THEORY OF INTERMOLECULAR FORCES FOR THE STUDY OF PROTEIN CONFORMATION*,†

G. N. RAMACHANDRAN

Molecular Biophysics Unit, Indian Institute of Science, Bangalore-12, India

AND

Department of Biophysics, University of Chicago, Chicago, Ill. 60637, U.S.A.

1. Introduction

IT is generally accepted nowadays that the formulae for various types of intermolecular interaction deduced from physico-chemical studies on small molecules can be generalised for application to large biopolymers and proteins in a semi-empirical manner[1,2]. However, the results so obtained from model compounds related to the peptide unit are not always consistent, and further theoretical examination is needed to find out which of these are the most satisfactory ones for the case of a "dipeptide", composed of two connected peptide units. Recently, several persons have used quantum chemical calculations to obtain the energy map of a dipeptide[3,4]. However, when making the quantum chemical calculations, the individual contributions from different types of interactions, such as non-bonded attractive and repulsive forces, electrostatic forces, torsional potentials, and so on, are not obtained separately but only as an overall effect. Therefore, when the study is extended to larger peptides and proteins, the results obtained for the dipeptide model from quantum mechanics is not capable of being extended in a semi-empirical manner, but the calculations have to be carried out for the larger molecule completely in terms of quantum chemical considerations. It is well known that the current techniques of quantum chemistry have a limitation in the number of atoms that can be included in the calculations with reasonable computer times. Therefore, the problem of the conformation of a large peptide or protein, or cyclic peptides like those which occur in the case of antibiotics, have to be calculated by dissecting the problem into smaller dimensions accessible for ready calculation. However, this sort of dissection cannot be easily done in the quantum chemical technique, but is readily possible by using the semi-empirical technique mentioned earlier. Therefore, it becomes necessary to find out which of the functions normally adopted in the semi-empirical technique are accurate and whether any of them needs any modification. It is therefore suggested that a careful study should be made of the degree of validity of the various functions used for the calculations of conformational energies.

2. Nonbonded Forces

The semi-empirical functions, adopted by Ramachandran and Sasisekharan[1] for nonbonded attractive and repulsive potential, have been applied to various problems in our laboratory and have been found to fit the observed facts reasonably well. These functions are not very different from those adopted by other workers in the field. However, it will be worthwhile examining whether these potentials give the correct atomic positions of a crystal structure if they are applied to such a problem. Therefore, the functions were tested in the case of the crystal structure of benzene and the minimum energy conformation found is shown in Table I in relation to the actual structure observed. A similar test in another simple example, namely, that of sulphur dioxide, is also shown in Table I. In both these cases, the electrostatic interactions, if any, were also taken into account, and the agreement between theory and observation was satisfactory. However, more examples will have to be tested before confirming the essential correctness of the potential functions adopted in our laboratory. It is, however, interesting that some of the functions adopted by Professor Kitaigorodsky have an appreciably larger value for the interatomic distances at which the energy is minimum, although

TABLE I

Comparison between theory of packing and observation in two crystal structures

Structure	Parameters	Theory	Observed
Benzene (Orthorhombic)	Eulerian angles (ϕ Ψ, θ)	$\phi = 106°$ $\Psi = 2°$ $\theta = 47°$	$\phi = 104{\cdot}4°$ $\Psi = 2{\cdot}3°$ $\theta = 46{\cdot}6°$
Sulphur dioxide (Orthorhombic)	Angle θ between plane of SO_2 and the (ac) plane	$\theta = 48°$	$\theta = 47{\cdot}2°$

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they have been applied and found to work in the prediction of a number of crystal structures[5]. A comparison is shown in Figs. 1 (*a*), (*b*) and (*c*).

