04-Crystallography of Biological Small Molecules

04.04 – Peptide Structure and Analysis

PS–04.04.01 THE STRUCTURE OF WATER AS ORGANIZED IN AN RGD PEPTIDE CRYSTAL: ENCAPSULATION OF A VALINE METHYL GROUP; COMMENTS ON "MELTING".

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As part of a structural study of a series of cyclic RGD peptides, one compound, cyclic[D-valyl-N^2-methyl-L-arginylglycyl-L-aspartyl-3-(aminomethyl)-benzoic acid], drew special attention because the asymmetric unit contained 12 water molecules per peptide. All of the waters are clustered in a channel which runs parallel to the a-axis. At 193K, the water molecules are rigidly held in a tight network of hydrogen bonds forming an "ordered" array containing 4-, 5-, 6- and 7-membered rings. All of the hydrogen atoms on the water molecules were found and refined. Projecting into the channel are Val methyl groups which are encapsulated with a series of rings in which the hydrogen-bonding requirements of every water molecule remain satisfied. Above 0°C, the crystal undergoes a phase transition in which some of the secondary waters appear to "melt".

PS–04.04.02 MOLECULAR DESIGN OF PEPTIDES: SYNTHESIS, MOLECULAR STRUCTURE AND ß-TURN II' FORMATION OF N-BOC-L-PHE-DEHYDRO-ABU-NH-CH_3 IN CRYSTALS

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The peptide N-Boc-L-Phe-dehydro-Abu-NH-CH_3 was synthesized by the usual workup procedure. The crystals grown from methanol at 4°C belong to the space group $P2_12_12_1$ with a = 7.589(2)Å, b = 13.690(4)Å, c = 21.897(6)Å, Z = 4, dc 1.149(5)gcm^{-3} for $C_{19}H_{29}N_3O_5.CH_3OH$. The peptide crystals were highly sensitive to radiation. The final agreement factor R was 0.055 for 1109 observed reflections ($I > 2\sigma$) with data extending to a 2θ value of 103°. The methanol oxygen atom is split into two occupancies. Both sites are involved in identical hydrogen bonding. As a result of substitution of a dehydro-Abu residue at (i+2) position the peptide adopts an ideal ß-turn II' conformation with torsion angles of corner residues as ϕ_1 63(1)°, ψ_1 = -127(1)°, ϕ_2 = -66(1)° and ψ_2 = -10(1)° and an intramolecular hydrogen bond N - H - - O of distance 3.01(1)Å. This shows that the conformational constraints produced by dehydro-Abu are similar in nature but different in magnitude than those produced by dehydro-Phe and dehydro-Leu. The methanol-peptide interactions show characteristic features of multiple hydrogen bond formations involving polar sites of participating peptide and methanol molecules. The packing of the molecules in the unit cell is stabilized by interactions through methanol molecules with the help of several hydrogen bonds.

PS–04.04.03 CRYSTAL STRUCTURE AND CONFORMATION OF THE TRIPEPTIDE L-LEUCYL-L-LEUCYL-L-LEUCINE.

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As part of our continuing studies on the relationship of sequences to folding preferences, we have been searching for sequences that will take up a folded conformation in the crystalline state. In this process, we have discovered earlier five tripeptide sequences GAF, GAV, GGV, GAL and GAI that exhibited helical conformation in the crystalline state (Ramasubbu, N. & Parthasarathy, R. (1989) *Biopolymers* **28**, 1259-1269; Parthasarathy, R., Chaturvedi, S. & Go, K. (1990), *Proc. Natl. Acad. Sci. U.S.A.* **87**, 871-875; Go, K., Chaturvedi, S. & Parthasarathy, R. (1992) *Biopolymers* **32**, 107-117). Leucine has a strong helical preference in proteins but short homooligo-L-leucines form β-sheet structures. In order to test the helical preference of short oligo-L-leucines, we crystallized the tripeptide L-Leucyl-L-Leucyl-L-Leucine (LLL) and carried out x-ray diffraction studies on it. (L-Leucyl-L-Leucyl-L-Leucine)$_2$. $3CH_3OH$. H_2O, ($C_{39}H_{84}N_6O_{12}$), crystallized in the monoclinic system, space group $P2_1$, cell parameters: a = 12.031(2), b = 15.578(3), c = 14.087(2) Å, α = 90°, ß = 97.29(1)°, γ = 90°, V = 2618.6 Å^3, M.W. = 829.1, Dc = 1.048 gcm^{-3}. Using a three circle diffractometer, 5332 unique reflections were measured to the end of the Cu-sphere ($\lambda_{CuK\alpha}$ =1.5418Å). We solved the structure by direct methods using the SHELXS program. The structure was refined to an R of 0.06 with 57 non-hydrogen atoms with anisotropic temperature factors and 65 hydrogen atoms with isotropic temperature factors for 3652 reflections > 3σ. Further refinement is in progress. The LLL molecules form anti-parallel β-sheet structure with hydrogen bonds between the N1 and N3 of molecule A to O3' and O1', respectively, of molecule B, and N1 and N3 of molecule B to O3' and O1', respectively, of molecule A. The molecules of LLL are not as extended as GGG (Srikrishnan, T., Winiewicz, N. & Parthasarathy, R. (1982) *Int. J. Pept. Prof. Res.* **19**, 103-113) but are less folded than AAA (Fawcett, K., Camerman, N. & Camerman, A. (1975) *Acta Cryst.* **B31**, 658-665). The three molecules of methanol and the water molecule form a network of hydrogen bonds to the LLL molecules and to one another. Adjacent molecules of A are connected by hydrogen bonds through two methanol molecules; N1A...O(MeA)...O(MeC)... O3'2A. Also, adjacent molecules of B are connected by hydrogen bonds through one methanol molecule; N1B...O(MeB)...O3'2B. The molecules A and B are connected through a water molecule; O3'2A...OW...O3'2B. It is interesting that though GGG, AAA and LLL form anti-parallel pleated β-sheet structures, GGG has no water molecule in the β-sheet, AAA has (1/2)H_2O per tripeptide and LLL has (1.5)CH_3OH and (1/2)H_2O per tripeptide. It is rather remarkable that though A and L have stronger helical preferences than G, neither AAA nor LLL form the crystalline helix but GAL does, indicating that the helical preferences depend on the sequence context. The side chain conformation (χ_1, χ_{12}, χ_{22}) of the three leucines (L1, L2 and L3 in LLL) in the two molecules are, respectively, (g^-, g^-, t) for L1, (t, g^+, t) for L2 and (g^-,g^-,t) for L3 of A, (t, g^+, t) for L1, (g^-, g^-, t) for L2 and (t, g^+, t) for L3 of B. For the side chain conformation χ_1, g^- is favored for forming helices. The residue L2 in molecule A and the residues L1 and L3 of molecule B do not show this preferred conformation for forming the helices. The (ω_1, ω_2) angles for the two molecules A and B are, respectively, (-178.8°, -175.3°) and (178.9°, -179.3°).