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EMPIRICAL TORSIONAL POTENTIAL FUNCTIONS FROM PROTEIN STRUCTURE DATA

Phi- and Psi-Potentials for Non-glycyl Amino Acid Residues

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The torsional potential functions $V_t(\phi)$ and $V_t(\psi)$ around single bonds $N-C^\alpha$ and $C^\alpha-C$, which can be used in conformational studies of oligopeptides, polypeptides and proteins, have been derived, using crystal structure data of 22 globular proteins, fitting the observed distribution in the (ϕ, ψ)-plane with the value of $V_{tot}(\phi, \psi)$, using the Boltzmann distribution. The averaged torsional potential functions, obtained from various amino acid residues in L-configuration, are $V_t(\phi) = -1.0 \cos(\phi + 60^\circ)$; $V_t(\psi) = -0.5 \cos(\psi + 60^\circ) - 1.0 \cos(2\psi + 30^\circ) - 0.5 \cos(3\psi + 30^\circ)$.

The dipeptide energy maps $V_{tot}(\phi, \psi)$ obtained using these functions, instead of the normally accepted torsional functions, were found to explain various observations, such as the absence of the left-handed alpha helix and the C_7 conformation, and the relatively high density of points near the line $\psi = 0^\circ$. These functions, derived from observational data on protein structures, will, it is hoped, explain various previously unexplained facts in polypeptide conformation.

Key words: torsional potential function from protein data; protein data analysis; PHI and PSI potentials in peptides.

A primary goal of theoretical, as well as experimental, studies on the conformation of biopolymers in general, and polypeptides in particular, is to make an accurate theoretical prediction of the tertiary structure of proteins, knowing only the primary structure and molecular environment. This problem of protein folding has been the subject of study by a number of groups in recent years (Pititsyn & Finkelstein, 1970; Wu & Kabat, 1973; Anfinsen & Scheraga, 1975; Levitt & Warshel, 1975; Kuntz *et al.*, 1976; Robson & Pain, 1976). The problem of protein folding also involves several other complex problems, the major one being the calculation of conformational energy of a protein as a function of its internal coordinates. At the outset, accurate potential energy functions are necessary to calculate correctly the total potential energy of the molecule.

At present, there is no unique set of potential functions which can predict correctly the observed conformations even at a dipeptide level. In fact, none of the dipeptide energy maps obtained using either semiempirical quantum chemical methods, such as CNDO/2, EHT, or PCILO (Pullman & Pullman, 1974). or empirical potential energy functions, as those mentioned in the review by Ramachandran &

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Sasisekharan (1968), could explain the occurrence of the large number of conformations near the line $\psi = 0°$ or the absence of the seven-membered hydrogen-bonded conformation at $\phi = -90°$, $\psi = +60°$, either in globular proteins, or in oligopeptide crystal structures. This suggests a need for a new set of potential functions which can be used in conformational studies on polypeptides and proteins with some degree of confidence. An attempt has been made to obtain such a set of potential functions, essentially by modifying the existing torsional potential functions (Kolaskar *et al.*, 1975 *a*, *b*).

In order to obtain better torsional potential functions which can be used directly in predicting conformations of polypeptide chains, the crystal structure data of globular proteins were analyzed. In part I (Kolaskar & Prashanth, 1977), the results of our studies mainly for Ala residue are discussed very briefly. In this communication, we present the method which we have developed to obtain the torsional potential functions $V_t(\phi)$ and $V_t(\psi)$, using the crystal structure data of 22 globular proteins, and the results obtained for various amino acid residues, such as Val, Leu, Ile, Phe and Ser. As indicated in part I, the averaged potential functions, $V_t(\phi)$ and $V_t(\psi)$, obtained from this study have a different form from those normally used in the literature (see calculations of these functions below).

