Stabilization of Helix-Turn-Helix Motif in Short Peptides

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Summary: Effective *De novo* designing of proteins is a great challenge and a critical test of our knowledge of protein structure. The main problem is the attainment of a protein with a defined fold for its specific function. This paper reports the synthesis and characterization of a series of helix - turn - helix (h-t-h) peptides with stable secondary and tertiary structures. In the order to optimize the stability of the anti-parallel coiled-coil structure of alpha helical hairpin peptides, all peptides with the same interacting helical regions but different number and sequences of residues in the turn region was examined. The turn region was incorporated between g and e^2 of leucine zipper heptad CD measurements showed that a four residue turn region was the best of stabilizing coiled coil structure in these peptides. The four residue turn sequence selected from h-t-h motifs of DNA binding proteins showed the highest helix stabilization in a short peptide of twenty nine amino acid residues.

Introduction

Recently several parallel as well as antiparallel coiled-coil peptides [1,2] and simplest supersecondary structure peptides with h-t-h motif [3-7] have been designed and characterized successfully, exhibiting the designed aspects of the secondary and tertiary structures in water. However the lengths, the sequences and the disposition of the turns were different in all these studies. Recent modifications of theoretical models for helix-coil transitions in peptides showed excellent accuracy in prediction of helical propensities of monomeric peptides with complicated non repetitive sequences in water [8-10]. These approaches provide useful tools for designing helical segments, however no data were reported about optimal length and sequence of the peptide segment that can fold into an appropriate turn to allow stable h-t-h formation at a minimum energy cost. Therefore in this study we attempt to find out the optimum number and sequence of residues required in the turn region

between fixed terminal positions to facilitate maximum helix stabilization by inter-helical interactions.

Results and Discussion

All peptides exhibited typical α-helical circular dichroism spectra with minima at 222 nm and 208 nm. The ellipticities of the peptides were found independent of concentration from 5 - 200 μM suggesting that the peptides are monomeric in this concentration range. Addition of 50% TFE showed that all peptides are between 65 - 80 % of their maximum helical potential in benign buffer. This is reasonably high helicity value for short helical segments of only 11 residues each. The sequences of peptides studied are given in Table-1 along with the mean residue ellipticity values at 222 nm in benign buffer and in 50% TFE buffer. The percent helicity as determined by the ratio of mean residue ellipticity at 222 nm in the benign buffer to that in 50% TEF buffer ($[\theta]_{222}$ benign/$[\theta]_{222}$ in 50% TFE buffer) for all the peptides designed to have hairpin structure is shown in figure 1. In order to find out the optimum number of residues required to form turn segment between positions g and e' of the two helices, we used peptides with all glycine residue in the turn region. As can be seen in the figure the percent helicity value first increases with the number of glycine residues in the turn segment, after attaining a maximum for a four residue turn segment, it starts decreasing for a longer turn segment. A turn segment of all glycine residues, though capable of acquiring the desired geometry of the tight turn because of absence of any side chain steric hindrances, require high entropy cost for fixation into the turn conformation due to its very high flexibility. Additional helical stabilization was achieved by turn sequence Gly-Ala-Gly as compared to Gly-Gly-Gly in a three residue turn segment and by turn sequence Leu-Gly-Val- Ser as compared to Gly- Gly- Gly- Gly in a four residue turn segment. Maximum α-helical stabilization by the turn sequence Leu- Gly- Val- Ser, which was designed from the most frequently found amino acid residues at their respective positions in the turn regions of the conventional family of h-t-h DNA binding gene regulatory proteins, suggests that the helical stabilizing tendency of these residues at their particular positions in the h-t-h motif might be one of the causes of their natural selection in these proteins.

The n-π* transition (CD band at 220 nm) is responsive to the α-helical content whereas the π-π* excitation at 205 nm polarizes parallel in the helix axis and is sensitive to whether the α-helix is single standard or is an interacting helix as in case of two stranded coiled-coils [11]. The decrease in the parallel band intensity, coupled with the red shift in the parallel band maximum, corresponds to the conversion of a rigid single stranded α-helix to an α-helical coiled-coil structure [12]. The magnitude of the ratio $[\theta]_{222}/[\theta]_{208}$ in many inter-molecular [11] and intra-molecular [1] coiled-coil peptides has been found in the range [1.01- 1.08]. In case of non-interacting helices, where the eliplticity at 208 nm is generally more negative than at 222 nm [12,13] this ratio is generally less than 1 and is typically about 0.9. The magnitude of the ratio ($[\theta]_{222}/[\theta]_{208}$] in the benign buffer was therefore considered a sensitive parameter to detect the coiled coil structural stabilization in the h-t-h peptides. As shown in figure 2 this ratio remains close to 0.9 (+ 0.03) for peptides with two, three and five residues in the turn region, but is noticeably high for four residue turn region peptides with turn sequences Gly- Gly- Gly-Gly and Leu- Gly- Val- Ser showing the stabilization

