\alpha_L}}{N_{\alpha_L}} \text{ and } F^{i}_{t} = \frac{n^{i}_{t}}{N_{t}}$$

here, $n^{i}_{\alpha_L}$ = the number of occurrences of the residue i in α_L conformation, n^{i}_{t} = the number of occurrences of the i^{th} residue at the helix termini in the entire data set of helices, N_{α_L} = total number of residues having α_L conformation and N_t = total number of residues found at the helix termini, in the data set.

3. RESULTS AND DISCUSSION

Fig. 1a shows the distribution of ϕ, ψ values at the 'terminator' residue. Of the 636 residues marked, 208 are Gly residues. Fig. 1b and 1c provide the observed ϕ, ψ distributions for the individual residues Gly and Asn. The clustering of Gly residues in α_L conformations at helix C-termini is dramatic and may be compared with overall distributions for Gly residues in proteins, reported earlier [7]. The clustering in the α_L region is less pronounced for Asn. Fig. 1d shows the propensity of each of the 20 amino acids to occur in the α_L conformation at the helix termini. Clearly the propensities of the non-Gly/non-Asn residues to occur in α_L conformations at the C-termini of helices is very low with several residues being practically unrepresented. Proline, of course, is stereochemically prohibited from adopting α_L conformation since ϕ for L-Pro is restricted to $-60° \pm 20°$. The