METHODS

The total potential energy of a dipeptide unit ($\overset{\alpha}{C}H_3$–CO–NH–$\overset{\alpha}{C}$HR–CO–NH–$\overset{\alpha}{C}H_3$) for a particular conformation can be written as

$$V_{tot}(\phi, \psi) = V_1(r_{ij}) + V_2(\phi, \psi) \quad (1)$$

where r_{ij}, the interatomic distance between a pair of nonbonded atoms i and j, will depend on values of dihedral angles ϕ and ψ. Thus, the value of the distance dependent term V_1 will depend on the disposition of pairs of interacting atoms while the value of the term V_2 will depend only on the values of the dihedral angles ϕ and ψ. Explicitly, the terms V_1 and V_2 are

$$V_1(r_{ij}) = V_{nb}(\phi, \psi) + V_{es}(\phi, \psi) \quad (2a)$$

and

$$V_2(\phi, \psi) = V_{tor}(\phi, \psi) = V_t(\phi) + V_t(\psi) \quad (2b)$$

where, V_{nb}, V_{es}, $V_t(\phi)$ and $V_t(\psi)$ are nonbonded, electrostatic, and torsional potentials around the single bonds N–C^α and C^α–C respectively. Other terms, such as hydrogen bond energy and solute-solvent interaction energy, are ignored in the present study, assuming that the (ϕ, ψ)-data from the crystal structures of globular proteins is random, so that the Boltzmann statistics developed by Flory (1969) for polypeptide chains in solution can be used. Some reasons for the validity of assumptions made in this study are discussed in the succeeding parts of this paper. Therefore,

$$P_{tot}(\phi, \psi) = P_1(r_{ij}) . P_{tor}(\phi, \psi) \quad (3a)$$

Since the variables ϕ and ψ are independent, and the influence of one on the other is negligible, we can write

$$P_{tot}(\phi, \psi) = P_1(r_{ij}) \times P_t(\phi) \times P_t(\psi) \quad (3b)$$

The term P_{tot} (ϕ, ψ) has been calculated using the crystal structure data of globular proteins, while the term $P_1(r_{ij})$ has been obtained from semiempirical potential energy calculations on $V_1(r_{ij})$ of dipeptide units having different side chains. The procedure used to calculate these terms is discussed below.

Calculation of $P_{tot}(\phi, \psi)$ or $P_{exp}(\phi, \psi)$

The values of (ϕ, ψ) for each type of amino acid residue such as Ala, Val, Leu, Ile, Phe and Ser were collected from the crystal structure data of globular proteins. The names of the proteins, along with the number of amino acid residues which were considered in our analysis, are given in Table 1. Only those amino acid residues which were found to lie outside the α-helical regions were considered for this analysis. In this way, we have ignored, to a certain extent, the long-range cooperative effects, which might be specific for each globular protein. We have not ignored the residues in β-structures, mainly because our conformational calculations on dipeptides using only nonbonded interaction energy and an

TABLE 1

Protein	Species	No. of residues						References
		Ala	Val	Ile	Leu	Phe	Ser	
Subtilisin	BPN'	25	25	9	12	3	21	Kraut *et al.* (1971)
Myoglobin	Sperm whale	4	2	4	5	3	2	Watson (1969)
Ferricytochrome C_2	Rhodospirillum	13	4	0	8	5	4	Salemme *et al.* (1973)
Cytochrome B_5	Calf liver	3	3	4	5	3	4	F.S. Mathews *et al.* (private communication)
Thermolysin	*Bacillus thermo-protelyticus*	12	13	16	11	7	23	Mathew *et al.* (1974)
Carboxypeptidase	Bovine	14	4	17	18	12	29	Lipscombe (private communication)
Human deoxy hemoglobin	Human	14	15	0	15	12	16	G. Fermi (private communication)
Ribonuclease-S	Bovine	8	9	3	2	3	15	Wycoff *et al.* (1970) (private communication)
D-glyceraldehyde 3-phosphate dehydrogenase	Lobster	22	32	15	11	16	24	M.G. Rossmann *et al.* (private communication)
Horse Met Hemoglobin	Horse	17	21	0	30	15	16	M.F. Perutz (private communication)
Oxidized clostridial flavodoxin	Clostridium MP	4	10	11	7	5	8	M.L. Ludwig *et al.* (private communication)
Tosyl-chymotrypsin	Bovine	21	22	9	17	6	23	Birktoft & Blow (1972)
Concanavalin A	Jack bean	19	16	15	18	11	33	Becker *et al.* (1975)
Staphylococcal nuclease	Staphylococcus	7	8	5	9	3	2	E.E. Hazen *et al.* (private communication)
Trypsin Inhibitor	Bovine	10	2	3	4	8	1	Huber *et al.* (1974)
Trypsin	Bovine	14	17	13	14	3	34	Huber *et al.* (1974)
Parvalbumin	Carp muscle	13	4	14	6	6	5	Kretsinger *et al.* (1972)
Oxidized chromatium (Hipip)	*Rhodopseudomonas gelationosa*	18	2	1	5	2	3	Carter *et al.* (1974)
Dogfish M_4 Apo-lactate dehydrogenase	Dogfish	25	24	13	22	6	27	Adams *et al.* (1973)
Bence Jones Protein (REI)	Human	6	2	16	16	6	28	Epp *et al.* (1974) (private communication)
Hen egg white lysozyme	Hen egg white	6	2	5	6	3	8	Imoto *et al.* (1972)
Dogfish lactate NAD-Pyruvate ternary complex	Dogfish	12	29	14	24	4	21	M.G. Rossmann *et al.* (private communication)
Total number		287	266	177	265	142	349	= 1486
Statistical wt. factors (W_k)		0.193	0.179	0.119	0.178	0.096	0.235	