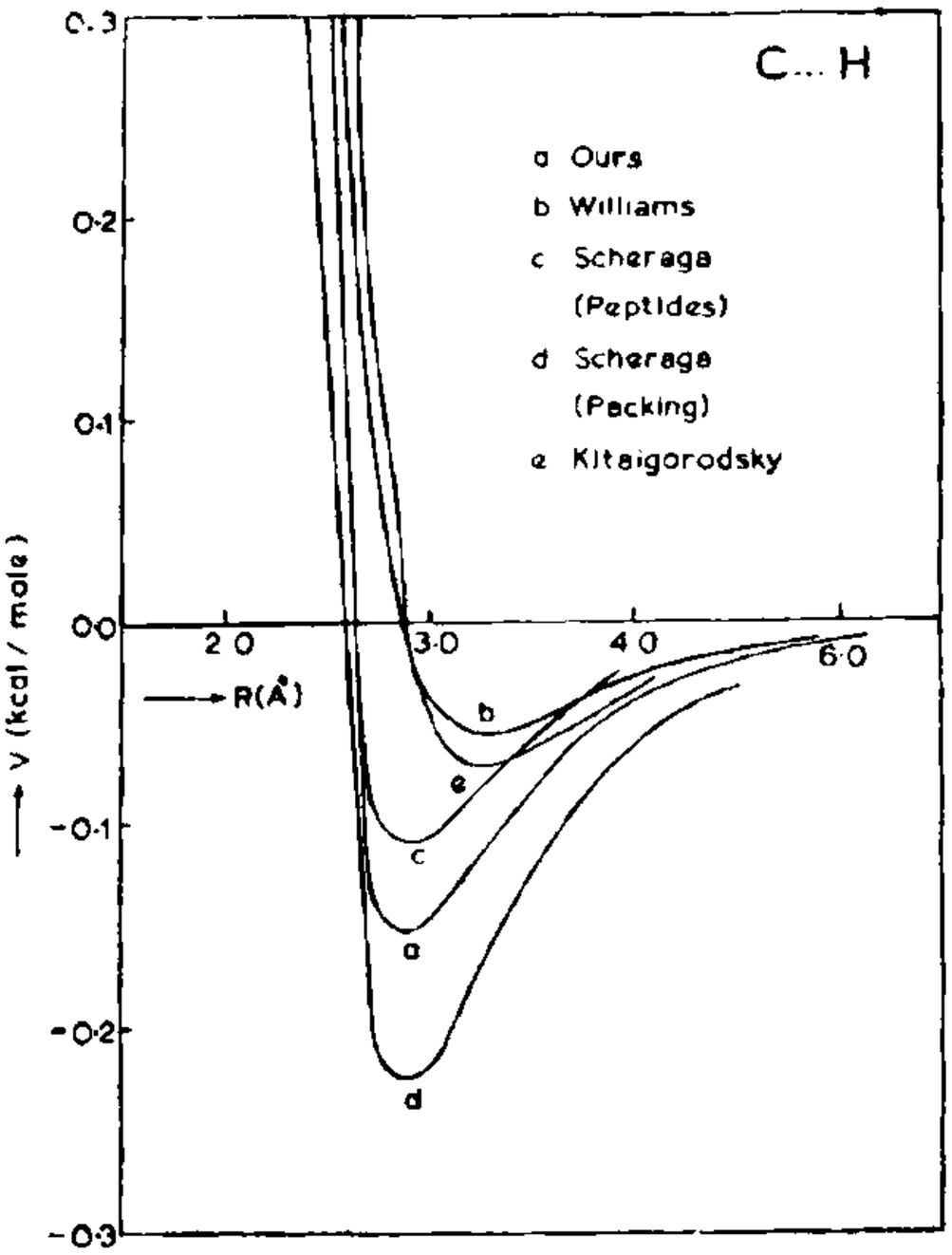

FIG. 1 (*a*)