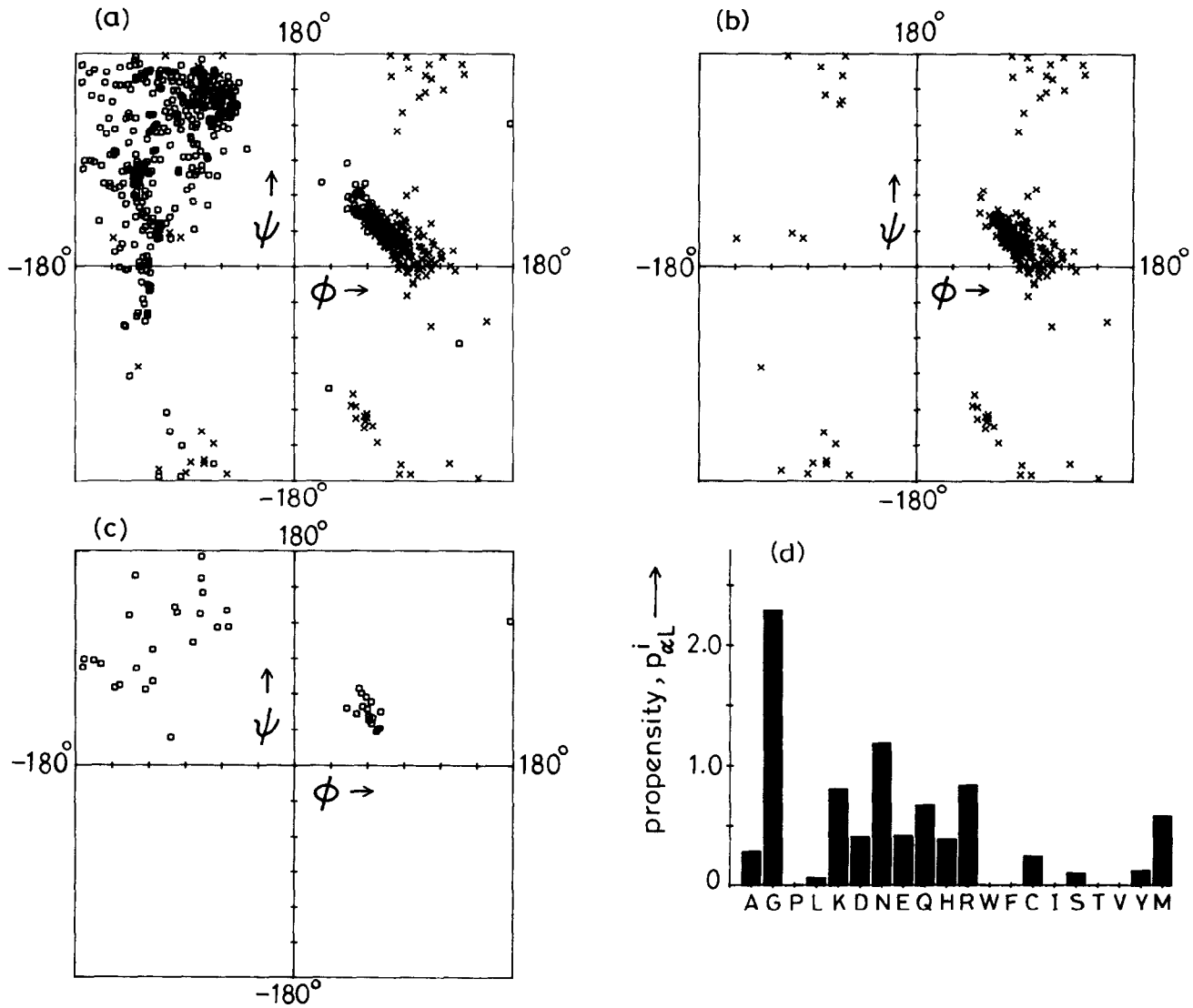


Fig. 1. (a) The ϕ, ψ distribution of 'terminator' residues that are found at the C-terminal end of the 636 helices. The non-glycyl residues are marked with 'o', while glycyl residues are marked with 'x'. (b) The ϕ, ψ distribution in the case of terminator glycyl residues. In the majority of the examples of Gly, being the terminator residue, the conformation of Gly is α_L (78%). (c) The ϕ, ψ distribution in the case of terminator Asn residues. Relatively equal distribution of points can be seen in the α_L region as compared to the rest of the map. There are 16 Asn examples which have α_L conformations, while 24 Asn residues occur elsewhere in the (ϕ–ψ) map. (d) The propensity value of each of the 20 aminoacids, to occur at the terminator position, having an α_L conformation. Notable peaks that are > 1.0 are due to Gly (2.29) and Asn (1.18). Residues which are totally absent are Pro, Trp, Phe, Ile, Thr and Val.

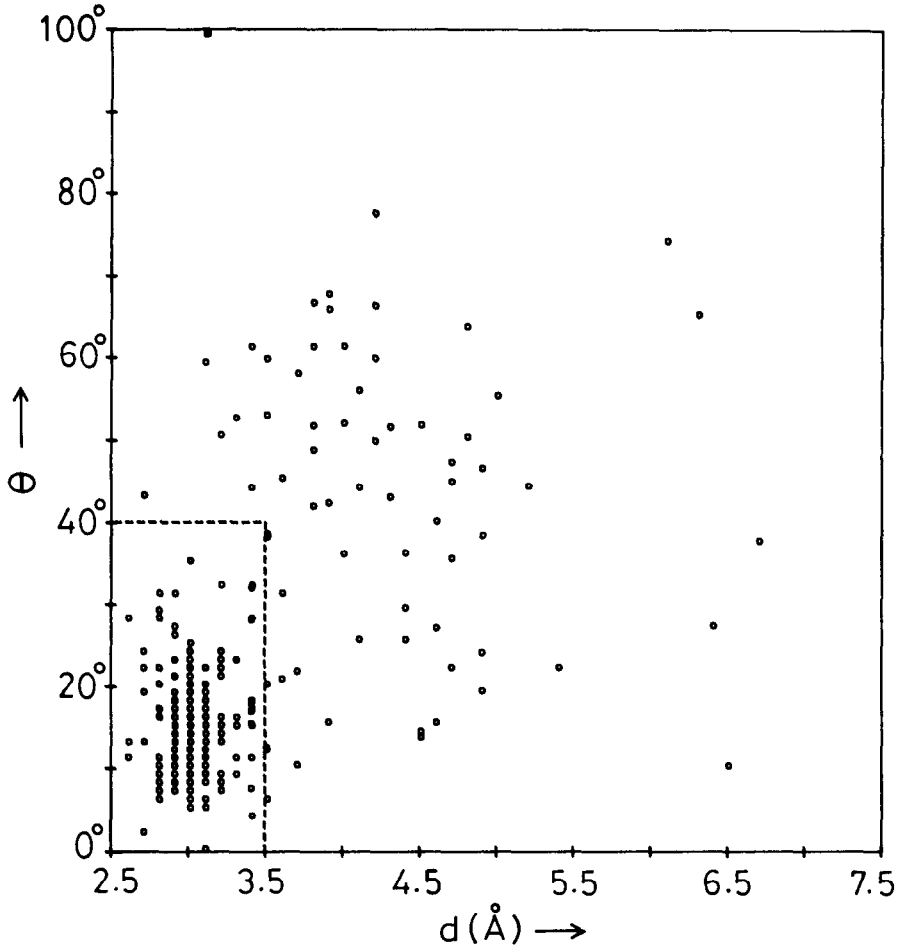


Fig. 2. The plot of (d–θ) parameters of the 6 → 1 hydrogen bond (π-type), where d is the distance between N_{i+5} to O_i and θ is the angle $H_{i+5} - N_{i+5}...O_i$. A rectangular box is drawn enclosing the region spanned by $2.5 \leq d \leq 3.5$ Å and $0° \leq \theta \leq 40°$. Values which lie within this box have been considered as hydrogen bonded (154 examples). The number of examples that fall outside the box is 62.