TABLE 2

The value of χ^1 and χ^2 used to fix the side chains in different amino acid residues as obtained from the analysis of proteins and oligopeptide crystal structure data

Residue name	χ^1 around C^α–C^β	χ^2 around C^β–C^γ or C^β–O^γ
Val	−60°, +60°, +180°	–
Ile	−60°, +60°, +180°	−60°, +60°, +180°
Leu	−60°, +60°, +180°	−60°, +60°, +180°
Ser	−60°, +60°, +180°	−120°, 0°, +120°
Phe	−60°, +60°, +180°	−120°, 0°, +120°

analysis of protein data (Ananthanarayanan & Bandekar, 1976) have indicated that the extended chains are stabilized mainly because of short-range interactions, and the additional stability because of hydrogen bonds is only of a secondary nature.

It can be seen from Table 1 that we have considered globular proteins with very different types of primary, secondary and tertiary structures. Therefore, the data collected from such a set are expected to be random. In fact, for a particular type of amino acid residue, having nearly the same conformation, the environment was found to be quite variable in the data used. For example, Ala residues having nearly the same values of (ϕ, ψ) were found to have different amino acid residues before and after them in most cases. So the distributions of conformations obtained in the (ϕ, ψ)-plane from such a set of data will be expected to be the same as that obtained from a random polypeptide chain in solution. This became more evident when we found that the (ϕ, ψ)-distribution remained practically unchanged when more data from proteins were added to the initial set of data from 15 proteins, indicating that the effect due to long-range or medium-range forces which may be present due to primary structure in a particular protein are completely masked in the data under analysis. Therefore, we feel that we are justified in applying the Boltzmann statistics, initially developed for only random polypeptide chains in solution, in this study.

The (ϕ, ψ)-values for each type of amino acid residues were plotted in a $(20^\circ \times 20^\circ)$ grid of the (ϕ, ψ)-plane;

$$P_{tot}(\phi, \psi) = P_{exp}(\psi) = \sum_{\phi} n(\phi, \psi)/N \qquad (4a)$$

and

$$P_{tot}(\phi, \psi) = P_{exp}(\phi) = \sum_{\psi} n(\phi, \psi)/N \qquad (4b)$$

were calculated, where $n(\phi, \psi)$ is the number of amino acid residues occurring in a particular grid, and N is the total number of amino acid residues of each type in (ϕ, ψ)-plane.

Calculation of $P_1(r_{ij})$

The potential energy $V_t(r_{ij})$ was calculated for each dipeptide unit with different side chains in the L-configuration, using the method described in the review by Ramachandran & Sasisekharan (1968). The constants for nonbonded interactions were adopted from Chandrasekaran & Balasubramanian (1969), except for those interactions which involve hydrogen atoms. The van der Waals radius for hydrogen atoms was reduced by 0.1 Å (Ramachandran, 1975; Hagler & Lapiccirella, 1976) and the constants were recalculated. For each dipeptide, the side-chain torsional angles χ^1 and χ^2 were fixed at values observed in crystal structure data of oligopeptides and proteins. The values used to fix the side chain-atoms are given in Table 2. A suitable torsional potential function was used to calculate the contributions from torsional energy for side chains. Thus,

$$\begin{aligned} V_1(r_{ij}) &= V_1(\phi, \psi, \chi) \\ &= V_{nb}(\phi, \psi, \chi) + V_{es}(\phi, \psi, \chi) \\ &\quad + V_{tor}(\chi^1) + V_{tor}(\chi^2) \end{aligned} \qquad (5)$$

$$P_1(r_{ij}) = \Sigma \exp\left[-V_1(\phi, \psi, \chi)/RT\right]/M \qquad (6)$$

M is the total number of conformations for which $V_1(r_{ij})$ has been calculated by using

