J|A|C|S COMMUNICATIONS

Published on Web 03/17/2010

Design of Short Linear Peptides That Show Hydrogen Bonding Constraints in Water

Benben Song,[†] Patrick Kibler,[†] Alpeshkumar Malde,[‡] Krishna Kodukula,[†] and Amit K. Galande*,[†]

Center for Advanced Drug Research (CADRE), SRI International, Harrisonburg, Virginia 22802, and School of Chemistry and Molecular Biosciences (SCMB), The University of Queensland, St. Lucia QLD 4072, Australia

Received June 29, 2009; E-mail: amit.galande@sri.com

Short linear peptides with 4 to 10 residues are considered flexible molecules with entropic limitations on achieving unique conformations in water.[1] Predominant conformation in these peptides can be achieved in water by incorporating amino acids that restrict access to conformational space, such as proline[2] and aminoisobutyric acid (Aib)[3] or by using noncovalent interactions,[4] such as π-stacking. In the water environment, however, hydrogen bonds are generally not considered to be the major driving force for folding and constraining short linear peptides into a distinct conformation.[5] We explored the possibility of designing linear tetrapeptides that can fold into a compact secondary structure through intramolecular hydrogen bonds in the water environment. We began the design by choosing ST[6] and Asx turns[7] as the structural motifs to model short linear peptides. In ST turns, side chain oxygen atoms of serine or threonine form a hydrogen bond with the backbone NH groups of the i+2 residues to create a 9-membered ring. The Asx turn is characterized by a similar hydrogen bonding pattern of Asp or Asn to create a 10-membered ring which can be further supported by an adjacent 7-membered ring as is the case in the N-glycosylation sequence (NXS/T) in proteins (Figure 1). These motifs provide a basis for modeling short linear peptides that can adopt a well-defined turn in water through side chain−main chain hydrogen bonds.

As indicated in Figure 1, we combined the features of ST and Asx turns to develop a novel motif referred to here as the Hse turn. The Hse turn was designed through two important amino acid substitutions. The first substitution involved replacing the i^{th} amino acid (Asn) of the natural Asx turn with an unnatural amino acid Homoser. The second substitution involved changing the configuration of the i+2nd amino acid from the natural L to the unnatural D form. We hypothesized that these two substitutions would maintain essentially the same conformation as the Asx turn including the 10-membered and the 7-membered rings but eliminate side reactions associated with Asx residues such as deamidation.[8] Based on this new motif of Homoser-Xaa-D-Yaa, we synthesized a series of tetrapeptides (Table 1) using standard Fmoc chemistry procedures. The tetrapeptides were studied using NMR spectroscopy and MD simulations to identify sequences that show a turn-like conformation in water. Initially, we incorporated three N-terminal amino acid residues—alanine (peptide **1**), β-alanine (peptide **2**), and 2-aminobenzoic acid or 2Abz (peptide **3**)—to evaluate their effect on stabilizing the turn mimicry. Ser (at the third position) and Glu (at the C-terminus) were chosen for these tetrapeptides, as their side chains are likely to form additional hydrogen bonds with each other or with the peptide backbone to stabilize the peptide conformation.[9] Conformational features of these peptides were evaluated using 1D and 2D NMR experiments conducted in 90% H_2O/10% D_2O. Specifically, rotating frame Overhauser effect spectroscopy (ROESY) and MD simulations in water were used to evaluate peptide secondary structures.

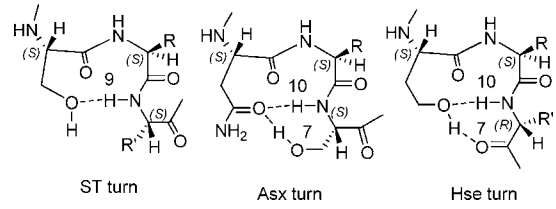

Figure 1. Hse turn was created by replacing the i^{th} amino acid with Homoser and by changing the spatial configuration of the i+2nd amino acid from L to D.

Table 1. Tetrapeptides Evaluated for Asx Turn Mimicry

Peptide sequence
H-Ala-Homoser-Ser-D-Glu-NH_2 (**1**)
H-β-Ala-Homoser-Ser-D-Glu-NH_2 (**2**)
H-2Abz-Homoser-Ser-D-Glu-NH_2 (**3**)
H-2Abz-Homoser-Ser-Glu-NH_2 (**4**)
H-2Abz-Homoser-Ser-D-Ala-NH_2 (**5**)
H-2Abz-Homoser-Ala-D-Glu-NH_2 (**6**)
H-2Abz-Homoser-Gly-D-Glu-NH_2 (**7**)
H-2Abz-Ala-Ser-D-Glu-NH_2 (**8**)
H-Homopro-Homoser-Ser-D-Glu-NH_2 (**9**)
H-Achc-Homoser-Ser-D-Glu-NH_2 (**10**)
H-Aib-Homoser-Ser-D-Glu-NH_2 (**11**)
1Nap-Homoser-Ser-D-Glu-NH_2 (**12**)
H-Phe-Homoser-Ser-D-Glu-NH_2 (**13**)
H-Abz-Homoser-Ser-D-Gln-NH_2 (**14**)