Table I

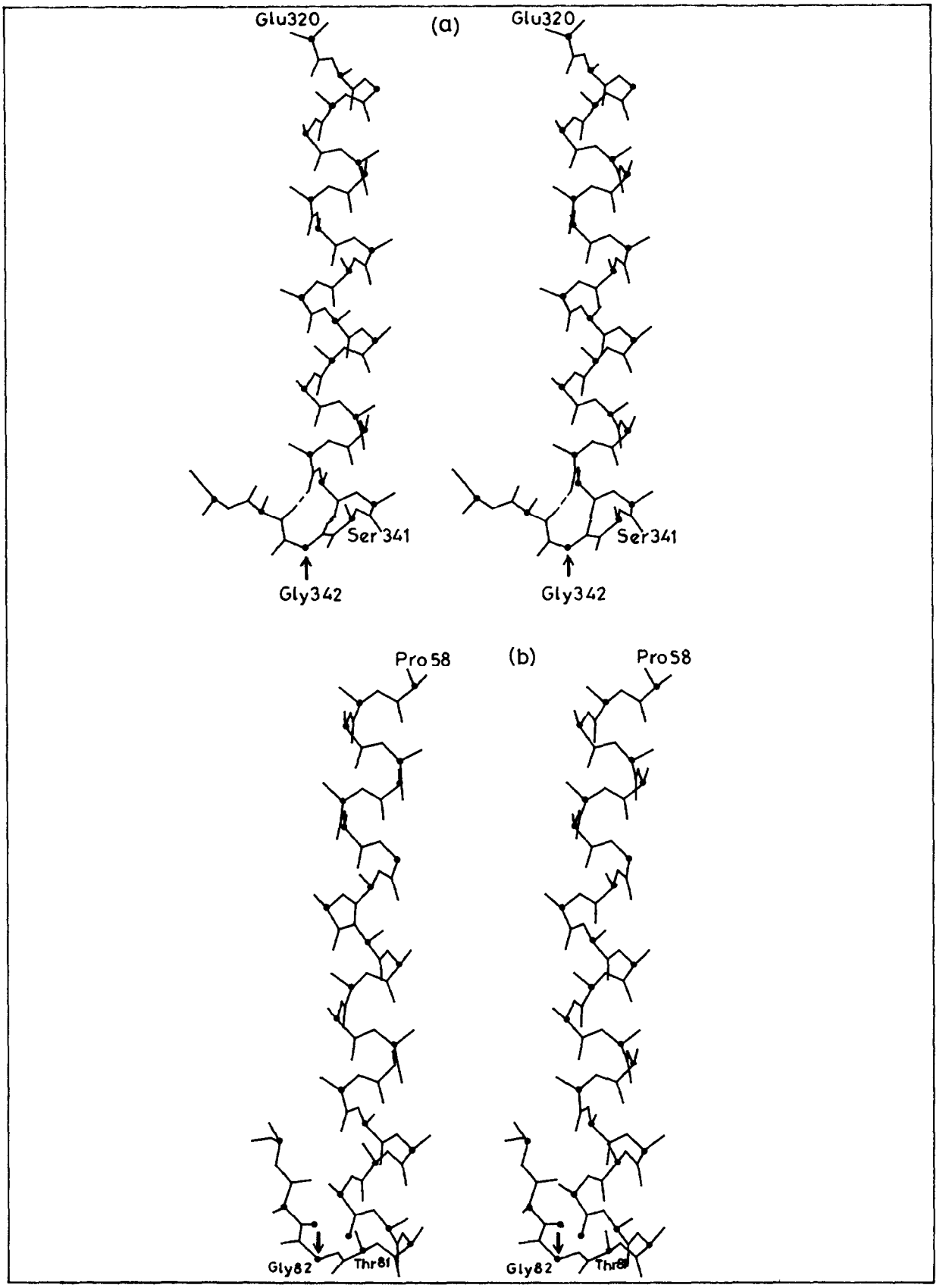
Distribution of 6 → 1 (π) hydrogen bonds at helix termini involving α_L terminators[a]

Residue ($p_{\alpha_L}^{i}$)[b]	Number of occurrences[c]		Residue ($p_{\alpha_L}^{i}$)[b]	Number of occurrences	
	With 6 → 1 hydrogen bond	No 6 → 1 hydrogen bond		With 6 → 1 hydrogen bond	No 6 → 1 hydrogen bond
Ala (0.28)	0	3	Arg (0.84)	5	1
Gly (2.29)	118	44	Trp (0.00)	0	0
Pro (0.00)	0	0	Phe (0.00)	0	0
Leu (0.06)	0	1	Cys (0.24)	1	0
Lys (0.81)	6	2	Ile (0.00)	0	0
Asp (0.41)	6	0	Ser (0.10)	0	1
Asn (1.18)	12	4	Thr (0.00)	0	0
Glu (0.42)	0	1	Val (0.00)	0	0
Gln (0.67)	3	2	Tyr (0.12)	0	1
His (0.39)	2	2	Met (0.58)	1	0

[a] Number of helices from 116 independent protein crystal structures.