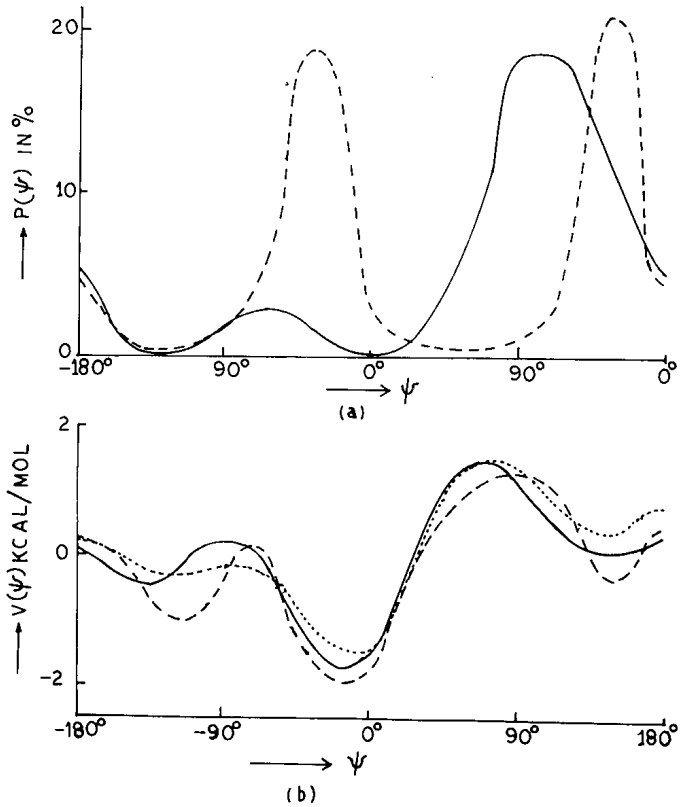

FIGURE 1

(a) The variation of values of $P_{exp}(\psi)$ (— — —) and $P_n(\psi)$ (———) versus ψ, for Ala residue. Note the disagreement between $P_n(\psi)$-and $P_{exp}(\psi)$-values, particularly in the region of $\psi = 0°$ and $\psi = 150°$. Similar disagreement was obtained for other types of amino acid residues. (b) The variations of $V_t(\psi)$ versus ψ for Ala residues are represented here. — — — curve obtained by using the relation $V_t(\psi) = 0.6 \ln (P_{exp}(\psi)/P_n(\psi)$. . . . . curve drawn using the first three terms of the series in the Fourier analysis of the above curve. ——— curve obtained using the torsional function $V_t(\psi)$ for Ala residue, as given in Table 3(b).

different values of χ^1 and χ^2, as given in Table 2. It should be mentioned here that in the present study the average over a parameter was always calculated for probability values assuming RT = 0.6 kcal/mol rather than for energy values.

The bond angle τ at C^α atom was found to vary over a range of values from 105° to 115° in globular proteins. Therefore, calculations on each dipeptide unit were repeated by varying τ from equilibrium value of $\tau = 110°$ by ± 5°. Hence the $P_1(r_{ij})$-values mentioned above were further averaged over τ-values and final $P_1(r_{ij})$-values were obtained that were used to calculate the $P_n(\psi)$ and $P_n(\phi)$ at an interval of 20°. Thus,

$$P_n(\psi) = \sum_{\phi} P_1(r_{ij}) / \sum_{\phi}\sum_{\psi} P_1(r_{ij}) \qquad (7a)$$

similarly,

$$P_n(\phi) = \sum_{\psi} P_1(r_{ij}) / \sum_{\phi}\sum_{\psi} P_1(r_{ij}) \qquad (7b)$$

Calculations of torsional potential functions $V_t(\phi)$ and $V_t(\psi)$

The values of $P_{exp}(\psi)$, obtained for each amino acid residue from the crystal structure data of proteins, and $P_n(\psi)$, obtained through calculations on dipeptides, were used to obtain the

values of $P_t(\psi)$ at intervals of 20°, using the relation

$$P_t(\psi) = P_{exp}(\psi)/P_n(\psi) \quad (8)$$

or

$$V_t(\psi) = -0.60 \ln P_t(\psi) \quad (9)$$

For the first cycle of calculation of $V_t(\psi)$, we have assumed $V_t(\phi) = 0$. The curve for $V_t(\psi)$ (Fig. 1), was analyzed by the method of Fourier analysis and the first three dominant terms were taken. The function $V_t(\psi)$, thus obtained was of the form:

$$V_t(\psi) = V_{\psi 1} \cos(\psi - \delta_1) + V_{\psi 2} \cos(2\psi - \delta_2) + V_{\psi 3} \cos(3\psi - \delta_3) \quad (10)$$