Conformational analyses of the first three peptides revealed that only **3** adopts the Hse turn in water. Peptides **1** and **2** remain unstructured as indicated by the lack of ROE cross peaks and clustering of backbone amide protons. Peptide **3**, however, indicated a well dispersed amide region and significant through-space interactions. Based on peptide **3**, a series of analogues were evaluated (**4**−**14**) to determine the contribution of each residue in peptide **3** toward the Hse turn formation. Of all the analogues, peptide **14** indicated the most compact Hse turn conformation (Figure 2). For peptide **14**, ROESY experiments indicated two sequential medium dNN (i, i+1) ROE contacts between the adjacent amide protons of Ser and D-Gln and also between D-Gln and the C-terminal amide, which are characteristic of the conformation. The ROE contacts between the gamma proton of the Homoser side chain and the amide protons of Ser and D-Gln further support that the conformation involves a Homoser side chain assisted turn. Temperature-dependent experiments indicated that resonance from a Homoser amide proton broadens (313 K) and eventually disappears (343 K) in response to fast exchange with water. In contrast, the resonances from Ser and D-Gln amide protons remain as prominent doublets at a high temperature (343 K), indicating that these protons

[†] SRI International.
[‡] The University of Queensland.

10.1021/ja905341p

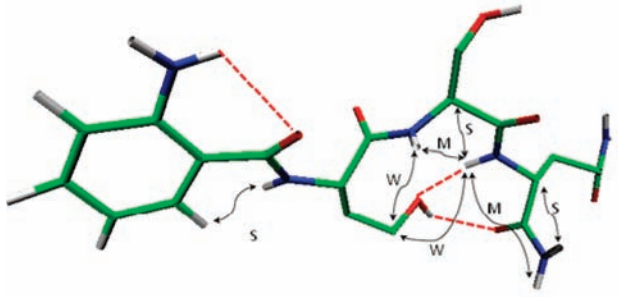

Figure 2. The major solution conformer of peptide **14** indicated by NMR experiments and MD simulations in water shows an Hse turn. The arrows indicate interactions through space observed in ROESY experiments. S, M, and W represent strong, medium, and weak ROE intensities.

are exchanging with water molecules more slowly because of their involvement in hydrogen bonding. Hydrogen/deuterium exchange experiments (Supporting Information, SI) on peptide **14** indicated that, among the four backbone amide protons, the exchange rate for the NH of D-Gln is the smallest further supporting the Hse turn conformation in Figure 2. A review of 1D NMR spectra from the concentration-dependent experiments (SI) showed that the chemical shifts of the amide protons of Ser and D-Gln remained consistent at various peptide concentrations; this pattern is consistent with intramolecular hydrogen bonding.

The MD simulation of peptide **14** indicates that the peptide conformation alternates between two states (SI). The peptide folds and unfolds several times in the 50 ns simulation. The maximum lifetime observed for the folded conformation was on the order of 10 ns. The conformation represented by Figure 2 was the central conformer of the major cluster being populated approximately 86% of the time in the simulation and fits the NMR data. The free energy of folding ($\Delta G_{\text{folding}}$) was estimated to be -3.9 kcal/mol at 298 K, indicating almost no barrier to folding. The ROE distances derived from the MD trajectory are within the upper bounds (0.5 nm) of the experimental ROEs and compare well with the NMR data. NMR spectroscopy and MD simulations indicated that peptides **3** and **14** show similar conformational features and **14** is more structured than **3** ($\Delta G_{\text{folding}}$ of -0.54 kcal/mol), most likely because of the larger conformational entropy of the C-terminal Gln as compared to that of Glu.[10]

We performed four additional amino acid substitutions (peptides **4**−**7**) to determine the contribution of each residue toward the Hse turn. When the D-Glu amide was changed to the L-Glu amide (peptide **4**) or D-Ala amide (peptide **5**), a critical dNN (i, i+1) ROE contact between the adjacent amide protons of Ser and Glu/D-Ala was eliminated. When Ser was substituted by Ala (**6**), or Gly (**7**), the peptides showed essentially the same conformation compared to peptide **3** as indicated by the ROESY spectrum. The ROESY spectrum of peptide **8**, in which Homoser was replaced by Ala, only indicated an ROE contact between the side chain proton of Ala and the amide proton of Ser as the side chain−main chain intramolecular hydrogen bonding was eliminated.

