Synthesis and Nuclear Magnetic Resonance Structure Determination of an α-Helical, Bicyclic, Lactam-Bridged Hexapeptide

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Attempts to generate short α-helical peptides in solution have so far met with limited success, due to the unfavorable nature of helix initiation.[1-4] This has led to the use of non-native amino acids, and side-chain or backbone bridges to serve as elements for stabilizing helices in short peptide sequences.[5-7] Rigid structures that maintain multiple hydrogen-bond donors and/or acceptors in the appropriate geometry should overcome the highly unfavorable entropic barrier for helix initiation.[5] Such structures would facilitate the more favorable propagation of helix into neighboring amino acid sequences in the appropriate direction. This communication outlines the synthesis and NMR structure determination of a conformationally constrained bicyclic hexapeptide containing two overlapping *i*, *i* + 4 side-chain lactam bridges and selectively cleavable protecting groups on the N-terminal and C-terminal ends (I, Figure 1). NMR characterization indicates that this hexapeptide adopts a rigid, α-helical conformation in solution. This extremely short helical structure should serve as a useful α-helical peptidomimetic scaffold or α-helix initiator.

Compound I was designed to include two Lys^{i}, Asp^{i+4} side chain to side chain lactam bridges, chosen for their helix-stabilizing properties[8,9] and arranged to constrain overlapping peptide structures in the shortest possible amino acid sequence. The synthesis of I followed oxime resin-based assembly and cyclization-cleavage methods reported earlier,[9] but modified to allow a second cyclization in solution by using temporary fluorenylmethyl-based protecting groups for Lys^{1} and Asp^{5}. This strategy, along with the use of N^{α}-Boc and C^{α}-phenacyl ester (OPac) protecting groups, allows peptide coupling to I in either direction, as well as future substitution of Ala^{3} and Ala^{4} by any other residues.

Conformational analysis of peptide I by NMR was performed in TFE-d_3/H_2O solutions, because the peptide showed limited solubility in water.[10] However, circular dichroism spectra of peptide I in water at lower concentrations matched very closely those measured in TFE/H_2O solution, indicating that solvent-induced perturbations of the peptide structure were slight.[11] Amino acid assignments of the NMR spectra of peptide I were made using standard 1D and 2D ^1H NMR methods.[12] Significant structural information was obtained from NOESY spectral data. A summary of the observed interresidue NOEs is shown in Figure 1. A string of $d_{NN}(i,i+1)$ NOEs are observed from Lys^{1} to Asp^{6} in addition to NOEs from $H\alpha(i)$–$HN(i+3)$ and $H\alpha(i)$–$H\beta(i+3)$. This pattern of NOEs is consistent with that generally seen in helical regions of proteins.[13] Side-chain cross-linkage through the two lactam bridges was also confirmed by NOEs between the $HN\epsilon(i)$ to $H\beta(i+4)$ protons for Lys^{1} to Asp^{5} and Lys^{2} to Asp^{6}. No NOEs were observed from the Boc and OPac end groups to the amino acids.

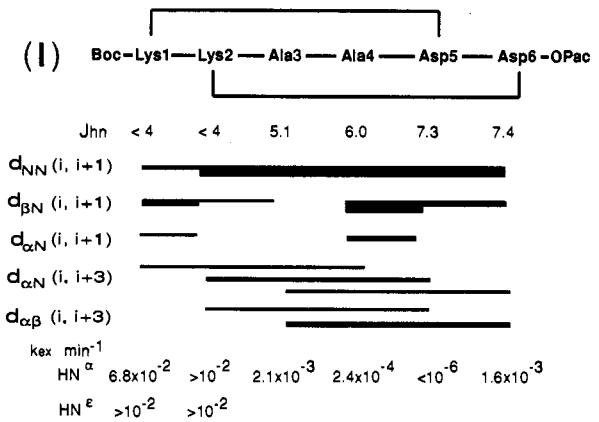

Figure 1. NMR data for compound I. Coupling constants and NOEs were measured at −10 °C in 50% (w/w) TFE-d_3/H_2O, pH 4.0. The thickness of the lines indicates relative intensities of the NOE cross peaks. Amide exchange rates (k_{ex}) were measured by 1D NMR at pH 3.7 at 22 °C in 50% (w/w) TFE-d_3/D_2O by measuring the decay of peak height.

The conformation of peptide I, shown in Figure 2, was determined by distance-restrained annealing and restrained molecular dynamics[14-18] using 85 NOE constraints measured at −10 °C. An average of fewer than one NOE violation (>0.5 Å) per structure generated was observed, excluding the Lys side chains (discussed below). Van der Waals overlaps (>0.1 Å) were also rarely observed. All of the structures were helical throughout the peptide backbone (Figure 2), and the pattern of calculated hydrogen bonds corresponded predominantly to the two Lys-to-Asp backbone hydrogen bonds expected for an α-helical conformation. H bonds between the N^{α}-Boc carbonyl and Ala^{3} (3_{10} helix) or Ala^{4} (α-helix) were observed in only a few of the structures, and other H bonds were also observed only rarely.