[b] Values in parentheses correspond to the propensity of a residue to adopt α_L conformations at helix termini. Values > 1.0 indicate a preference for α_L helix terminator conformations.

[c] The number of occurrences refers to the number of α_R-α_R-α_R-α_L conformations at helix termini.


Glu320
(a)
Glu320
Ser341
Gly342
Ser341
Gly342
Pro58
(b)
Pro58
Gly82
Thr81
Gly82

←
Fig. 3. Stereo diagrams of the two representative examples of helices that terminate with a residue in α_L conformation (the side chains are shown only up to the C^β atom). (a) The helix-spanning residues Glu-320 to Ser-341 in glycolate oxidase (PDB code 1GOX). Gly-342 ($\phi = +84°$, $\psi = +41°$) is the terminator (indicated by an arrow). There are two hydrogen bonds, formed between the residues Met-338 to Cys-343 (π-type) and Ala-339 to Gly-342 (β-type) (shown by broken lines). (b) The helix spanning residues Pro-58 to Thr-81 in leghemoglobin (PDB code 2LH7). Gly-82 ($\phi = +99°$, $\psi = -11°$) is the α_L terminator (indicated by an arrow). In contrast to the helix shown in Fig. 3a, there is no π-type hydrogen bond.

β-branched residues Val, Ile and Thr are totally missing from the list of α_L terminators, as are the aromatic residues Phe and Trp.

A further examination of the C terminus local structural feature in helices, α_R-α_R-α_R-α_L conformations for the i + 1 to i + 4 segment, revealed that a large fraction of the observed examples did possess a 6 → 1 hydrogen bond between the $(CO)_i$ and $(NH)_{i+5}$ groups (Table I). Fig. 2 shows the distribution of the structural parameters viz., d (the distance from N_{i+5} to O_i) and θ (the angle H_{i+5}–N_{i+5}...O_i) used to evaluate hydrogen bond geometry for the 6 → 1 (i.e., i + 5 → i) π-type hydrogen bond. Points lying outside the rectangular box correspond to cases where a good hydrogen bond is not formed. Of the 162 examples involving Gly at position i + 4 (α_L), 118 can be classified as 6 → 1 hydrogen bonded. In the case of Asn, 12 out of the 16 cases possessed 6 → 1 hydrogen bonds. Fig. 3 provides illustrations of both types of helix terminating segments. Of the 636 helices examined there are 216 examples where the terminating residue is in an α_L conformation. In 75% of these cases the terminating residue is Gly and in 7.4% the residue is Asn. This suggests that C-terminal helix termination signals are well defined and algorithms for their identification might be usefully incorporated into protein secondary structure prediction strategies, particularly for the identification of helix stop signals [8,9]. The incorporation of unusual residues with high propensities for α_L conformations (cf. D-residues) at appropriate sites might facilitate predictable helix termination in de novo protein design approaches and in protein engineering [10].

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REFERENCES

[1] Baker, E.N. and Hubbard, R.E. (1984) Prog. Biophys. Molec. Biol. 44, 97–179.
[2] Schellman, C. (1980) in: Protein Folding (R. Jaenicke, ed.) Elsevier, Amsterdam, pp. 53–61.
[3] Milner-White, E.J. (1988) J. Mol. Biol. 199, 503–511.
[4] Flippen-Anderson, J.L., Karle, I.L., Uma, K. and Balaram, P. (1993) Int. J. Peptide Protein Res. (submitted).
[5] Bernstein, F.C., Koetzle, T.K., Williams, G.J.B., Meyer, E.F., Brice, M.D., Rogers, J.R., Kennard, O., Shimanouchi, T. and Mitsuo, T. (1979) J. Mol. Biol. 112, 535–552.
[6] Srinivasan, N., Sowdhamini, R., Ramakrishnan, C. and Balaram, P. (1991) in: Molecular Conformation and Biological Interactions (P. Balaram and S. Ramaseshan, eds.) Indian Academy of Sciences, Bangalore, India, pp. 59–73
[7] Ramakrishnan, C. and Srinivasan, M. (1987) Int. J. Peptide Protein Res. 29, 629–637.
[8] Richardson, J S. and Richardson, D.C. (1988) Science 240, 1648–1652.
[9] Presta, L.G. and Rose, G.D. (1988) Science 240, 1632–1641.
[10] Balaram, P. (1992) Curr. Opin. Struct. Biol. 2, 845–851.