To further evaluate the effect of the N-terminal amino acid on the Hse turn, 2Abz was substituted with its cyclic but nonaromatic analogues—homoproline (**9**) and 2-aminocyclohexane carboxylate (**10**)—as well as with Aib (**11**). None of the three peptides showed a distinct conformation in water. However, **12** with N-terminal 1-napthoate and **13** with N-terminal Phe adopted an Hse turn where **12** showed a more compact turn than **13**. These results confirmed that a planar aromatic N-terminal residue is essential for inducing a turn in the Homoser-Xaa-D-Yaa motif. Peptides **3**, **6**, **12**, and **14** exhibit populations containing two hydrogen bonds (Homoser_γO−D-Glu/n_NH and Homoser_OH− D-Glu/n_CO, SI) that are essential for the Hse turn while the rest of the peptides did not show such a characteristic pattern. Our studies show that analogous to proline which promotes beta turns when present in the i, i+1 position, planar aromatic amino acids when present at the N-terminus can promote the Asx turn. A statistical analysis of glycoproteins in the Protein Data Bank also reveals a marked preference for aromatic amino acids immediately before the Asn in N-glycosylation sites.[11]

The Hse turn represents a novel structural motif with a topologically similar conformation[12,13] to that of ST and Asx turns. It is important to note that while ST and Asx turns are formed in proteins under aqueous conditions, most conformational studies[7] involving such turns in short peptides have been carried out in organic solvents ($CDCl_3$ or DMSO-d_6) that have completely different hydrogen bonding properties and a much lower dielectric constant as compared to those of water. Peptide **14**, however, adopts a well-defined and hydrogen bond-assisted conformation in the water environment. Considering the high pK_a values and nonionizable nature of the side chain groups in **14**, we expect the turn to be stable under physiological pH (SI) as well as in organic solvents.

Short peptides with distinct secondary structures have substantial usefulness as catalysts for asymmetric organic synthesis, as supramolecular synthons for self-assembly, and as bioactive molecules for therapy. The sequence of **14** provides a new structural motif (Hse turn) that can be used for designing short synthetic peptides with a compact structure in water.

Acknowledgment. CADRE was established through funding support from the Commonwealth of Virginia to SRI International. The authors thank Drs. Richard Foust and David Brakke, Department of Chemistry at James Madison University, for access to the NMR facility and the NCI National Facility, Australia for the computation time provided through project n63 to A.M., who is an ARC Postdoctoral Fellow. Encouragement and guidance from Dr. Walter Moos, Vice President of Biosciences at SRI are gratefully acknowledged.

Supporting Information Available: Experimental details on peptide synthesis, NMR spectroscopy, and MD studies of all the peptides in Table 1. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Perczel, A.; Hollosi, M. In *Circular Dichroism and the Conformational Analysis of Biomolecules*; Fasman, G. D., Eds.; Plenum Publishing Corporation: New York, 1996; Vol. 28, pp 5−380.
(2) Imperiali, B.; Fisher, S. L.; Moats, R. A.; Prins, T. J. *J. Am. Chem. Soc.* **1992**, *114*, 3182–3188.
(3) Karle, I. L.; Balaram, P. *Biochemistry* **1990**, *29*, 6747–6756.
(4) Bruch, M. D.; Dhingra, M. M.; Gierasch, L. M. *Proteins: Struct., Funct., Genet.* **1991**, *10*, 130–139.
(5) Ragone, R. *Protein Sci.* **2001**, *10*, 2075–2082.
(6) Duddy, W. J.; Nissink, J. W. M.; Allen, F. H.; Miller-White, E. J. *Protein Sci.* **2004**, *13*, 3051–3055.
(7) Imperiali, B.; O'Connor, S. E.; Hendrickson, T.; Kellenberger, C. *Pure Appl. Chem.* **1999**, *71*, 777–787.
(8) Geiger, T.; Clarke, S. *J. Biol. Chem.* **1987**, *262*, 785–794.
(9) Chellgren, B. W.; Creamer, T. P. *Proteins* **2006**, *62*, 411–420.
(10) Eswar, M.; Ramakrishnan, C. *Protein Eng.* **2000**, *13*, 227–238.
(11) Petrescu, A. J.; Milac, A. L.; Petrescu, S. M.; Dwek, R. A.; Wormald, M. R. *Glycobiology* **2004**, *14*, 103–114.
(12) Abbadi, A.; Mcharfi, M.; Aubry, A.; Premilat, S.; Boussard, G.; Marraud, M. *J. Am. Chem. Soc.* **1991**, *113*, 2729–2735.
(13) Xu, T.; Werner, R. M.; Lee, K.; Fettinger, J. C.; Davis, J. T.; Coward, J. K. *J. Org. Chem.* **1998**, *63*, 4767–4778.

JA905341P