The average φ angles in the models grow increasingly negative from the N terminus to the C terminus, starting at around −50° for Lys^{1} and Lys^{2} and increasing to −70° to −80° for residues 3–6. These φ angles agree well with the small experimental values

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(1) Zimm, B. H.; Bragg, J. K. *J. Chem. Phys.* **1959**, *31*, 526–535.
(2) Lifson, S.; Roig, A. *J. Chem. Phys.* **1961**, *34*, 1963–1974.
(3) Scheraga, H. A. *Pure Appl. Chem.* **1978**, *50*, 315–324.
(4) Marqusee, S.; Robbins, V. H.; Baldwin, R. L. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 5286–5290.
(5) (a) Kemp, D. S.; Boyd, J. G.; Muendel, C. C. *Nature* **1991**, *352*, 451–454. (b) Chiang, L.; Cabezas, E.; Noar, B.; Arrhenius, T.; Lerner, R. A.; Satterthwaite, A. C. In *Peptides 1990: Proceedings of the 21st European Peptide Symposium*; Giraldt, E., Andreu, D., Eds.; ESCOM: Leiden, The Netherlands, 1991; pp 465–467. (c) Jackson, D. Y.; King, D. S.; Chmielewski, J.; Singh, S.; Schultz, P. G. *J. Am. Chem. Soc.* **1991**, *113*, 9391–9392. (d) Pease, J. H. B.; Storrs, R. W.; Wemmer, D. E. *Proc.Natl. Acad. Sci. U.S.A.* **1990**, *87*, 5643–5647.
(6) Rizo, J.; Gierasch, L. M. *Annu. Rev. Biochem.* **1992**, *61*, 387–418.
(7) Burgess, A. W.; Leach, S. J. *Biopolymers* **1973**, *12*, 2599–2605.
(8) Felix, A. M.; Heimer, E. P.; Wang, C.-T.; Lambros, T. J.; Fournier, A.; Mowles, T. F.; Maines, S.; Campbell, R. M.; Wegrzynski, B. B.; Toome, V.; Fry, D.; Madison, V. S. *Int. J. Pept. Protein Res.* **1988**, 32, 441–454.
(9) Osapay, G.; Taylor, J. W. *J. Amer. Chem. Soc.* **1992**, *114*, 6966–6973.
(10) Spectra were collected in 40% (w/w) TFE-d_3/H_2O at 0 °C and in 50% (w/w) TFE-d_3/H_2O at 0 °C and −10 °C; all NMR solutions were buffered with 10 mM sodium acetate-d_3 adjusted to pH 4.0.
(11) CD spectra (180–250 nm) were compared using 0% and 50% (w/w) TFE/H_2O buffered with 10 mM sodium phosphate at pH 6.0, at temperatures ranging from −10 °C to 70 °C.
(12) Wuthrich, K. *NMR of Proteins and Nucleic Acids*; Wiley: New York, 1986.
(13) Wuthrich, K.; Billeter, M.; Werner, B. *J. Mol. Biol.* **1984**, *180*, 715–740.
(14) Randomized coordinates were used to generate 500 starting structures for distance-restrained annealing using DSPACE.[15] The 10 lowest energy structures (backbone root mean square deviation 0.88 Å) were refined further.[17]
(15) Hare Research Inc., Woodinville, WA, 1990.
(16) Clore, G. M.; Gronenborn, A. M. *Crit. Rev. Biochem. Mol. Biol.* **1989**, *24*, 479–564.
(17) Each of the 10 distance restrained annealing models were refined using five separate runs of restrained molecular dynamics using DISCOVER.[18] Each run employed a different distance-dependent dielectric function. The backbone root mean square deviation for the 50 resultant structures was 0.44 Å. The structures displayed in Figure 2 resulted from molecular dynamics runs employing a 10-Å cutoff, with the two highest energy structures omitted.
(18) Biosym Technologies Inc., 10065 Barnes Canyon Rd., San Diego, CA, 1991.

