The Structure of Water As Organized in an RGD Peptide Crystal at −80 °C

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Since the discovery that peptides containing the Arg-Gly-Asp sequence can serve as antagonists against human platelet aggregation, synthetic and structural studies of cyclic RGD peptides have been initiated in an effort to develop structure–activity relationships and to derive information about the conformation of the binding site.[1,2] The activity of cyclic[D-valyl-(N^2-methyl-L-arginyl)glycyl-L-aspartyl-3-(aminomethyl)-benzoic acid], hereafter referred to as **VRGDB**, as such an antagonist led to an X-ray diffraction study to determine its structure.

It is, however, not the intent of this communication to discuss the cyclic peptide in any detail, because the molecular geometry, shown in Figure 1, has a "turn-extended-turn" conformation similar to that found for the nitrate salt of (2-mercaptobenzoyl)-(N^{α}-methylArg)-Gly-Asp-2-mercaptoanilide cyclic disulfide.[1] Instead, this communication focuses on the structural aspects of the large number of water molecules which cocrystallize with the peptide. At 193 K, these water molecules, using the peptide structure as a template, organize into a fully ordered, infinite hydrogen-bonded network. In fact, all of the hydrogen atoms were refined and thus the nature of the network was revealed in intricate detail.

The peptide exists as a zwitterion, with the proton transferred from the Asp carboxylic group to the Arg guanidyl moiety as shown in Figure 1. The guandinium cation hydrogen bonds into a hydrophilic "pocket" of C=O groups in the center of a neighboring ring. The Asp carboxylate anion meanwhile accepts two hydrogen bonds from the Gly and Asp amide nitrogens of a neighboring molecule. Figure 2 shows the packing of the peptide molecules, which leave large infinite voids running parallel to the *a*-axis. In projection, the corners of these parallelogram-like voids are the locations of the peptide–peptide intermolecular hydrogen bonds. Of the peptide's nine N–H moieties, six hydrogen bond to neighboring peptides, one forms an intramolecular hydrogen bond (Figure 1), and only two bond to water molecules. The seven peptide oxygen atoms accept seven hydrogen bonds from N–H groups of neighboring peptides and only five bonds from the water molecules. It thus seems reasonable to conclude that it is the peptide interactions that drive the crystallization process with only a little assistance from the 12 water molecules (per peptide) which fill the channels.

Of the 34 hydrogen bonds listed in Table I, seven are between the water molecules and the peptide, eight are intra- or intermolecular peptide bonds, and 19 are among the water molecules themselves. The large number associated with the water molecules is a direct result of the fact that the majority of the peptide's hydrophilic groups are tied up in peptide–peptide hydrogen bonds, leaving mostly hydrophobic groups to line the channels. With only limited interactions between the peptides and the water molecules, the water molecules are reasonably free to self-assemble. The "backbone" of the resulting water structure is an infinite set of 6-rings with chair conformations which run along the channel parallel to the *a*-axis. This same backbone can be found in the two polymorphs of ice, Ih and Ic, which exist at atmospheric pressure. The complete water structure is shown in Figure 3 and is seen to consist of one 4- and several 5-, 6-, 7-membered "fused" rings. The Val methyl groups, seen

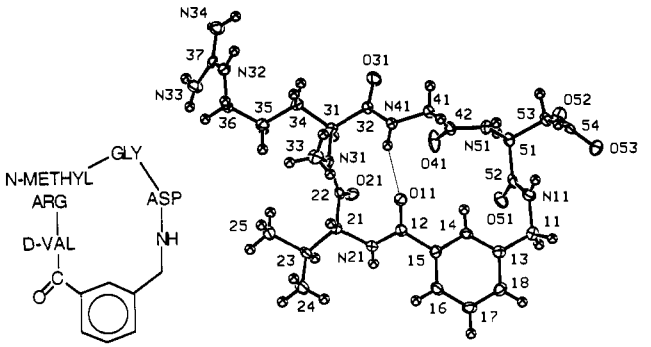

Figure 1. Molecular conformation and atom labeling scheme for **VRGDB**. The carbon atoms are labeled by number only. The thermal ellipses encompass 50% probabilities. There is one intramolecular N—H···O hydrogen bond (thin line) which helps to maintain the structure in the observed conformation.