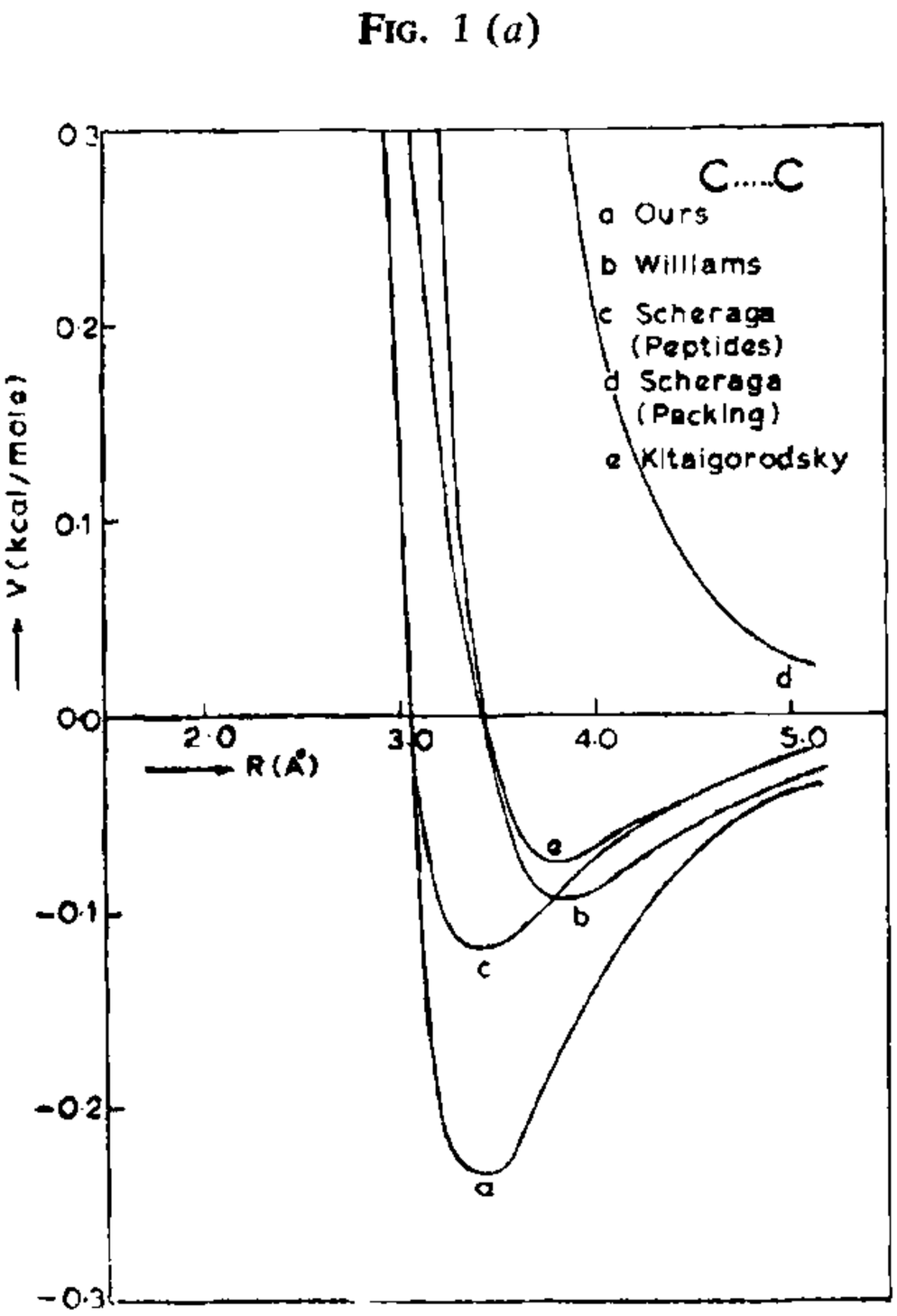

FIG. 1 (*b*)