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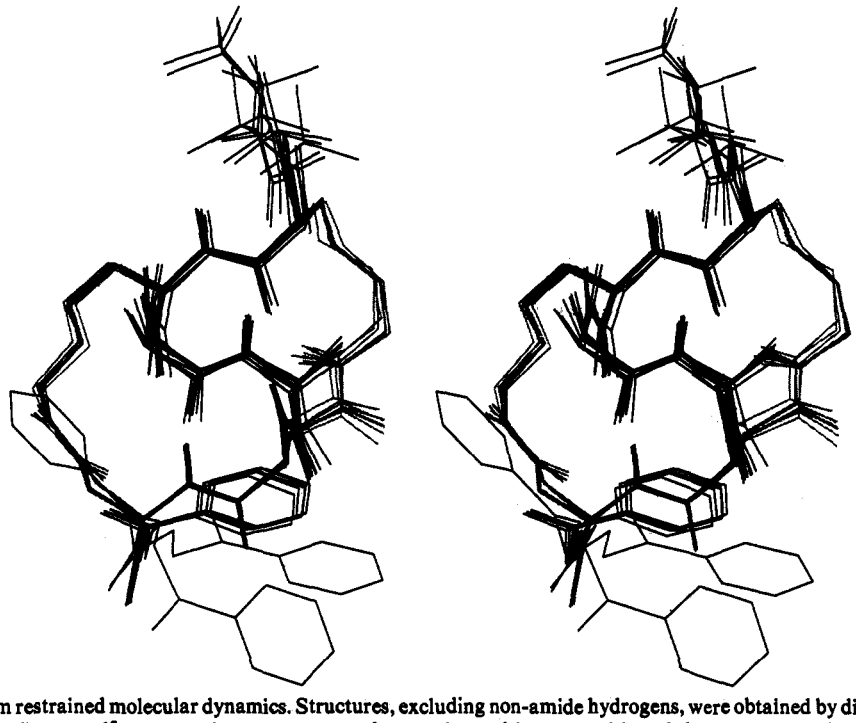

Figure 2. Structures from restrained molecular dynamics. Structures, excluding non-amide hydrogens, were obtained by distance-restrained annealing[14] and molecular dynamics refinement.[17] They are shown as cross-eyed stereoviews with superposition of all atoms except those of the Boc and OPac groups.

of $J_{hn-h\alpha}$ (<4 Hz) seen at the N terminus and the larger $J_{hn-h\alpha}$ values found at the C-terminal end (Figure 1). The changing ϕ values result in a moderate narrowing of the helical structure in the N-terminal direction. However, all ϕ and φ values in the models lie within the normal range found in α-helices.[19]

The Lys^i, Asp^{i+4} lactam bridges showed one predominant conformation of the Asp side chains through the amide bonds, with the Asp β-methylenes adopting $\chi 1$ angles of g^- ($\sim$–60°). Both bridging amides were in the trans conformation and oriented on the helix surface with the Lys^i N$^\epsilon$ hydrogens directed toward the $i+1$ residue. The alternative orientation (N$^\epsilon$–H toward the $i+3$ residue) was not observed in any model. In contrast, the conformations of the bridging Lys side chains are less certain. Most of the NOE violations observed in the models centered around the Lys Cγ positions of the lactam bridges. This pattern, and an examination of Corey–Pauling–Koltun models, suggests that a conformational averaging of the bridges, predominantly involving movement of the Cγ and Cδ methylene groups, may be occurring.

Conformational stability and solvent accessibility of the amide protons were estimated directly by measuring hydrogen–deuterium exchange rates at 22 °C (Figure 1).[20] By this measure, the Asp^5 amide appears to be the most solvent protected, with an exchange rate comparable to those observed for the core residues of globular proteins. Intermediate exchange rates were observed for Ala^4, Asp^6, and Ala^3 amides (in order of increasing exchange rate), and the rates for Lys^1 and Lys^2 were as rapid as those for the side-chain bridging amides. These data suggest that slow exchange results from the presence of a stable helical conformation which offers significant solvent protection to the slowly exchanging amides. In support of a rigid α-helical structure for peptide **I**, CD spectra measured in H_2O or TFE/H_2O[11] indicated only partial melting at high temperature.

The solution NMR data indicate that our design goal of creating a stable α-helical structure for use as an α-helix scaffold and/or initiator has been achieved. Peptide **I** shows most of the characteristic NOEs found in helical structures, and the slow amide exchange rates observed at 22 °C indicate that this helical structure is very stable. The calculated structures show a predominant α-helical H-bonding pattern, and the orientations of the free N-terminal H-bond donor and C-terminal H-bond acceptor groups should serve equally well in facilitating helix propagation in either direction. This offers the possibility of detailed physical studies of helix structure and propagation, as well as the rapid generation of small helical peptides for use as models for biological processes, including helix recognition or electron transfer, or for the generation of helical peptide libraries.

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Supplementary Material Available: Experimental details of peptide synthesis, spectral assignments, structure refinement data, and CD spectra (4 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(19) Creighton, T. E. In *Proteins; Structures and Molecular Properties;* W. H. Freeman and Co.: New York, 1984.

(20) Englander, S. W.; Kallenbach, N. *Q. Rev. Biophys.* **1984**, *16*, 521–655.