Using this function for the torsional potential around C^α–C single bond, the total energy values $P_{th}(\psi)$ for a dipeptide unit were obtained. These values of $P_{th}(\psi)$ were compared with the values of $P_{exp}(\psi)$ and the reliability index R, as is usually calculated in crystallography, was determined. The calculated values of R were found to be quite high. This was mainly because the function obtained from Fourier analysis was highly inaccurate in regions where either $P_{exp}(\psi)$ or $P_n(\psi)$ was nearly zero. Therefore, the technique of curve-fitting was adopted and $V_t(\psi)$ was refined by finding the best fit between $P_{th}(\psi)$ and $P_{exp}(\psi)$, as judged by the value of the R-factor (Fig. 1b)

Using this refined function $V_t(\psi)$, the values of $P_t(\psi)$ were obtained. The entire procedure was repeated to get the best $V_t(\phi)$ function. This completed one cycle of refinement. In a similar way, a few more cycles of refinement were carried out, first by summing over ϕ and analyzing $V_t(\psi)$, and then by summing over ψ and analyzing $V_t(\phi)$ each time. At the end of the third cycle of refinement, the variations in the values of coefficients $V_{\theta k}$ and $\delta_{\theta k}$ (where $\theta = \phi, \psi$ and $k = 1, 2, 3$), were found to be practically zero. These are given in Tables 3 and 4 for different side chains attached to the middle C^α atom in dipeptide unit.

RESULTS AND DISCUSSION

The values of $V_{\theta k}$ and $\delta_{\theta k}$ reported in Tables 3 and 4 for different amino acid residues such as Ala, Val, Leu, Ile, Ser and Phe are only marginally different from each other. These values are for the best function obtained by varying $V_{\theta k}$ at intervals of 0.25 kcal/mol and $\delta_{\theta k}$ at intervals of 30°. As mentioned in part I, the accuracy of these parameters is only of the order of 0.5 kcal/mol for $V_{\theta k}$ and 30° for $\delta_{\theta k}$. Thus it can be seen that the parameters for all the different amino acid residues occur within the range of the expected standard deviation. This indicates that the side-chain effect on torsional potential function is small and if the nonbonded interactions are calculated properly, as we have done in this study, the torsional potential will be practically independent of side chain. The small differences in the constants, $V_{\theta k}$ and $\delta_{\theta k}$, may be partly because of the inaccuracy of protein data and partly due to

TABLE 3

Torsional potential function $V_t(\phi)$ obtained from 22 protein structures for six different amino acid residues

$V_t(\phi) = V_{\phi_1} \cos(\phi - \delta_{\phi_1}) + V_{\phi_2} \cos(2\phi - \delta_{\phi_2}) + V_{\phi_3} \cos(\phi - \delta_{\phi_3})$

Residue	V_{ϕ_1}	V_{ϕ_2}	V_{ϕ_3}	δ_{ϕ_1}	δ_{ϕ_2}	δ_{ϕ_3}	R%
Ala	−1.0	−0.5	0.0	−60°	−30°	—	28
Val	−1.0	0.0	−0.25	−60°	—	−30°	27
Ile	−1.0	−0.25	−0.25	−60°	−30°	−30°	11
Leu	−1.0	0.0	0.0	−60°	—	—	20
Phe	−1.0	0.0	0.0	−60°	—	—	27
Ser	−0.5	−0.5	−0.25	−60°	−30°	−30°	24
Average	−1.0	0.0	0.0	−60°	—	—	27

TABLE 4

Torsional potential $V_t(\psi)$ obtained from crystal structure data of 22 proteins for six different amino acid residues

$V_t(\psi) = V_{\psi 1} \cos(\psi - \delta_{\psi 1}) + V_{\psi 2} \cos(2\psi - \delta_{\psi 2}) + V_{\psi 3} \cos(3\psi - \delta_{\psi 3})$

