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Effect of variations in peptide parameters and charges on the unperturbed dimensions of polypeptide chains

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Abstract. The net charges on various atoms of poly (L-alanine), polyglycine, poly (N-methyl-L-alanine) and poly (N-methylglycine) were computed using the MO-LCAO method of Del Re for σ charges and the Hückel MO method for π charges. The characteristic ratios C_∞ were computed for all the above polypeptide chains, with different sets of parameters for the peptide unit. The calculated values of C_∞ are found to be very sensitive to the input peptide geometry. The calculated value of 2·2 for C_∞ of poly (N-methylglycine) obtained with set-3 parameters (derived from a crystal structure containing prolyl residue) is closer to the experimental value of $1{\cdot}8 \pm 0{\cdot}2$ than the value of 2·7 obtained with set 1 (Pauling-Corey parameters), suggesting that the peptide parameters of N-substituted aminoacids have close similarity to set 3 rather than to set 1. The calculated values of C_∞ of the polypeptide chains show no correlation with the number of allowed conformations, suggesting that the ratio of C_∞/C_t need not always provide information about the flexibility or freedom of rotation of chain units.

Keywords. Polypeptides; unperturbed dimensions; characteristic ratio.

1. Introduction

Recently Flory and coworkers (Flory 1969; Brant and Flory 1965 *a*; Brant *et al* 1967) have developed theoretical methods to calculate the unperturbed dimensions of polypeptide chains. These methods have been followed to obtain the characteristic ratios of a number of polypeptide chains (Flory 1969; Tanaka and Nakajima 1970, 1971). However, all the earlier investigators have used Pauling Corey parameters for fixing the atoms in the peptide unit. But the x-ray crystal structure studies (Davies 1965) on di- and tripeptides in the solid state have indicated significant deviations from the Pauling-Corey parameters. Similar deviations in the peptide parameters may be expected in poly (aminoacid) chains having different substituents or in different solvent systems. Another objection to the earlier studies is the assignment of zero charge to the α-carbon atom and other side groups in computing the electrostatic energy. Thirdly, it is known that the fractional charges assigned for various atoms in the polypeptide chain by different proce-

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dures differ significantly (tables 1A and 1B). It is, therefore, advisable to use the charges derived by the same procedure for all the polypeptides while considering the effect of various groups on their unperturbed dimensions. Hence, in the present study, we have attempted to assign the charges to various atoms i poly (L-alanine), Poly (N-methyl-L-alanine) poly glycine and poly (N-methylglycine) and also to compute their unperturbed dimensions with different sets of parameters for the peptide unit, in order to bring out the effect of small variations in the peptide parameters and charges on their dimensions.

2. Charge calculations

The fractional charges on the atoms in the peptide residues of various polypeptide chains were obtained by using the LCAO-MO method of Del Re (1958) and Del Re *et al* (1963) for σ charges and Hückel method for π charges. The values of the parameters used for the σ charge calculations are due to Berthod and Pullman (1965) and for the π charges due to Pullman and Pullman (1963). The net charges so obtained on various atoms in different polypeptides are shown in tables 1A and 1B together with the values assigned by the earlier investigators.

Table 1A. Partial charges on the atoms in polyglycine and polyalanine

Atom	Polyglycine				Polyalanine			
H	0·281	0·272	0·204	0·204	0·281	0·272	0·204	0·204
N	−0·281	−0·305	−0·202	−0·198	−0·281	−0·305	−0·202	−0·200
C′	0·394	0·449	0·318	0·368	0·394	0·449	0·318	0·367
O	−0·394	−0·416	−0·422	−0·470	−0·394	−0·416	−0·422	−0·471
C^{α}	0·0	0·0	0·0	0·0	0·0	0·0	0·046	0·046
H^{α}	0·0	0·0	0·0	0·048	0·0	0·0	0·046	0·046
$H^{\alpha 2}$	0·0	0·0	0·0	0·048	..		..	..
C^{β}					0·0	0·0	−0·110	−0·110
$H^{\beta 1}$							0·04	0·04
$H^{\beta 2}$							0·04	0·04
$H^{\beta 3}$							0·04	0·04
ref.	1	2	3	4	1	2	3	4

1. Brant *et al* (1967) 2. Scott and Scheraga (1966)
3. Poland and Scheraga (1967) 4. Present study.

Table 1B. Partial charges on the atoms in poly (N-methylglycine) and poly (N-methylalanine). [Present study]

Atom	Poly (N-methylglycine)	Poly (N-methylalanine)
N	−0·042	−0·044
C′	0·359	0·357
O	−0·472	−0·472
C^{α}	−0·007	0·039
$H^{\alpha 1}$	0·047	0·046
$H^{\alpha 2}$	0·047	..
C^{β}		−0·111
$H^{\beta 1}$		0·04
$H^{\beta 2}$		0·04
$H^{\beta 3}$		0·04
C^{N}	−0·071	−0·072
H^{N1}	0·046	0·046
H^{N2}	0·046	0·046
H^{N3}	0·046	0·046

