Crystal Structure of Cyclo(Adm-Cyst)$_3$: Example of a Topologically Defined Double-Helical Cystine Cyclic Peptide

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While topologically chiral motifs are commonly observed in nucleic acids,[1] there are hardly any examples of proteins and polypeptides that exhibit knots or links in their structures.[2,3] A recent survey[4] of the X-ray structures deposited in the Brookhaven Protein Data Bank has brought out the significance of the presence of multiple disulfide bonds in proteins and polypeptides with topologically important features. Our recent report[5] of a family of novel macrocyclic membrane ion carriers containing cyclic repeats of adamantane and cystine units in 26-, 39-, 52-, and 65-membered rings and the presence of increasingly large number of disulfide linkages in the rings suggested that the higher members of this family may exhibit topologically significant features.[6]

In this paper, we report the "figure-eight" (double-helical arrangement) motif in the solid state structure of cyclo(Adm-Cyst)$_3$, [Adm = 1,3 adamantane dicarbonyl unit and Cyst = L-cystine dimethyl ester] where the 39-membered [3 + 3] cyclic peptide **1** contains three S−S linkages.[7] Moderate and large macrocycles formed by cyclization of peptides have rarely been observed to form large open pores.[8] Much more frequently, the interior space in the macrocycle collapses to some minimum space, accompanied by folding of the backbone and formation of intracyclic NH···OC hydrogen bonds.[9,10] The present molecule **1** assumes the topologically defined, "figure-eight" motif,[11] as shown in Figure 1.[12,13] The molecule contains a 2-fold rotation axis of symmetry that passes through atoms C(1) and C(6) and between S(3) and S(3a) (numbering shown in Figure 1b). While the two symmetry equivalent adamantane units have their carbonyl units anti to each other, the carbonyl units in the central adamantane unit adopt a syn conformation that is in a favorable orientation to form intramolecular NH···OC bonds with the amide NHs of the middle cystine unit, Figure 2. The symmetrical pair of hydrogen bonds

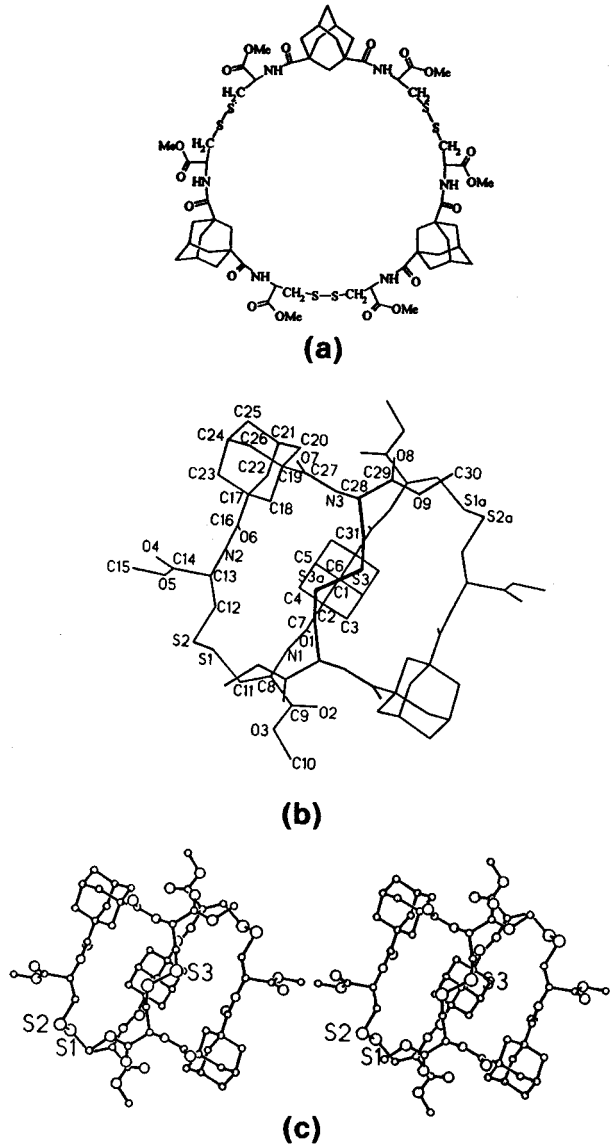

Figure 1. (a) Chemical formula. (b) The "figure-eight" conformation of cyclo(Adm-Cyst)$_3$ **1** in the crystal. A 2-fold rotation axis passes through C(1) and C(6) and halfway between S(3) and S(3a). The view is down the *x* axis. Several interatomic distances that indicate the size of the cavities are as follows: C(1)−S(3), 5.80 Å; N(2)−C(4), 4.36 Å; N(2)−S(3a), 6.20 Å; N(1)−C(12), 3.94 Å; C(18)−C(3a), 4.77 Å; O(6)−C(5), 3.85 Å. (c) Stereodiagram of (b). The dotted lines indicate the only two intramolecular hydrogen bonds, N(3)···O(1a) and N(3a)···O(1).