Residue	$V_{\psi 1}$	$V_{\psi 2}$	$V_{\psi 3}$	$\delta_{\psi 1}$	$\delta_{\psi 2}$	$\delta_{\psi 3}$	R%
Ala	−0.5	−1.0	−0.5	−60°	−30°	−30°	40
Val	−0.5	−0.5	−0.5	−60°	−30°	−30°	40
Ile	−0.5	−0.5	−0.5	−60°	−30°	−30°	40
Leu	−0.75	−0.75	−0.5	−60°	−30°	−30°	26
Phe	−0.75	−0.75	−0.5	−60°	−30°	−30°	37
Ser	−0.5	−1.0	−0.5	−60°	−30°	−30°	36
Average	−0.5	−1.0	−0.5	−60°	−30°	−30°	30

exclusion of terms such as hydrophobic interaction in our energy calculations. However, it is clear that the average torsional potential functions $V_t(\phi)$ and $V_t(\psi)$ can be derived in a similar fashion, using the data of all residues put together. The values of $V_{\theta k}$ and $\delta_{\theta k}$ thus obtained are given in the last row of Tables 3 and 4. These averaged potential functions are not very different from those obtained for individual amino acid residues.

The (ϕ, ψ)-total energy maps, using these newly obtained torsional functions, are given in Figs. 2 and 3, along with the similar maps drawn using data from proteins. Fig. 3(b), in particular, contains the recommended formulae for $V_t(\phi)$ and $V_t(\psi)$. The theoretical dipeptide energy maps of different types of amino acid residues seem to agree well with the corresponding protein data energy map, indicating that the method of treating each parameter ϕ and ψ separately is an essentially reasonable procedure. In fact, the values of constants $V_{\theta k}$ and $\delta_{\theta k}$ obtained after the first cycle of refinement for $V_t(\phi)$ and $V_t(\psi)$ were found to vary only over a small range in the successive cycles of refinement, further proof that the assumption that ϕ and ψ are independent variables is quite valid.

The results presented in Figs. 2 and 3 clearly show that the new torsional potential functions, $V_t(\phi)$ and $V_t(\psi)$, when used along with the usual nonbonded and electrostatic interaction energy terms, explain to a large extent some of the unaccounted features in polypeptide chain conformations, the most important of which is the absence of left-handed alpha helices. All the previously used potential functions indicate that the left-handed α-helix is energetically as stable as right-handed α-helix, though in practice one observes only the right-handed α-helix. However, the energy maps in Fig. 3 clearly show that the left-handed α-helical region is much higher in energy than the right-handed α-helical region, which explains why the left-handed α-helix is never observed to occur in a protein structure.

Secondly, there has been a lot of dispute regarding the C_7 conformation of dipeptides in solution. Early literature on solution studies of dipeptides, done using i.r. and n.m.r. techniques, shows the occurrence of the C_7 conformation (Marraud & Neel, 1973), but this is very rarely observed in crystals of oligopeptides or globular proteins. Almost all previous (ϕ, ψ)-energy maps have shown that the $C_7(-90°, +60°)$ conformation for a dipeptide is energetically favorable. However, this C_7 conformation in our new energy map is quite high in energy and this explains the absence of this conformation in polypeptides or proteins. Recent solution studies, where the concentration of solute was slightly increased, indicated that the C_7 conformation is absent even in such a solution and the molecules generally take up extended conformations (private communication from J. Neel). Similarly, our preliminary calculations on tripeptides of Ala residues indicate that they will explain explicitly the formation of LL