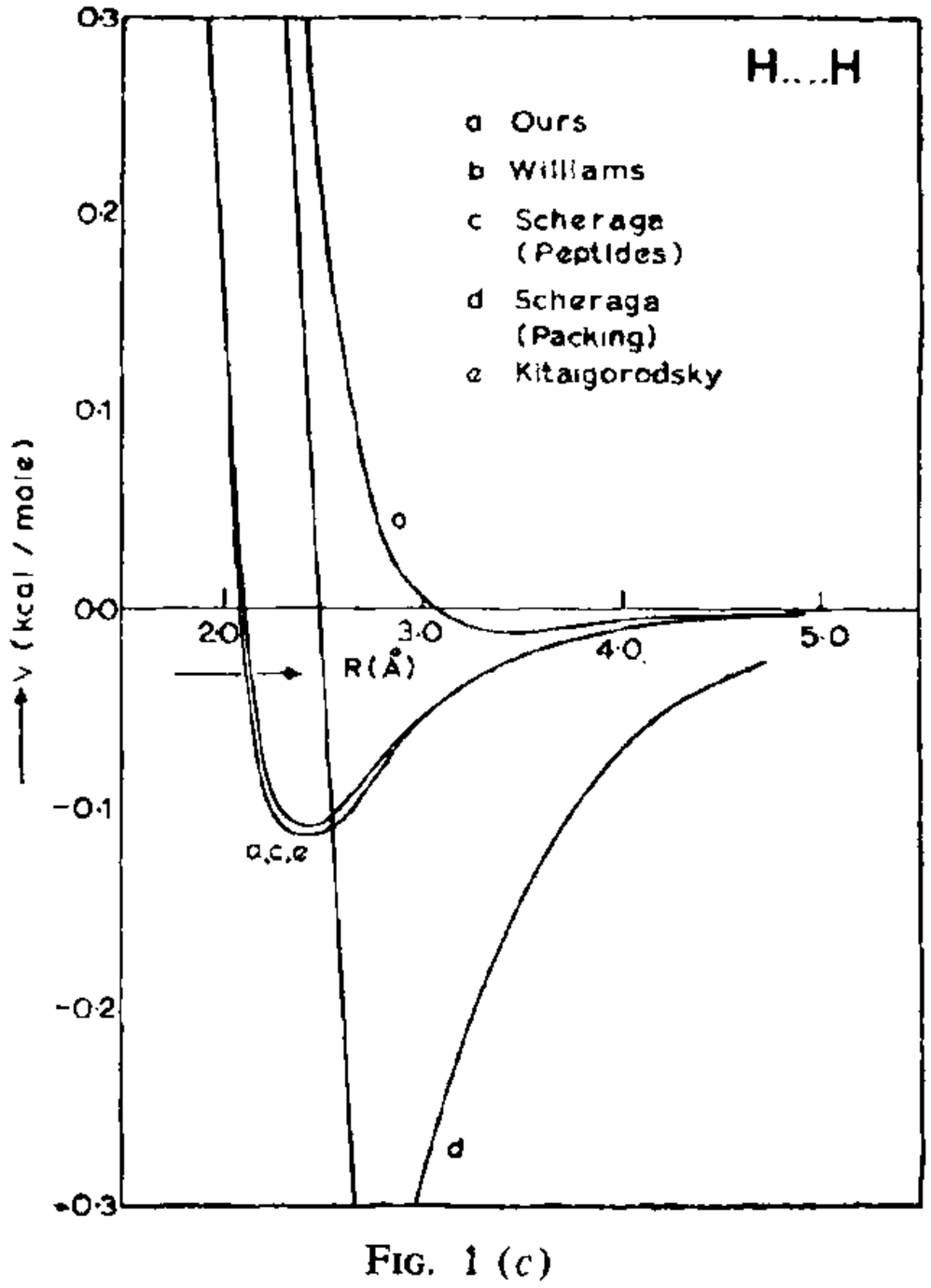

FIG. 1 (*c*)

FIG. 1. Variation of nonbonded potential energy with distance for three examples, taken from different sources. It will be seen that there is appreciable variation between different authors.