Table-1

Peptide Name	Sequence of the peptide: Helix (d e f g a b c d e f g)	Turn	Helix (e' f' g' a' b' c' d' e' f' g')	No. of Residues	Mean Reisue Ellipticity $[\theta]_{222}$ Benign	with 50% TFE
2G	N E E E L R R L A A A —	CG	— N E A L E R L L R L Q G G Y	27	-15701	-23260
3G.	N E E E L R R L A A A —	GGG	— N E A L E R L L R L Q G G Y	28	-15249	-21784
GAG	N E E E L R R L A A A —	GAG	— N E A L E R L L R L Q G G Y	28	-15504	-21888
4G	N E E E L R R L A A A —	GGGG	— N E A L E R L L R L Q G G Y	29	-14921	-19790
GTDS	N E E E L R R L A A A —	GTDS	— N E A L E R L L R L Q G G Y	29	-15056	-22584
LGVS	N E E E L R R L A A A —	LGVS	— N E A L E R L L R L Q G G Y	29	-17739	-22633
5G	N E E E L R R L A A A —	GGGGG	— N E A L E R L L R L Q G G Y	30	-12424	-17890

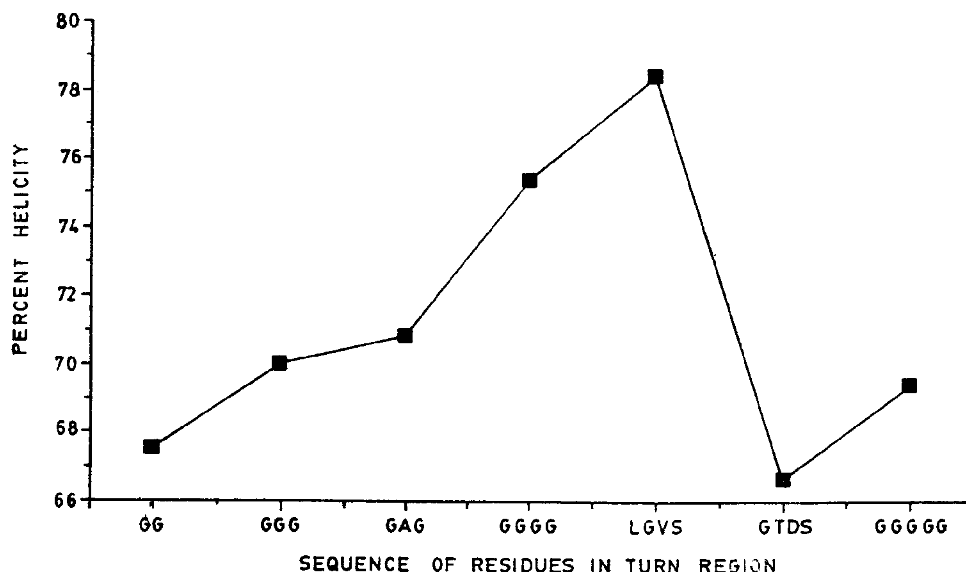

Fig. 1: Percent helicity of the peptides as calculated from the ratio of mean reisude ellipticity at 222 nm in benign buffer to that in 50% TFE buffer.

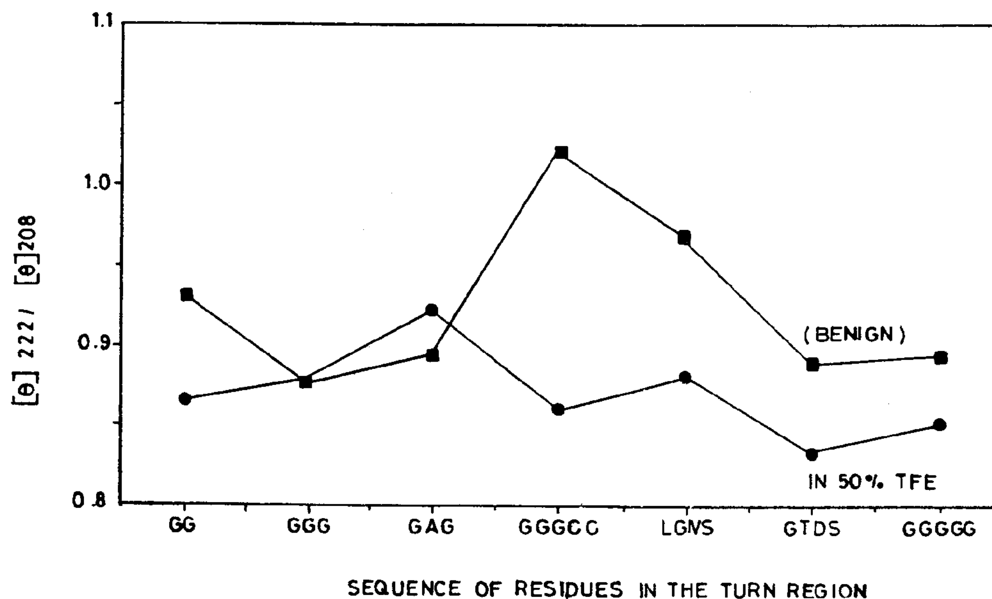

Fig. 2: Ratoi of mean residue ellipticity of the peptides at 222 nm to that at 208 nm IN ■ benign buffer and in ● 50% TFE buffer