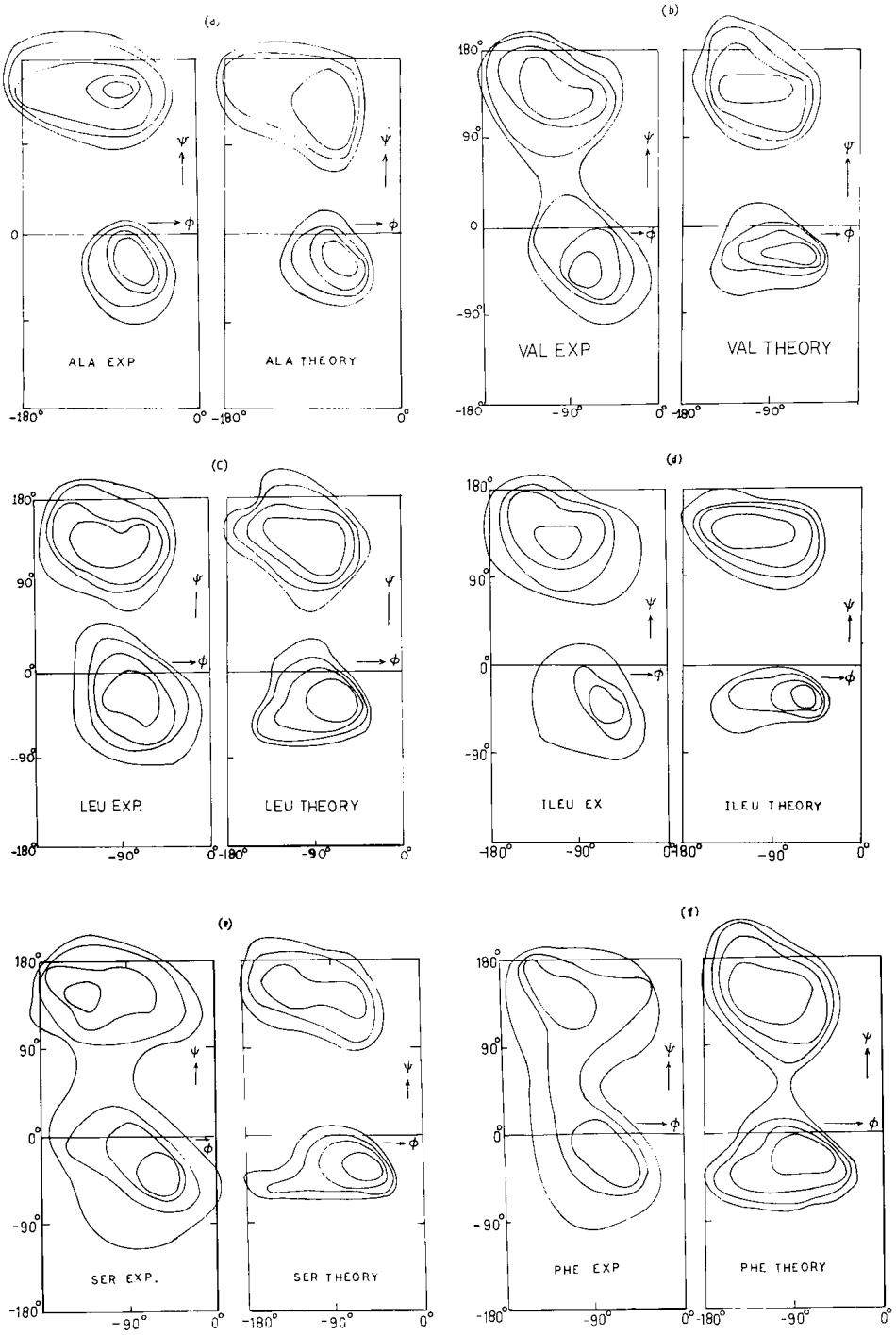

FIGURE 2

(a)–(f) The isoenergy curves, for various types of amino acid residues, drawn at intervals of 0.5 kcal/mol from the minimum. In this figure, only the left half of the (ϕ, ψ)-energy map is presented, both from theory and experiment, since the energy values in the right half region are above 2.0 kcal/mol from minimum. The comparison of theoretical energy maps with those drawn using experimental data indicates a good agreement, which has never been obtained before.