Table I. Hydrogen-Bonding Geometries at 193 K[a]

peptide donors:	N···O	H···O	N—H···O
N11—H11N···O52	2.834(3)	2.00(3)	163(2)
N21—H21N···O1W	2.848(3)	2.04(3)	175(2)
N32—H32A···O11	2.869(3)	2.03(3)	163(2)
N33—H33A···O51	2.973(3)	2.16(3)	163(2)
N33—H33B···O21	3.070(3)	2.28(3)	142(2)
N33—H33B···O41	3.105(3)	2.52(3)	121(2)
N34—H34A···O3W	2.936(3)	2.11(3)	156(3)
N34—H34B···O21	2.825(3)	1.95(3)	165(3)
N41—H41N···O11	2.974(3)	2.16(2)	157(2)
N51—H51N···O53	2.779(3)	1.93(3)	169(2)
water donors	**O···O**	**H···O**	**O—H···O**
O1W—H1A···O3W	2.868(3)	1.92(3)	170(2)
O1W—H1B···O7W	2.870(3)	2.10(4)	164(4)
O2W—H2A···O31	2.782(3)	1.96(4)	163(4)
O2W—H2B···O8W	2.761(3)	1.89(4)	166(3)
O3W—H3A···O2W	2.758(3)	1.98(3)	165(3)
O3W—H3B···O10W	2.713(3)	1.84(3)	171(3)
O4W—H4A···O41	2.805(3)	1.98(4)	166(3)
O4W—H4B···O6W	2.839(3)	2.05(4)	159(3)
O5W—H5A···O52	2.699(3)	1.79(4)	157(4)
O5W—H5B···O12W	2.978(4)	2.18(5)	154(4)
O6W—H6A···O5W	2.839(3)	2.04(4)	161(3)
O6W—H6B···O53	2.729(3)	1.91(4)	160(4)
O7W—H7A···O4W	2.717(3)	1.80(3)	167(3)
O7W—H7B···O9W	2.797(3)	1.95(4)	172(3)
O8W—H8A···O7W	2.764(3)	1.94(4)	174(3)
O8W—H8B···O11W	2.817(3)	1.93(4)	173(3)
O9W—H9A···O2W	2.822(3)	2.04(4)	158(4)
O9W—H9B···O8W	2.845(3)	2.04(3)	177(4)
O10W—H10A···O5W	2.811(4)	1.95(4)	175(3)
O10W—H10B···O11W	2.884(3)	2.01(4)	166(3)
O11W—H11A···O6W	2.741(3)	1.89(4)	169(3)
O11W—H11B···O12W	2.863(4)	2.04(4)	164(3)
O12W—H12A···O9W	2.838(3)	2.02(4)	166(3)
O12W—H12B···O31	3.161(3)	2.32(4)	151(4)

[a] Bond distances are in Å units; angles are in degrees. The N-H distances ranged from 0.81(3) to 0.94(3) Å; the O-H distances, from 0.79(3) to 0.96(4) Å.

(1) Kopple, K. D.; Baures, P. W.; Bean, J. W.; D'Ambrosio, C. A.; Huges, J. L.; Peishoff, C. E.; Eggleston, D. S. *J. Am. Chem. Soc.* **1992**, *114*, 9615.
(2) McDowell, R. S.; Gadek, T. R. *J. Am. Chem. Soc.* **1992**, *114*, 9245.
(3) VRGDB was recrystallized by slow evaporation of a water solution. A rather large crystal, 0.50 × 0.20 × 0.61 mm, was placed in a glass capillary and mounted on a Syntex R3 diffractometer equipped with Mo radiation (λ = 0.710 69 Å). Crystallographic information: $C_{26}H_{38}N_8O_7.12H_2O$, FW = 790.82 (27.3% water by weight) orthorhombic, space group $P2_12_12_1$; at −80 °C, a = 8.655(3), b = 18.031(6), and c = 25.905(8) Å, V = 4042.7 Å^3, Z = 4, d_c = 1.286 g cm^{-3}, μ = 1.03 cm^{-2}. The intensities of three full octants ($4° < 2\theta < 56°$) were averaged and yielded an R(merge) of 0.038. Standard reflections showed an intensity variation of less than 1% over the course of the data collection. No absorption correction was applied. The refinement of 726 variables (O, N, C with anisotropic thermal parameters; H with isotropic) using 3857 reflections with $I > 2\sigma(I)$ converged at R = 0.034 and R_w = 0.025.

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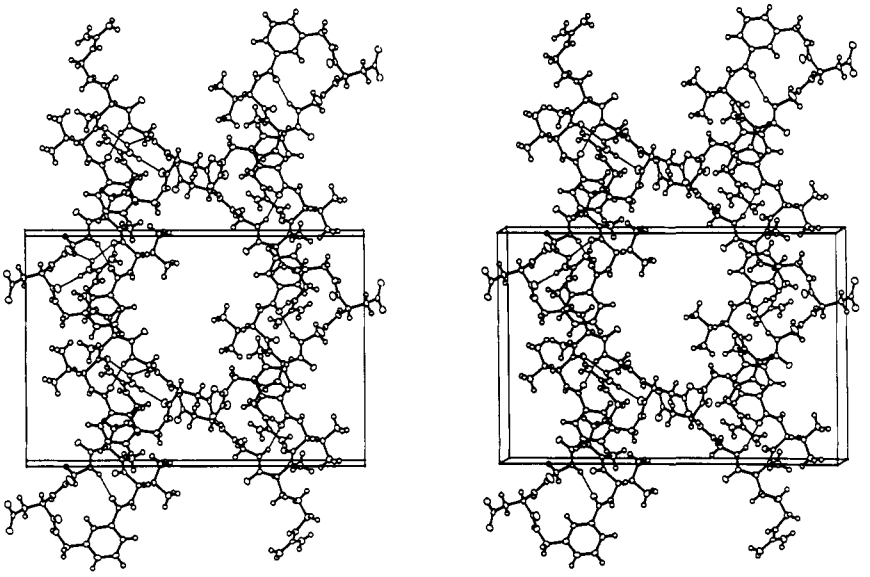