Therefore a more critical test of the nonbonded potential function in well-established examples is necessary in order to obtain the best functions. In this connection, attention may be drawn to the studies made in Professor Scheraga's laboratory[6] for deriving potential functions by taking into account the observed data in a number of crystal structures. It is understood that these are being refined at present (personal communication).

As regards the electrostatic potential, the charges involved in the problem are generally capable of being calculated from simple quantum chemical considerations, for the participating groups in the total structure. Thus, the only unknown part is the effective dielectric constant (ϵ'), which is different from the true dielectric constant (ϵ), of the medium. Some calculations made in our laboratory[7] indicate that a value between 3 and 5 for ϵ' is satisfactory for protein solutions in water. This agrees with the general practice in the field.

3. TORSIONAL POTENTIALS

In a recent paper submitted to the Jerusalem symposium[8] held in April 1972, it was pointed out that the torsional potential for the dihedral angle

ϕ has been taken to have two different forms by different workers

$$V(\phi) = \frac{V_0}{2}(1 + \cos 3\phi) \quad (1)$$

and

$$V(\phi) = \frac{V_0'}{2}(1 - \cos 3\phi) \quad (2)$$

By a comparison with the observed NMR coupling constant J(NH-C$^\alpha$H) with those calculated using both these potential functions, it was shown in that paper that equation (1) for $V(\phi)$ is the one that fits best with the observed data, leading to minima for potential energy at $\phi = 60°$, 180° and 300°.

As regards the potential function $V(\psi)$, it is normally supposed that this has three-fold minima, while the observation of the distribution of observed conformations in proteins seems to indicate that the function has probably two-fold minima at 0° and 180°. We are not basing this on any theoretical considerations, but rather on the fact that a large number of conformations (ϕ, ψ) in various proteins occur close to $\psi \sim 0°$ and $\psi \sim 180°$, values for which the torsional potential $V(\psi)$ is expected to be a minimum for the individual dipeptide interactions in the two-fold potential.

4. Hydrogen Bond Potential Function

This raises a very important question namely, that it should be possible to derive an empirical potential function by examining the *distribution* of the observed conformations with regard to the parameters involved in the particular interaction. Thus we postulate that if $P(\alpha)$ is the probability distribution of a particular parameter, then

$$P(\alpha) = Ke^{-V(\alpha)/RT}. \quad (3)$$

Although an assumption of this type is not completely provable, this type of relation between $P(\alpha)$ and $V(\alpha)$ is to be expected when the other interactions are somewhat independent of the interaction determined by the parameter α. This approach was used by us for determining the hydrogen bond potential function. On examining the observed distribution of hydrogen bonds NH...O in peptides in relation to the two parameters, hydrogen bond length R, and angle θ (Fig. 2), it