of the coiled-coil structure in these peptides. In the presence of 50% TEF all peptides showed $[\theta]_{222}/[\theta]_{208}$ ratio of 0.83 - 0.92 suggesting that the helices are dissociated. This is in accordance with established behaviour of TFE in disrupting interhelical hydrophobic interactions and stabilizing unassociated a-helices in solution.

Experimental

Design of h-t-h

Peptide sequences of the helical segments were designed by the global optimization of intra-helical interaction by a self developed computer

program of sequence optimization using statistical mechanical model of helix-coil transition in peptides [9]. Only hydrophobic interactions between leucine residues at positions a-d' and d-a' of leuzine zipper heptad were considered as inter-helical interactions. Peptides with 2,3,4 and 5 glycine residues in the turn region were synthesized to study the effect of the length of the turn segment. Two h-t-h peptides were synthesized with turn sequences already reported to provide stable tertiary turn in various de novo designed peptides. These turn sequences include Gly-Ala-Gly [10] and Gly-Thr-Asp-Ser [4,5]. A turn sequence Leu-Gly-Val-Ser was selected from frequently found residues at same positions in the turn region of well known family of bacterial and phage gene regulatory proteins with the conventional h-t-h- DNA binding motif [14].

Synthesis of peptides

The peptides were synthesized on an automated solid phase peptide synthesizer (Shimadzu PSSM-8) using Tenta Gel TG-RAM resin and Fmoc chemistry with benzatriazole-1-yl-oxy-tris-pyrrolidinophosphonium hexafluorophos-phate and N-hydroxybenzotriazole as coupling reagents. Peptides were cleaved from the resin by trifluoro-acetic acid and purified by reverse phase HPLC on a C_{18} column. Molecular masses were confirmed by mass spectrometry on a time of flight mass spectrometer (Shimadzu/Kratos Kompact MALDI II) with matrix assisted laser desorption ionization.

CD measurements

CD measurements were made with a spectropolarimeter (Jasco, model 1-500 A, equipped with data processor, DP-501). All measurements were carried out at 5°C in a thermostatically controlled cell with a pathlength of 0.1 cm. The begngn buffer used for CD measurements was 5mM 7,N-[tris (hydroxymethyl)methyl-2-aminomethanesulphonate (TES-NaOH) at pH 7.5 and peptide concentration range from 20 - 100 μM Percent halicity here refers in helicity of the peptide in benign buffer expressed as percentage of the maximum attainable helicity in 50% trifluoroethanol (TFE) buffer. This was calculated from the ratio of mean residue ellipticity of the peptide at 222 nm in benign buffer solution to that in 50% TFE buffer.

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References

1. O.D. Monera, N.E. Zhou, C.M. Kay and R.S. Hodges, *Biol. Chem.*, **268**, 19218 (1993).
2. N.E. Zhou, C.M. Kay, and R.S. Hodges, *J. Mol. Biol.*, **237**, 500 (1994).
3. D.G. Myszka and M.C. Irwin, *Biochemistry*, **33**, 2363 (1994).
4. Y. Fezoui, D.L. Weaver, J.J. Osterhout, *Proc. Natl. Acad. Sci., USA* 91, 3675 (1994).
5. Y. Fezoui, D.L. Weaver, and J.J. Osterhout, *Protein Science*,4, 286 (1995).
6. Y. Kuroda, *Protein Engineering* **2**, 97 (1995).
7. Y. Kroda, T. Nakai, and T. Ohkubo, *J. Mol. Biol.*, **236**, 862 (1994).
8. A. Doing, A. Chakrabarty, T. Klingler, and R.L. Baldwin, *Biochemistry*, **33**, 3396 (1994).
9. V. Munoz, and L. Serrano, *J. Mol. Biol.*, **245**, 275 (1995).
10. S.K. Conrad, H. Lee, A.M. DiGeorge and P.T.P. Kaumaya, *The J. Biol. Chem.*, **268**, 25285 (1991).
11. W.D. Kohn, C.M. Kay and R.S. Hodges, *Protein Science*, **4** 237 (1995).
12. T. Cooper and R.W. Woodhy, *Biopolymers*, **30**, 657 (1990).
13. N.E. Zhou, C.M. Kay, B.D. Sykes, R.S. Hodges, *Biochemistry*, **32** 6190 (1993).
14. S.C. Harrison, A.K. Aggarwal, *Annu. Rev. Biochem.*, **59**, 933 (1990).