Short Communications

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Crystal structure from packing studies: application to the triclinic structure of the cyclic hexapeptide cyclo-(gly-tyr-gly)$_2$.*

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The paper describes a novel method of finding the position and orientation of a relatively rigid molecule in the unit cell from criteria concerning allowed contact distances between atoms. On application to the crystal structure of a hexapeptide, $C_{25}H_{31}N_6O_8.2H_2O$, it was possible to solve the structure from this starting point, by a series of SFLS refinements with an increasingly larger number of reflexions at successive stages. The packing analysis succeeded, even though the water molecules were not included to start with.

Introduction

The use of packing methods for the solution of the structure of fibrous proteins is well known, *e.g.* the α-helix (Pauling, Corey & Branson, 1951) and collagen (Ramachandran, 1967). The criteria for minimum contact distances were put forward by Ramachandran, Ramakrishnan & Sasisekharan (1963), particularly for amino acid and protein structures, and used by them for predicting ranges of the dihedral angles (φ, ψ) of two linked peptide units. It is reasonable to assume the criteria thus worked out for a pair of peptide units would work equally well for the packing of other peptides and related compounds in their crystal structure. Recently, the structure of the polypeptide $(Gly\text{-}Pro\text{-}Hyp)_n$ has been worked out in our laboratory with contact-criteria considerations and suitable computer programs (Bansal, Ramakrishnan & Ramachandran, 1975). Although a similar technique has been used by Kitaigorodsky (1973) and Williams (1969), unlike their analyses in which the energy is minimized, we have used simple contact-distance criteria to predict the probable trial structure.

Briefly, the packing analysis gave two or three possible trial structures, out of which the correct one was picked out by finding which refined. It may be mentioned that, for a molecule of this size occurring in a triclinic cell, the usual methods, such as Patterson or direct methods, would be difficult to apply.

Method

The unit-cell dimensions of the crystal, the space group, and the approximate shape of the molecule, are prerequisites. The parameters varied are the three Eulerian angles (φ, θ, ψ), designating the orientation of the molecule, and three translational parameters (X, Y, Z). In practice, we place one molecule of known shape and stereochemistry at a suitable position in the unit cell, and the translated and symmetry-related molecules are generated as required by the space group. The intermolecular contacts are calculated. The three Eulerian angles and the translational parameters are then varied in steps until the chosen molecule is free of short contacts with all its neighbours. If the molecule is stabilized by hydrogen bonds, the formation of good hydrogen bonds between neighbours is an additional requisite for choosing a trial model. These various steps can be programmed for a computer.

Application of the method to the cyclic hexapeptide

For the cyclic hexapeptide under consideration, the cell constants are

$a = 6{\cdot}270\ (2)$, $b = 8{\cdot}808\ (2)$, $c = 13{\cdot}350\ (2)$ Å,
$\alpha = 104{\cdot}18\ (1)$, $\beta = 97{\cdot}19\ (1)$, $\gamma = 98{\cdot}46\ (1)°$.
Space group $P1$, $Z = 1$.
Molecular formula: $C_{25}H_{31}N_6O_8.2H_2O$; M.W. 588.

The intensities of the 1972 observed reflexions, obtained with Cu $K\alpha$ radiation, were measured in an automatic four-circle diffractometer (there were 204 non-observed reflexions in the region surveyed).

The advantage of $P1$ is that the molecule has full translational degrees of freedom and any atom in the molecule can be chosen to be at the origin of the cell. There are only three degrees of rotational freedom, which may be represented by the Eulerian angles (φ, θ, ψ).

The conformation of this hexapeptide in solution is known from NMR studies (Kopple, Go, Logan & Savrda, 1972). A cyclized structure with dihedral angles close to those reported by Kopple *et al.* was worked out from theory. This molecule was put in the unit cell and its orientation varied by changing the Eulerian angles. At the start, it was not clear how many water molecules were present, because of the uncertainty in the measured density. Therefore, only the hexapeptide molecule was assumed to be present in the unit cell. The packing was checked by the contact criteria of Ramachandran *et al.* (1963), and those orientations were selected which had less than seven contacts (this number was chosen arbitrarily) beyond the 'extreme limits' for the corresponding pair of atoms. The method was successful

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Table 1. *R and shifts at different stages of refinement*

Stage	1*	2*	3*	4*	5*	6†	7‡	8§
Number of reflexions included	300	600	900	1200	all (2176)	all	all	all
Resolution	2 Å	1·5 Å	1·2 Å	0·9 Å	0·7 Å	0·7 Å	0·7 Å	0·7 Å
R Before L.S. refinement	0·540	0·441	0·484	0·504	0·440	0·294	0·130	0·081
R After 1 cycle	0·437	0·423	0·420	0·382	0·356	0·182	0·092	0·054
R After 2 cycles	0·402	0·400	0·394	0·360	0·300	0·131	0·081	0·051
Average shift in coordinates for the second cycle	0·6 Å	0·3 Å	0·10 Å	0·02 Å	0·01 Å	<0·01 Å	<0·01 Å	0·005 Å

* With hexapeptide molecule alone, and an increasing number of reflexions.
† Including the two water molecules as located in the difference synthesis.
‡ Including H atoms and isotropic temperature factors for non-hydrogen atoms.
§ With anisotropic temperature factors for non-hydrogen atoms.

and two regions which were practically free of bad contacts were detected (less than four such contacts, but none seriously bad).

Comparison with X-ray intensities and refinement of structure

The two possible orientations of the molecule were tested by comparing their calculated and observed structure factors. Both showed large R values of 0·594 and 0·613, when all 2176 reflexions were used. A preliminary attempt at least-squares refinement, with all the reflexions, did not show any reduction in R. However, when intensities going down to only 2 Å resolution were used (there were 300 reflexions of this type), one of the structures gave an R greater than 0·60, while the other gave a value of 0·54. Also, after two cycles of least-squares (L.S.) refinement, the former did not converge, while for the latter, R fell to near 0·40 (Table 1, stage 1). In view of this, the second structure was taken to be the one close to the correct structure. Further refinement of the structure was made with intensities of increasing ranges of $(\sin\theta)/\lambda$. The different steps are shown in Table 1. In this way, a structure with an R of 0·30 and with shifts in coordinates of only 0·01 Å per least-squares cycle was obtained.

When R had dropped to 0·30, a difference synthesis was calculated, which indicated the presence and the positions of two water molecules which had not been taken into account in the packing analysis. When these were added the structure continued to refine. Further refinement of the structure, with Hughes's (1941) weighting scheme and including H atoms, brought down R to 0·081 (Table 1). On inclusion of anisotropic temperature factors for the non-hydrogen atoms, R dropped to the final value of 0·051. The shifts of the atoms in the last cycle of refinement were less than $\frac{1}{20}$ of their standard deviations.

Details of the study and the discussion of the structure are expected to be published in the *Journal of the Indian Institute of Science*.

Conclusion

It is believed that the method adopted here could be used for molecules which are rigid, or which have only small regions which have large freedom of motion. The stepwise process of applying the least-squares refinement is likely to be useful, for example, to the refinement of protein structures, when larger amounts of data are used to obtain higher resolution.

The packing programme was written for an IBM 360/44 computer with 64 K memory. The full-matrix least-squares refinements *etc.* required for the closing stages were carried out on an IBM 370/155 with 512 K memory, available in Madras.

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