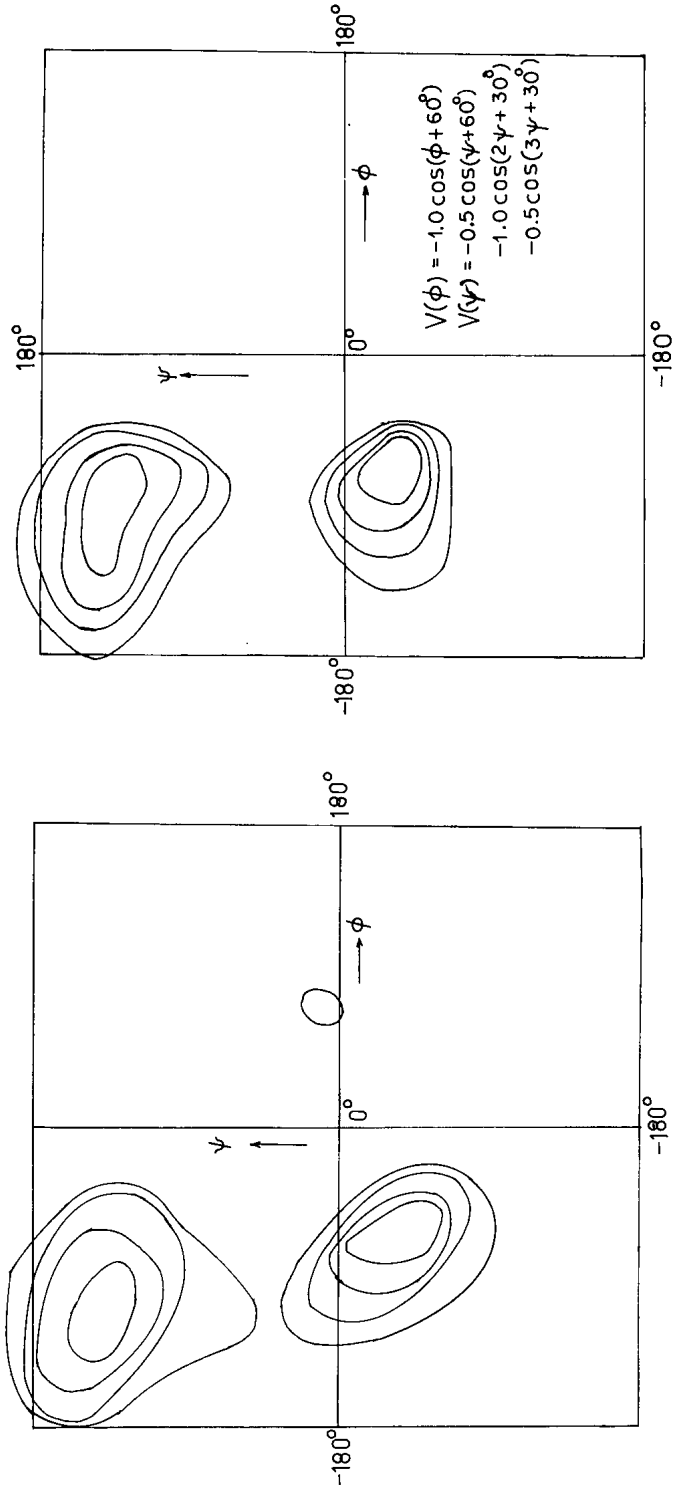

FIGURE 3
(a) Isoenergy contours at intervals of 0.5 kcal/mol using the data of six types of amino acid residues from 22 globular proteins, as mentioned in Table 1 and text. (b) Theoretical energy map obtained using the constants shown for the parameters $V_{\theta k}$ and $\delta_{\theta k}$ for the potentials $V_t(\phi)$ and $V_t(\psi)$ (Table 3). Note the very good agreement between (a) and (b), even though the isoenergy contours are at intervals of 0.5 kcal/mol.

bends and helices in proteins. The theoretical dipeptide energy maps shown in Figs. 2 and 3, obtained using the newly developed potential functions, have low energy near $\psi = 0^\circ$ and high energy near $\psi = 60^\circ$; this explains the observed density of points in the former region, which was not explained by any of the earlier dipeptide maps.

The results presented above indicate that the form of the functions $V_t(\phi)$ and $V_t(\psi)$ is quite different from those obtained from studies on small molecules having only one single bond, in which the environment of the single bond is different in contrast to those considered here. This indicates that the effect of environment on single bond rotational functions in macromolecules is quite important, and to get similar torsional potential functions one has to choose the model system very carefully. Secondly, because of the inherent approximations and drawbacks of the method used here, these torsional potential functions have absorbed into them a solute-solvent interaction term to a certain extent. Thus what are given here are not strictly intrinsic torsional potential functions, or the torsional potential functions one expects to get from quantum mechanical calculations on model compounds. However, these functions are compatible with the experimental data, when used with the other potential functions mentioned in this paper, and therefore we accept them as good torsional potentials and suggest that they could be used in semiempirical potential energy calculations, not only on dipeptides but also on polypeptides and proteins. These functions, as pointed out in part I, are for L-amino acid residues, except glycine. The treatment developed to obtain the potential functions for glycine will be discussed in a separate communication. For D-residues, the functions will be the same, except that $\delta_{\theta k}$ will have the opposite sign.

In this study we have developed a simple technique to obtain the torsional functions from observed protein data, rather than from data from small molecules, and thus focused the attention on the rapidly accumulating data of macromolecules which can be fruitfully used to derive empirical potential functions. These functions can then be used to study macromolecules and small molecules.

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