CHARACTERISATION OF PROTEIN-CHAIN-FOLDING USING PEPTIDE PLANE NORMALS

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THE general problem of representation of the structural data from X-ray analysis of protein crystals has recently been considered in this laboratory and some new methods have been proposed[1-2]. Broadly two types of representations have been proposed, namely, one-parameter and two-parameter representations which are aimed at extracting certain specific types of information about the chain folding. The basis of the new parameters itself is on the consideration of a set of four consecutive C^α atoms, C^α_{i-1}, C_i^α, C^α_{i+1}, C^α_{i+2} and defining angles involving the virtual bonds connecting the consecutive C^α atoms (such as the virtual bond-angle $C^\alpha_{i-1}-\hat{C}_i^\alpha-C^\alpha_{i+1} = \delta$ and the virtual-bond dehedral angle θ around the virtual bond $C_i^\alpha-C^\alpha_{i+1}$) and also defining a local helical axis of the segment $C^\alpha_{i-1}-C_i^\alpha-C^\alpha_{i+1}-C^\alpha_{j+2}$. It has been demonstrated that the use of these new parameters in different types of one-parameter representations (θ and δ-chain plots) and two-parameter representations such as η_{ij} plot, as well the two-dimensional representation such as stereographic projection lead to useful information on chain folding characteristics. It may be noted that in contrast to the above, the conventional two-parameter representation, namely, the (ϕ, ψ) diagram[3] deals with torsion angles around real bonds, namely $N-C^\alpha$ and $C^\alpha-C$ of a pair of peptide planes linked at a given C^α atom. The basis of the (ϕ, ψ) representation is the simple fact that the peptide units are planar* and any two successive planes take their relative orientation by virtue of the freedom of rotation along the single bonds involving the linking C^α atom. It would appear that the physical reality that the peptide unit, for all practical purposes, is planar and rigid could be exploited further to devise parameters which would lead to more such representations. Thus, one may define vectors which are normal to these planes and use these for the purposes of representations. The object of this communication is to point out that these vectors appropriately defined enable us to carry out many of the types of analyses discussed earlier. For instance the chain plots can be obtained using ν_i which is the angle between successive plane normals, say, $\boldsymbol{n}_{i-1}$ and $\boldsymbol{n}_i$**. So also a two-parameter representation, namely, ν_{ij} similar to η_{ij} is possible. Lastly the direction n_i can be used for a stereographic projection.

Chain-folding Characterisation Using Peptide-plane Normals:

Consider the polypeptide chain as in Fig. 1. For all practical purposes it is assumed that all the backbone atom C_i^α, C_i, O_i, N_{i+1}, H_{i+1} lie in a plane, whose unit normal is denoted by n_i. It is readily obtained by taking the cross-product of the vectors $\overrightarrow{C_i^\alpha - C_i}$ and $\overrightarrow{C_i - N_{i+1}}$ (Fig. 1) and normalising the resultant

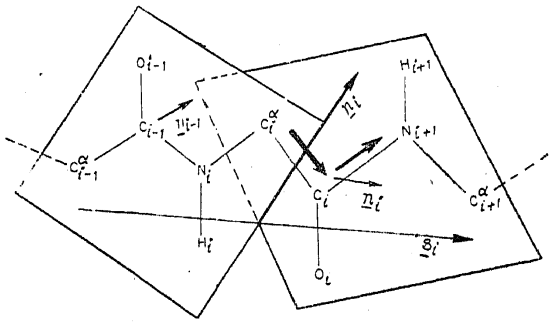

FIG. 1. Schematic diagram of a peptide chain showing vectors used for the characterisation of chain folding (*see* text for details).

vector. The dot product of two such unit vector $\boldsymbol{n}_{i-1}$ and $\boldsymbol{n}_i$ leads to the angle between the pair of peptide planes. Following our earlier conventions[2] this may be denoted by ν_i. It is interesting to note that this angle, being the angle between two planes, can be considered as a dehedral angle and hence in principle it should be possible to have the full range of $-180°$ to $+180°$. This is readily visualised by the fact that for any relative orientation of the two planes one can imagine an axis (which is actually along the direction of the line of intersection of the two planes) about which the two planes may be assumed to be rotated by the torsion angle. We may thus proceed to assign a sign to the angle ν by a suitable method as is done normally for torsion angles. Thus let $\boldsymbol{n}_{i-1} \times \boldsymbol{n}_i = \boldsymbol{r}_i$, so that $\boldsymbol{r}_i$ may be seen to lie along the line of intersection of the two planes. The vectors s_i defined by $\overrightarrow{C^\alpha_{i-1}-C^\alpha_{i+1}}$ may be taken as a reference direction. Thus for assigning a sign we may stipulate a rule based on comparison of the vector $\boldsymbol{r}_i$ and s_i. In the special case of $\boldsymbol{r}_i$ and s_i being collinear, one may take ν_i to be positive if $\boldsymbol{r}_i$ and s_i are parallel and negative if they are antiparallel. However since $\boldsymbol{r}_i$ and $\boldsymbol{s}_i$ need not always be collinear, one may find the angle between the vector $\boldsymbol{r}_i$ and s_i by taking dot product $s_i - s_i = \mu_i$.