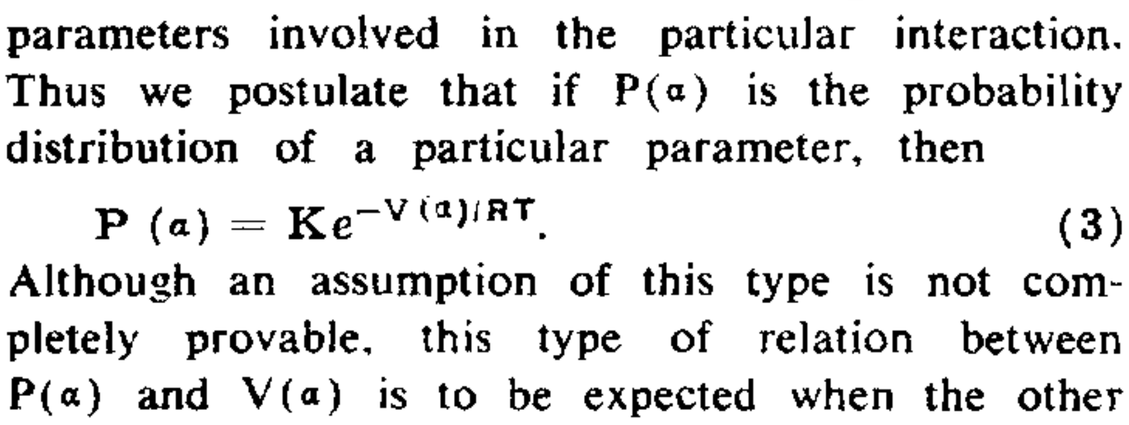

Fig. 2. Definition of the hydrogen bond length R and angle θ.

was found that the distribution could be described by an equation of the type,

$$V_{hb} = V_{min} + p_1\Delta^2 + q_1 e^{p_2\Delta\theta^2}. \quad (4a)$$

The best values of the parameters, as obtained by trial and error, were, for Δ in A,

$$V_{min} = 4{\cdot}5 \text{ kcal/mole},$$
$$p_1 = 25,\ p_2 = -2$$
$$q_1 = 0{\cdot}001. \quad (4b)$$

This potential function has been tested in the case of the alpha-helix. Using the various interactions already mentioned and also the hyrogen bond interaction as given by the equation 4(a) and 4(b), the energy contours of the alpha-helix were drawn in the (ϕ, ψ) plane (Fig. 3). The absolute minimum