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(3) The only topological link known to date that is composed entirely of amino acid residues is the catenated structure of β-subunit of human chorionic gonadotropin (see ref 6 in Liang, C.; Mislow, K. *J. Am. Chem. Soc*. **1994**, *116*, 11189).

(4) Metal atoms and disulfide bonds are found to be integral constituents of knots and links in proteins exhibiting extraordinary topological features (Liang, C.; Mislow, K. *J. Am. Chem. Soc*. **1989**, *111*, 6132).

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(6) A rotaxane-like structural motif of three cystine residues in various growth factors and related proteins has been widely referred to as a knot (McDonald, N. Q.; Hendrickson, W. A. *Cell* **1993**, *73*, 421. Schlunegger, M. P.; Grutter, M. G. *J. Mol. Biol*. **1993**, *231*, 445. Murray-Rust, J.; McDonald, N. W.; Blundell, T. L.; Hosang, M.; Oefner, C.; Winkler, F.; Bradshaw, R. A. *Structure* **1993**, *1*, 153).

(7) For **1**: mp 166−168 °C; ^{1}H NMR (300MHz, $CHCl_3$) δ 1.61, 2.11 (m, 36H), 2.23 (br s, 6H), 3.15 (m, 12H), 3.76 (s, 18H), 4.80 (m, 6H), 6.72 (d, J = 7.0 Hz, 6H); FAB MS, *m/z* (%) 1369 (100) $[M + H]^+$, 913 (44) $[M^+ - Adm-Cyst + H]$.

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(11) X-ray structure analysis: $C_{60}H_{84}N_6O_{18}S_6 \cdot C_4H_8O_2$, space group $C222_1$, a = 16.587(1) Å, b = 16.945(1) Å, c = 25.882(1) Å, V = 7274.6, d_c = 1.331 g/cm^3, Z = 4, Cu radiation, λ = 1.5418 Å. Crystals were obtained from chloroform and ethyl acetate. The molecule lies on a 2-fold axis. The structure was solved by direct phase determination and refined by full-matrix anisotropic least squares. Distance restraints were applied to the disordered ethyl acetate molecule that lies on a 2-fold axis, to one methyl ester group with high mobility and one C−S−S−C group highly disordered around a crystallographic 2-fold axis. R = 9.1 for 433 parameters and 2130 independent data ($>3\sigma(F)$).

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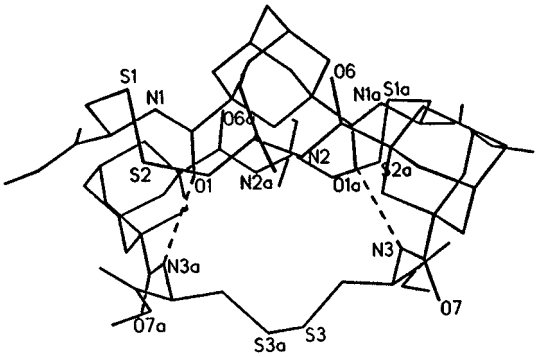

Figure 2. View of **1** down the *z* axis. Only the most prevalent of the disordered sites for S(3)−S(3a) and the bonded CH_2's (at the bottom of the macrocycle) are shown in all the diagrams.

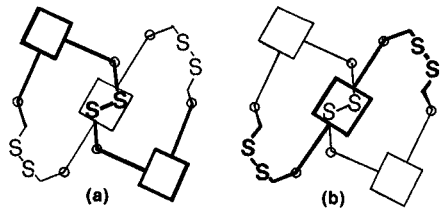

Figure 3. The presence of L-substituents on the chiral C^{α} atoms (designated by ○) results in the preference of (a) for the figure-eight conformation of the backbone rather than (b). The heavy lines are above the plane of the paper. If there were no chiral atoms in the macrocycle, then conformation (a) could be twisted into (b).