Figure 2. Packing of the **VRGDB** peptide molecules. The intra- and intermolecular hydrogen bonds have been drawn with thin lines. The left-hand column of molecules shows the strong interactions between the guanidinium cation and the carbonyl pocket of the central ring. The right-hand column shows that the guandinium cation makes only one hydrogen bond to a carbonyl on the more hydrophobic side of the molecule. The top and bottom of the central channel are formed by hydrogen bonds between the Asp carboxylate anions and two amide N–Hs from neighboring peptides.

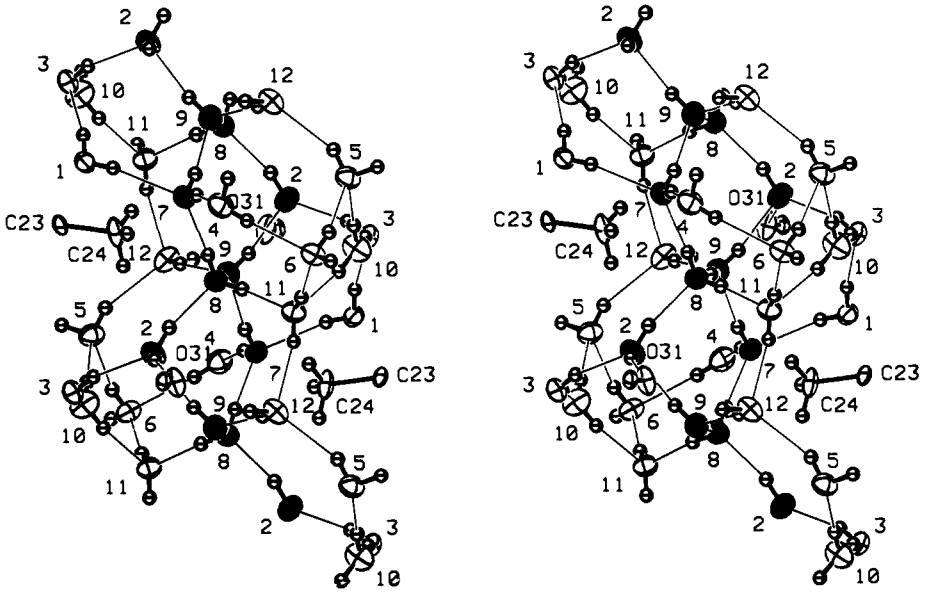

Figure 3. Twelve water molecules plus sufficient crystallographic equivalents to reveal the complete network of 4-, 5-, 6-, and 7-membered rings. The water molecules are labeled by number only. The darkened atoms represent the "backbone", a six-ring which is infinitely repeated by a combination of 2_1-screw and translational operations. The Val methyl group (C24) which protrudes into the water channel is also included, as is one of the "tie" points between the water structure, W2, and the peptide carbonyl oxygen O31.

protruding into the channel of Figure 2, have been included in Figure 3 to show in detail the formation of the "clathrate-like" structure which has developed adjacent to this hydrophobic segment of the peptide.[4]

Ring structures of various sizes, the cooperative hydrogen-bonding as noted in the "backbone", and even the self-organization of water molecules around hydrophobic moieties have been commonly found in clathrates and inclusion compounds.[5] Such structures have not been systematically observed in peptides, however, because most peptide structures have been determined from room-temperature crystals where the water molecules are not likely to be ordered. Even so, the present case may be somewhat unique, the self-assemblage of the water molecules being permitted to occur by the nature of the hydrophobic channel but being assisted by the appropriate placement of a limited number of hydrogen-bonding "tie" points to the peptide. In this sense, the present structure appears to provide some insight into the behavior of the ice nucleation proteins which serve as templates for the formation of ice crystals in freeze-tolerant organisms.[6]

Supplementary Material Available: Tables of atomic coordinates, tables of anisotropic thermal parameters and complete interatomic distances and angles (15 pages); tables of structure factor amplitudes (10 pages). Ordering information is given on any current masthead page.

(4) The clathrate-like structure is reminiscent of the water cluster found around a leucine moiety of the protein crambin (Teeter, M. M. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 6014).

(5) Jeffrey, G. A.; Saenger, W. *Hydrogen Bonding in Biological Structures*; Springer-Verlag: New York, 1991; and a multitude of references therein.

(6) Hew, C. L.; Yang, D. S. C. *Eur. J. Biochem.* **1992**, *203*, 33.