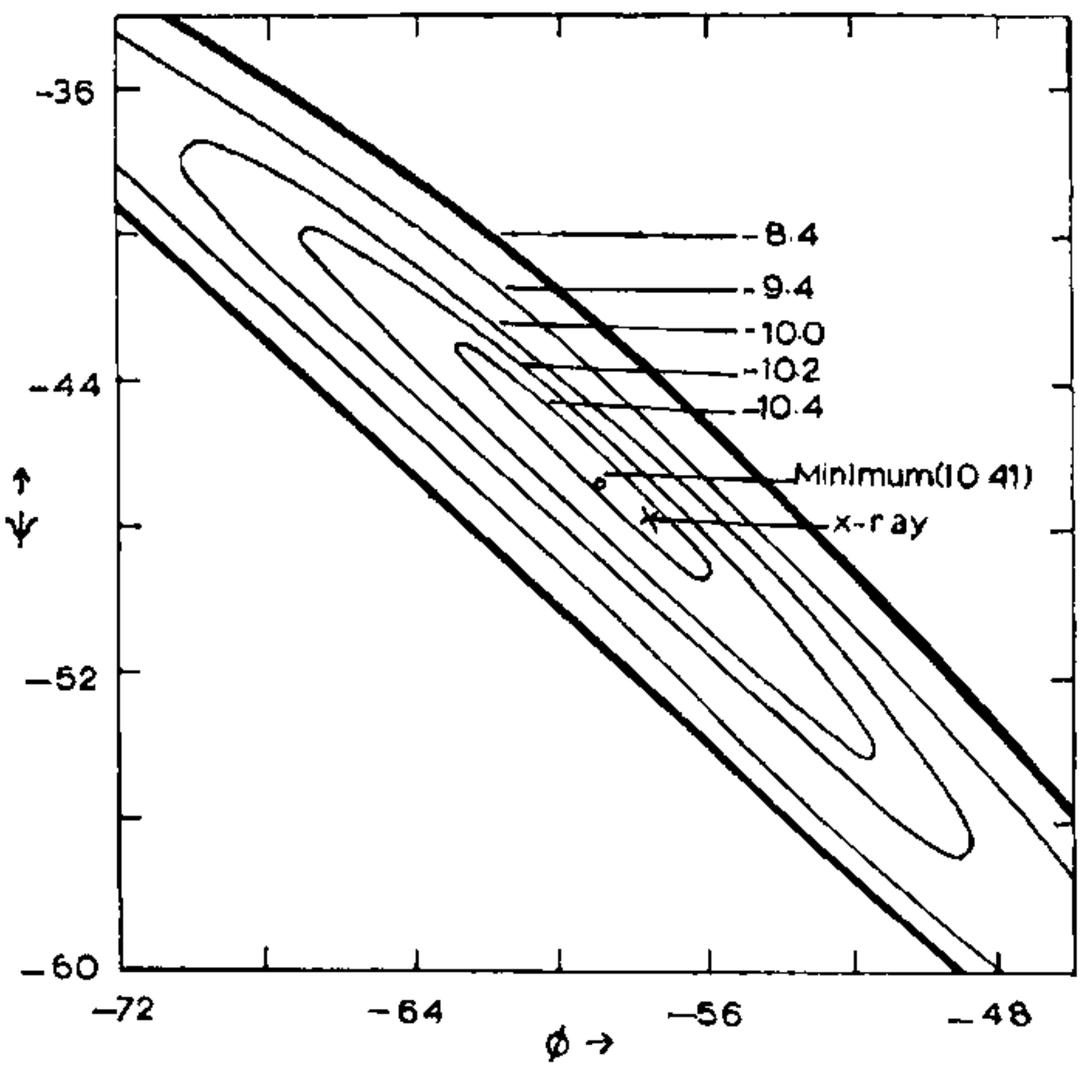

Fig. 3. Energy contours of alpha-helix in the (ϕ, ψ) plane, including hydrogen bond interaction. Note that the theoretical minimum is close to the observed minimum.

thus obtained agreed[9] to less than 2 with the observed conformation as deduced by Arnott and coworkers[10]. This clearly indicates that the various potential functions adopted for this purpose are reasonably satisfactory.

5. Potential Function Depending on the Angle Between the Normals of Two Neighbouring Peptide Units

Recently some studies made by Dr. V. Sasisekharan in our laboratory have indicated the possible existence of an interaction arising out of the interplanar angle between two neighbouring peptide units. Figure 4 shows a (ϕ, ψ) plot with contours drawn for various values of the angle θ between two neighbouring peptides. It will be seen from the distribution of the observed angle in various proteins that this angle is near 90°, in a number

of cases. In other words, the two neighbouring peptide units have a tendency to be at right angles to each other. This may probably be due to the interaction of the π-electrons in the two peptide units. Obviously this requires further careful study.

