

# THE NEED FOR A MODIFIED PSI-POTENTIAL IN THE DIPEPTIDE MODEL†‡

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## ABSTRACT

The need for modifying the potential function  $V(\psi)$  corresponding to the rotation about the bond  $C^\alpha-C$  in peptides and polypeptides, from three-fold minima and a low barrier, to  $V(\psi) = 2.0 (1 - \cos 2\psi)$  is stressed. Taking the new expression for  $V(\psi)$  it is shown that the theory for a pair of peptides is in good agreement with the conformational data obtained from the crystal structures of peptides and proteins. Applications of the new form of  $V(\psi)$  to LL and LD bends in peptides and proteins also improve the agreement between theory and observation.

## INTRODUCTION

IN connection with the calculations of the energy of a dipeptide unit corresponding to the conformation  $(\phi, \psi)$ , suitable potential functions for various types of interactions have been used by different laboratories. Although differing in minor details, all of them are essentially similar<sup>1-4</sup>. Considering in particular, the potential functions associated with the torsion angles  $\phi$  and  $\psi$ , the functions in common use have the following forms:

$$V(\phi) = \frac{1}{2} V_\phi (1 \pm \cos 3\phi) \quad (1)$$

with  $V_\phi$  varying between 0.6 and 1.5 kcal/mole; and

$$V(\psi) = \frac{1}{2} V_\psi (1 - \cos 3\psi) \quad (2)$$

with  $V(\psi)$  having values between 0.25 and 1.0 kcal/mole.

As a justification for using a low barrier for  $V(\psi)$ , it is stated that the barrier to the internal rotation about the  $C^\alpha-C$  bond in compounds of the type  $CH_3C(X)O$  ( $X=H, F, Cl, Br, N, OH$ ) is found to be very small and having three-fold minima<sup>5</sup>. However, for the rotation  $\psi$  about the bond  $C^\alpha-C$  in the peptide group, in which the nitrogen atom is attached to the C-atom on one side and to the  $C^\alpha$ -atom on the other side, as shown in Fig. 1, the nature and height of the barrier could be different. A recent analysis of the distribution of observed conformations in small peptides and in the non-helical regions of proteins indicated that the barrier to rotation about the bond  $C^\alpha-C$  is not three-fold<sup>6</sup>. These observed conformations are found to cluster within  $\pm 30^\circ$  around

$\psi \approx 0^\circ$  and  $\psi \approx 180^\circ$ , corresponding approximately to the *trans* and *cis* positions of the nitrogen atoms, as shown in Fig. 2<sup>6</sup>. Infrared studies of amides made by Shimanouchi<sup>7</sup> also indicated that the stable conformations that occur corresponded to either  $\psi \approx 0^\circ$ , or  $180^\circ$ . These observations suggest that the potential  $V(\psi)$ , for rotation about the bond  $C^\alpha-C$ , can be of the form,

$$V(\psi) = \frac{1}{2} V_\psi (1 - \cos 2\psi) \quad (3)$$

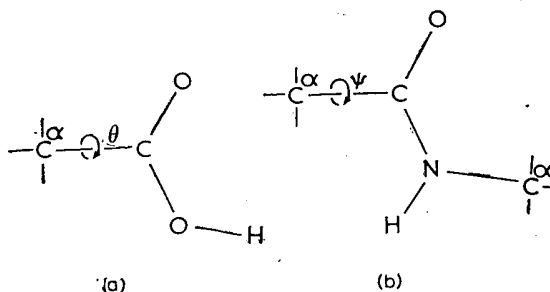


FIG. 1. Diagrams illustrating the differences in geometry related to the rotation about the bond  $C^\alpha-C$ , in the case of (a) a carboxylic acid and (b) a peptide.

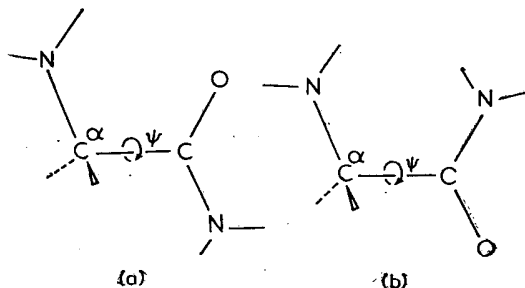


FIG. 2. (a) and (b) show respectively, the *trans* and *cis* positions of the nitrogen atoms in a dipeptide.