* In actual cases the peptide units are slightly non-planar [*see*, for example, references (4) and (5)].

** Sasisekharan[6] has considered the angle between two peptide planes in a different context but has taken only the acute angle. It may be noted that, in principle, since we are dealing with the angle between two planes a full range of $-180°$ to $+180°$ can be realised which preserves the sense of folding similar to the angle parameter θ considered earlier[1,2].

The sign for ν_i is chosen positive or negative depending on the angle μ_i being acute or obtuse, respectively.

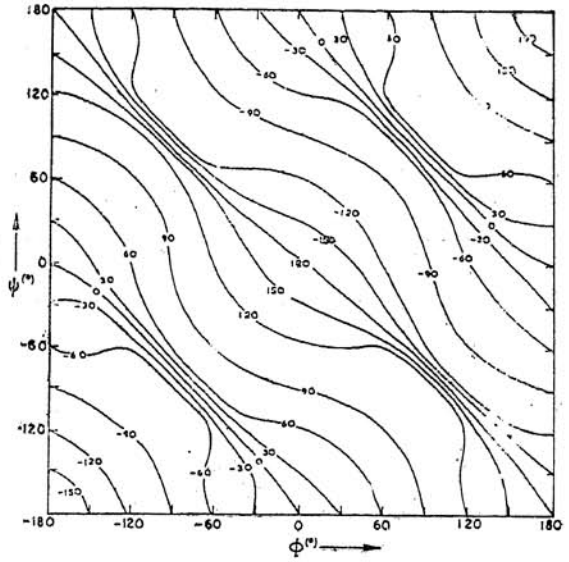

FIG. 2

FIG. 2. Equi ν_i-contours (*see* text for definition) in the (ϕ, ψ) plane.

Physically it may be seen that the above method of assigning the sign is equivalent to projecting the point C^{α}_{i-1} and C^{α}_{i+1} on to the line of intersection of the two planes as given by the vector r_i, and comparing the projected vector of $\overrightarrow{C^{\alpha}_{i-1}—C^{\alpha}_{i+1}}$ with r_i. While it might be possible to adopt for the above comparison any vector other than $\overrightarrow{C^{\alpha}_{i-1}—C^{\alpha}_{i+1}}$, we have chosen this here, since, as far as backbone atoms are concerned C^{α} atoms act as convenient reference points and also the progress of the chain is readily reckoned by tracing their path.

Figure 2 gives the equi-ν contours in the (ϕ, ψ) plane. The 'centrosymmetric' pattern of the contours is also readily understood. For example, the conformation $\phi = -120°$, $\psi = -60°$ and $\phi = +120°$, $\psi = +60°$ have ν values of $-60°$ and $+60°$ respectively.

A more detailed application of these ideas along the lines of our earlier analysis, including statisticaa. distribution of the angle ν_i is in progress and will be reported in due course.

1. Srinivasan, R and Balasubramanian, R., *Curr. Sci.*, 1975, **44**, 649.
2. —, — and Rajan, S. S., *J. Mol. Biol.*, 1975, **98**, 739.
3. Ramachandran, G. N. and Sasisekharan, V., *Adv. Protein Chem.*, 1968, **23**, 323.
4. Balasubramanian, R. and Ramakrishnan, C., *Int. J. Peptide Protein Res.*, 1972, **4**, 91.
5. Ramachandran, G. N. and Kolaskar, A. S., *Biochim. Biophys. Acta*, 1973, **303**, 385.
6. Sasisekharan, V., In: *The Jerusalem Symposia on Quantum Chemistry and Biochemistry* (Bergmann, E. D. and Pullman, B., Eds.), Jerusalem Academic Press, 1973, **5**, 36.