N(3)H···O(1a) and N(3a)H···O(1) have N···O = 3.027 (±0.025) Å and H···O = 2.21 Å. These are the only two hydrogen bonds within the macrocycle. There also is only one pair of symmetric NH···OC bonds between molecules, N(1)H···O(7) where N(1)···O(7) = 2.839 (±0.025) Å and H···O(7) = 2.05 Å. The N(2)H moiety does not participate in hydrogen bonding.

All ester substituents extend outward from the macrocycle. The disulfide moieties are on the periphery of the molecule. The torsion angle around S(1)−S(2) is +102°. The third disulfide S(3)−S(3a) has a different environment than the other two. There is considerable space about the C(31)−S(3)−S(3a)−C(31a) moiety that allows these atoms to occupy a number of disordered sites. For the most prevalent position (occupancy about 0.4), the torsion angle for S(3)−S3(a) is near +84°. Disordered S−S moieties in crystals are not uncommon.[15]

The need for maintaining a near orthogonal value for the S−S dihedral angle and the ring size of the macrocycle, or in other words the number of S−S linkages in the ring, appear to be crucial factors for the attainment of a "figure-eight" motif in a cystine-containing cyclic peptide. The macrocycle **1** with three cystine units may have the optimum ring size required for the adoption of this double-helical arrangement. This notion was supported by the observation that the 26-membered [2 + 2] macrocycle containing alternating repeats of *meta*-phenylene and cystine units adopted a collapsed open ring structure, as shown by its single crystal X-ray structure.[16]

It is of interest to note that the "figure-eight" motif could have a left or a right disposition of the intercrossing helices, Figure 3. The fact that only one arrangement is seen in **1** suggests control by the chirality of the cystine in the macrocycle formation. The choice of a over b (Figure 3) is analogous to the choice of right- or left-handed helices in linear peptides. The backbone atoms form a right-handed helix when the side chains have the L-configuration at the C^{α} atoms and a left-handed helix when the side chains have the D-configuration at the C^{α} atoms. In cases where the C^{α} atoms are rendered achiral by the presence of two CH_3 groups, both right- and left-handed helices occur as shown, for example, in crystal structure analysis of X-$(Aib)_n$-Y peptides.[17] Similarly, in the present case, there are six chiral C^{α} atoms, two for each L-cystine residue, that predispose the figure-eight backbone to assume the conformation a in Figure 3, rather than conformation b. Conformation b is not favored, if not impossible, because the six $COOCH_3$ moieties that extend outward (Figure 1) would have to turn inward for b and cause considerable steric interference.

The demonstrated ion transport through a model bilayer membrane[5] suggests that **1** should either form a complex by encapsulating a K^+ ion or aggregate to form an ion channel. Thus far, a K^+ complex has not been crystallized.

In summary, the present work provides conclusive evidence for the novel figure-eight motif that occurs only in one antipodal form in a cyclic peptide. The present findings should prove useful in the design of biologically important receptors with predefined shapes. A detailed study pertaining to the design of conformationally constrained templated cyclic peptides containing multiple cystine units and a host of other amino acids is in progress in our laboratories.

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Supporting Information Available: ^{1}H NMR and 2-D ROESY spectra of **1** in $CDCl_3$, CD spectra of **1** in trifluroethanol, and structural details, including coordinates, bond lengths, bond angles, anisotropic thermal parameters, and torsional angles for crystal **1** (13 pages). See any current masthead page for ordering and Internet access instructions.

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(12) Although a "figure-eight" motif has been observed in the solid state structures of some molecules, for example, in turcasarin, an expanded porphyrin macrocycle containing 10 pyrrole rings (Sessler, J. L.; Weghorn, S. J.; Lynch, V.; Johnson, M. R. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1509), and in a copper(I) complex of macrocyclic bis(dithiadiimine) ligands (Comba, P.; Fath, A.; Hambley, T. W.; Richens, D. T. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1883), to our knowledge the present report is the first example of a cyclic cystine peptide exhibiting a chiral "figure-eight" motif.

(13) The appearance of only a single set of resonances for cystine and adamantane protons in the ^{1}H and ^{13}C NMR spectra[7] coupled with the observation that these remain unaffected in the temperature range of 20−90 °C indicated that the molecule has a single conformation in solution. The anti arrangement for the 1,3 adamantyl dicarbonyl functions seen for the two symmetry equivalent adamantane units in the crystal structure of **1** was also suggested by the enhanced ROE between the NH and the adamantane methylene protons in the 2D ROESY spectrum of **1** (Supporting Information). The occurrence of a strong positive band at 213 nm in the CD spectrum of **1** (Supporting Information) indicated the presence of type II β-turns[14] which augurs well with the figure-eight conformation in the solid state.

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