In view of this, preliminary quantum chemical calculations, using an *ab-initio* method, were carried out by us sometime ago on a model compound  $NH_2CH_2CONH_2$ . For  $\phi = 90^\circ$ , the total energy

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was found to be more by as much as 6.0 kcal/mole for  $\psi$  near  $90^\circ$  as compared to the energy at  $\psi = 0^\circ$  or  $180^\circ$ . Another set of calculations using IEHT method developed by Rein *et al.*<sup>8</sup> and using the Cusach approximation, on the same model compound, indicated that the total energy variation with respect to  $\psi$  has minima at  $\psi = 0^\circ$  and  $\psi = 180^\circ$  with a maximum at  $\psi = 90^\circ$ . The total energy difference between maxima and minima was found to be of the order of 4.5 kcal/mole. Recent calculations of Pople and Radom<sup>9</sup>, for the same model compound using an *ab-initio* procedure, have indicated minima at  $\psi = 0^\circ$  and  $\psi = 180^\circ$ , for  $\phi = 90^\circ$  (since the calculations have been done only at intervals of  $60^\circ$ , no precise value for maxima, and the difference between maximum and minimum energy, could be obtained). In performing these calculations, the total energy was first minimized with respect to  $\phi$ , and then with respect to  $\psi$ . Therefore, the total energy variation with respect to  $\psi$  which gives minima at  $\psi = 0^\circ$  and  $\psi = 180^\circ$  is indicative of the fact that the form of the internal rotational potential function  $V(\psi)$  around C—C bond has two-fold minima. These quantum-chemical calculations also suggest that the barrier to the internal rotation about C $^\alpha$ —C bond has a large value compared to the currently accepted value of 1.0 kcal/mole or less. Though the quantum-chemical calculations give the total energy difference, the contributions from other interactions in this range is expected to vary little and therefore, the barrier to internal rotation about the bond C $^\alpha$ —C is taken to be as large as 4.0 kcal/mole.

#### APPLICATION OF THE MODIFIED $\psi$ -POTENTIAL

The potential energy variation, for a pair of linked peptide units having a C $^\beta$ -atom, using the 6-exp non-bonded potential function, the electrostatic interaction energy as given in Ref. 1 and the torsional potential  $V(\psi)$  as

$$V(\psi) = 2.0(1 - \cos 2\psi) \quad (4)$$

is shown in Fig. 3 (b). In view of our recent quantum chemical calculations made on simple amides which indicated that the barrier height to the internal rotation around the N—C $^\alpha$  bond is very small, we have neglected the term  $V(\phi)$ . The hydrogen bond energy contribution is also not included. The convention used for drawing the potential energy maps is as suggested by IUPAC-IUB Commission<sup>11</sup>, and the dihedral angles ( $\phi, \psi$ ) are defined as given in their recommendation. By making the only change

$$V(\psi) = 0.25(1 - \cos 3\psi) \quad (5)$$

the variation in energy is shown in Fig. 3 (a). The main difference between Fig. 3 (a) and Fig. 3 (b) is that the region between  $\psi = 60^\circ$  and  $120^\circ$  is

practically forbidden for all conformations of an alanine dipeptide unit according to the new  $\psi$ -potential, whereas it is not so with the old potential. This is because of the fact that the  $\psi$ -potential rises by 4.0 kcal/mole for  $\psi = 90^\circ$ .

In Fig. 3 (b) are also plotted the ( $\phi, \psi$ ) values for non-glycyl linear oligo-peptides (tri-, tetra- and hexa-peptides) as well as cyclic peptides. These points lie in the low energy regions of the map. The low energy regions shown in Fig. 3 (b) thus become the allowed conformations for a dipeptide. In a polypeptide chain, though some of the low energy regions of a pair of peptide unit may become disallowed, it is obvious that the disallowed conformations for dipeptide unit will not be a low energy conformation for a polypeptide chain. Thus, if a comparison of Fig. 3 (a) or 3 (b) with the empirical plot obtained from the protein data of Pohl<sup>10</sup>, and shown in Fig. 3 (c) (to facilitate ready comparison) is made, much better agreement is obtained for Fig. 3 (b) than for Fig. 3 (a). However, while making the comparison of Fig. 3 (c) with either Fig. 3 (a) or 3 (b), it should be remembered that the  $\alpha$ -helical type of conformations near ( $-60^\circ, -60^\circ$ ) and the  $\beta$ -structure conformations have been included in Pohl's diagram, although they are not particularly favoured for a dipeptide conformation. This explains the essential difference between Fig. 3 (b) and Fig. 3 (c).