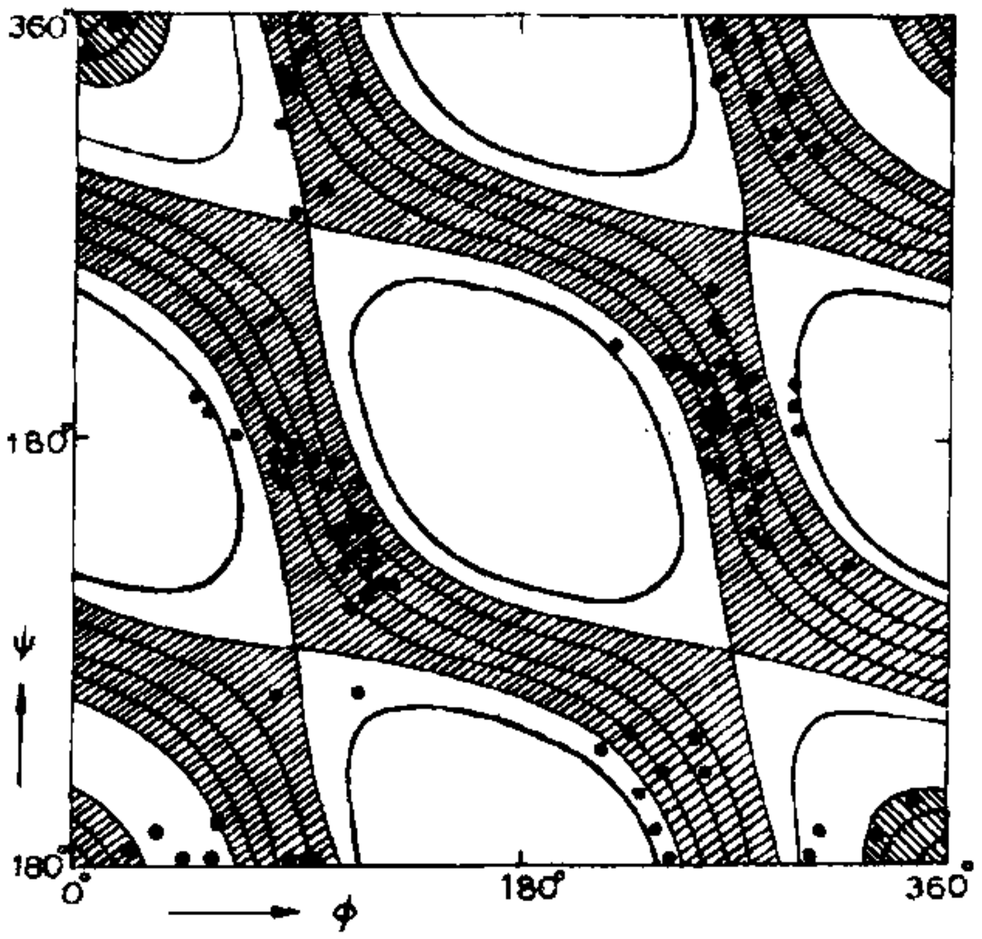

FIG. 4. Distribution of conformations in the (ϕ, ψ) map with regard to the parameter θ, namely, the interplanar angle between two neighbouring peptide units. The shaded region corresponds to $\theta = 90° \pm 20°$. Note the large concentration of points in this region.

Another factor which also requires study is the possible nonplanarity of the peptide unit. Winkler and Dunitz[11] have recently reviewed the nonplanarity of peptide units in cyclic peptides and showed that such a situation is quite common in cyclic compounds having peptide units. From the known fact that there is appreciable nonplanarity at the nitrogen atom in analine[12], there is good reason for the hydrogen atom in the NH group of a peptide unit being out of plane with the rest of the atoms. A preliminary quantum chemical calculation made in our laboratory indicates that such nonplanarity is quite likely to occur. However, this also requires careful examination.

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THE HALF-LIFE OF ^{155}Eu

B. N. SUBBA RAO
Tata Institute of Fundamental Research, Bombay-5, India

A FOURTEEN year old source of ^{155}Eu had larger activity, as determined in terms of the 105 keV γ-ray intensity, than it should have had if its half-life were $1{\cdot}811 \pm 0{\cdot}002$ yrs[1] as it has been believed to be hitherto. These earlier values in the range of 1·70 to 2·0 yrs were based on a few months decay curve. Herein, it is found to be $4{\cdot}53 \pm 0{\cdot}14$ yrs from the relative γ-ray intensities of the same source at different times during these fourteen years.

Figure 1 shows the decay curves with (I) 28·8 mgm/cm^2 Al absorber and (II) 521 mgm/cm^2 Al absorber recorded for six months from January 1958 through July 1958, using an end-window G.M. Counter. These curves were meant mainly to verify the existence of the 15-day ^{156}Eu and longer lived ^{155}Eu, produced in the neutron capture of enriched ^{154}Sm as Sm_2O_3, as supplied by the Oak Ridge National Laboratory and to prove that it was not ^{59}Fe as it had turned out to be in the 1957 supply of the sample under the label of ^{155}Eu. Curve II clearly follows a half-life of $15{\cdot}1 \pm 0{\cdot}9$ days (^{156}Eu). Curve I has this 15-day component and a residual component