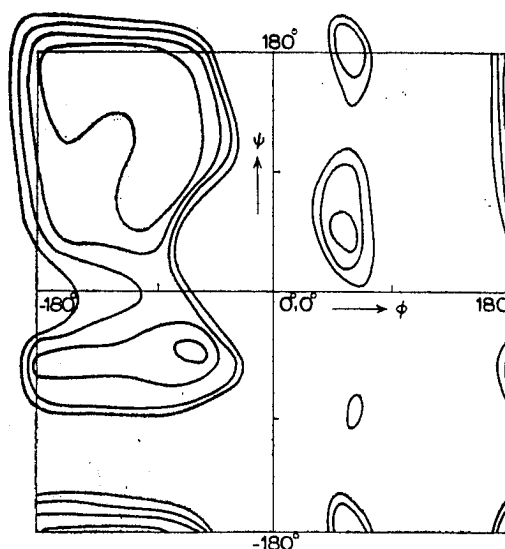


Fig. 3 (a). Isoenergy curves for a pair of peptide units linked at C $^\alpha$  ( $V_{tot} = V_{nb} + V_{cs} + V(\psi)$ ) at intervals of 1.0 kcal/mole, for an alanyl dipeptide, with  $V(\psi)$  having three-fold minima at  $-180^\circ$ ,  $-60^\circ$  and  $+60^\circ$  and a small barrier of 0.5 kcal/mole.

In all our calculations, we have considered the geometry for the peptide unit having  $\tau(C^\alpha-C-O) = 115.6^\circ$  and  $\tau(N-C_2^\alpha-C) = 112.5^\circ$ .

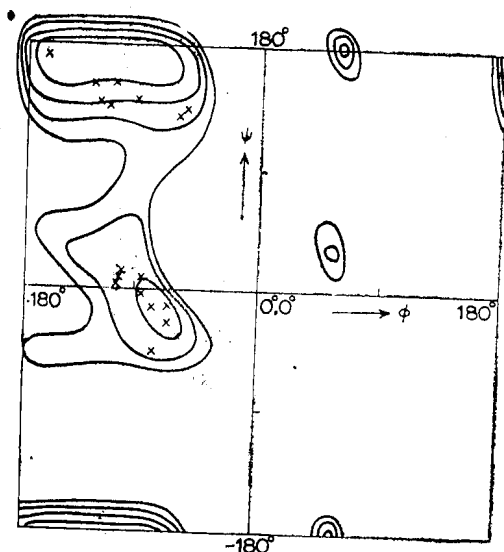


FIG. 3 (b). A similar diagram for an alanyl dipeptide unit, but with  $V(\psi)$  having two-fold minima at  $\psi = 0^\circ$  and  $180^\circ$  and a barrier of 4.0 kcal/mole in between them at  $\psi = 90^\circ$ . The values of  $(\phi, \psi)$  as obtained from crystal structure data of small peptides for non-glycyl residues are shown by cross (x).

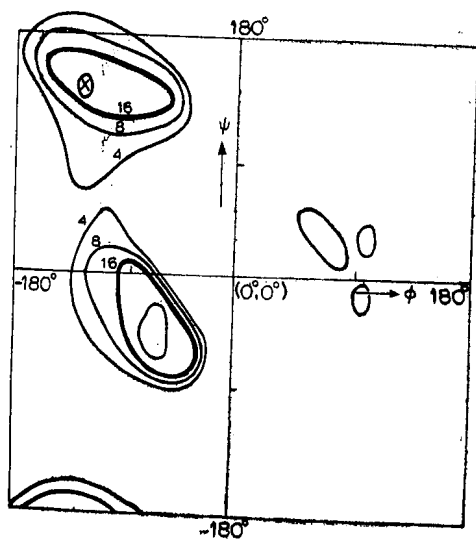


FIG. 3. (c) Isoprobability curves in the  $(\phi, \psi)$ -plane, reported by Pohl<sup>10</sup> (including glycyl examples). The curves at intervals of factors of 2. Note the similarity between Figs. (b) and (c).

The energy for a glycyl dipeptide unit, using the new potential function for  $V(\psi)$  is shown in Fig. 3 (d). In Fig. 3 (d) are also plotted the

$(\phi, \psi)$  values for linear oligo-peptides and cyclic peptides for glycyl residues along with the  $(\phi, \psi)$  values for glycyl residues in the non-helical regions of lysozyme, myoglobin and chymotrypsin as obtained from crystal structure data. The distribution of points clearly shows that the energy map drawn by using the new potential function for  $V(\psi)$  is in much better agreement with the observed data than the energy maps reported so far. Similarly, if the isoenergy contours of Fig. 3 (d) are compared with the iso-probability curves given by Pohl<sup>10</sup> for glycyl residues (not shown here), as obtained from protein crystal data, the agreement is found to be excellent.

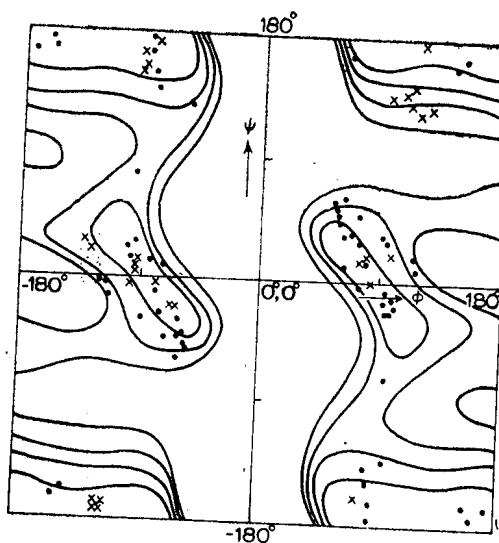


FIG. 3 (d). Diagram similar to (b), but for a glycyl dipeptide unit, using  $V(\psi)$  having two-fold minima and a barrier of 4.0 kcal/mole. The values of  $(\phi, \psi)$  for glycyl residues only, in peptides and proteins are plotted. x—denotes data from peptides; ●—denotes data from lysozyme, chymotrypsin and myoglobin.

#### APPLICATION TO BETA-BENDS

The theory of these bends was published by Chandrasekaran *et al.*<sup>13</sup>. This theory predicted minima for an LL bend for values of  $(\phi_2, \psi_2)$ ;  $(\phi_3, \psi_3)$  close to  $(-50^\circ, -50^\circ)$ ;  $(-110^\circ, 40^\circ)$  and to  $(-60^\circ, 100^\circ)$ ;  $(60^\circ, 40^\circ)$ . Thus,  $|\psi_3|$  was always greater than  $30^\circ$ . However Table II and Table III of Ref. 13 show that, for most of the observed conformation, the value of  $|\psi_3|$  lies between  $0^\circ$  and  $20^\circ$ . The mean of  $|\psi_3|$  for observed data as obtained from these tables of Ref. 13 in lysozyme and chymotrypsin is  $14^\circ$  and for small peptides is  $9^\circ$ , thus leading to a discrepancy between theory and observation. When the calculations were repeated using the new potential

$V(\psi)$ , the minimum energy conformations of the LL bend come close to ( $-60^\circ$ ,  $-30^\circ$ ), ( $-90^\circ$ ,  $20^\circ$ ) and ( $-60^\circ$ ,  $140^\circ$ ); ( $60^\circ$ ,  $10^\circ$ ), agreeing well with the observations.

Thus, the data presented in this note indicate that a form of the  $\psi$ -potential with two-fold minima and a relatively high barrier of 4.0 kcal/mole is the one that may have to be adopted for the classical energy calculations.

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1. Ramachandran, G. N. and Sasisekharan, V., *Adv. Protein Chem.*, 1968, 23, 283.
2. Maigret, B., Pullman, B. and Dreyfus, N., *J. Theor. Biol.*, 1970, 26, 321.
3. Brant, D. A. and Flory, P. J., *J. Am. Chem. Soc.*, 1965, 87, 2791.
4. Scheraga, H. A., *Adv. Phys. Org. Chem.*, 1968, 6, 103.
5. Dale, J., *Tetrahedron*, 1966, 22, 3373.
6. Sasisekharan, V., "Conformation of biological molecules and polymers" (Ed. Bergmann, E. D. and Pullman, B.), *The Jerusalem Symposium on Quantum Chemistry and Biochemistry*, 1973, 5, 36.
7. Shimanouchi, T., *Discuss. Far. Soc.*, 1970, 49, 60.
8. Rein, R., Clarke, G. A. and Harris, F. E., "Quantum aspects of heterocyclic compounds in chemistry and biochemistry" (Ed. Bergmann, E. D. and Pullman, B.), *The Jerusalem Symposium on Chemistry and Biochemistry*, 1970, 3, 86.
9. Pople, J. A. and Radom, L., "Conformation of biological molecules and polymers" (Ed. Bergmann, E. D. and Pullman, B.), *The Jerusalem Symposium on Quantum Chemistry and Biochemistry*, 1973, 5, 747.
10. Pohl, F. M., *Nature New Biol.*, 1971, 234, 277.
11. IUPAC-IUB "Commission of Biochemical Nomenclature," *J. Mol. Biol.*, 1970, 52, 1.
12. Ramachandran, G. N., Kolaskar, A. S., Ramakrishnan, C. and Sasisekharan, V., *Biochim. Biophys. Acta*, 1974, 359, 298.
13. Chandrasekaran, R., Lakshminarayanan, A. V., Pandya, U. V. and Ramachandran, G. N., *Ibid.*, 1973, 303, 14.