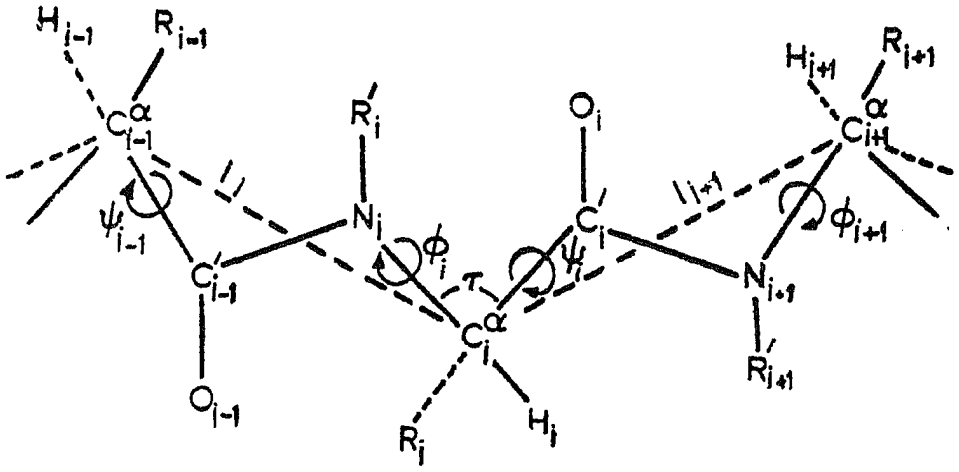

Figure 1. Section of a polypeptide chain consisting of two peptide units. The rotational angles (θ_i, ψ_i) are marked. R′ = H or CH_3.

Table 2. Bond lengths and bond angles used to fix various atoms in a polypeptide unit

Bonds and Bond angles	Set 1	Set 2	Set 3
C^{α}—C′	1·53	1·531	1·531
C′—N	1·32	1·331	1·331
N—C^{α}	1·47	1·461	1·461
C′—O	1·24	1·233	1·233
N—H	1·00	1·000	1·000
N—C^{N}	1·47	1·461	1·461
C^{α}—C′—N	114·0	115·8	119·0
C^{α}—C′—O	121·0	120·8	119·0
O—C′—N	125·0	123·4	122·0
C′—N—C^{α}	123·0	121·2	121·0
C′—N—H	123·0	123·9	126·0
C′—N—C^{N}	123·0	123·9	126·0
C^{α}—N—H	114·0	114·9	113·0
C^{α}—N—C^{N}	114·0	114·9	113·0
N—C^{α}—C′	110·0	110·8	110·8

Bond lengths are given in Å units and bond angles in degrees.

Set 1: Pauling-Corey parameters. Set 2: Averaged parameters. Set 3. Conti and DeSantis parameters.

3. Fixing of atoms and calculation of potential energy

A segment of a polypeptide chain in its fully extended conformation for which the torsional angles ϕ and ψ are defined to be (180°; 180°) is shown in figure 1. The three sets of parameters used to fix the atoms in the peptide unit are shown in table 2. Set 1 are the Pauling-Corey parameters; set 2 are the mean values of the peptide unit, averaged from x-ray crystal structure data. Set 3 are the parameters used by Conti and DeSantis (1971) in conformational energy calculations of poly (N-methyl-L-alanine). The side groups were fixed by the method suggested by Ramachandran and Sasisekharan (1968), assuming C^{α}—H^{α} and C^{α}—C^{β} bond lengths to be 1·0 and 1·54 Å respectively.

The conformational energy was computed using the expression

$$V(\phi_i, \psi_i) = \frac{V_{\phi}^0}{2}(1 + \cos 3\phi_i) + \frac{V_{\psi}^0}{2}(1 + \cos 3\psi_i) + \sum_{k,l} V_{k,l}(\phi_i, \psi_i) + \sum_{k,l} V_{c,k,l} \qquad (1)$$

The expressions and constants used for computing the torsional, non-bonded, and electrostatic energies are the same as reported by Brant *et al* (1967). Because of the difference in the definition of initial conformation we have used $(1 + \cos 3\theta)$ type function for the torsional potential. The characteristic ratios (C_∞) of an infinitely long chain were obtained by using the expression (Brant and Flory 1965, Srinivasan and Rao 1972)

$$C_\infty = [(\mathbf{E} + \langle \mathbf{T} \rangle)(\mathbf{E} - \langle \mathbf{T} \rangle^{-1})]_{22} \tag{2}$$

where **E** is the identity matrix of order 3 and $\langle \mathbf{T} \rangle$ is the statistical-mechanical averaged matrix. The subscript 22 denotes the (2, 2) element of the final matrix. The transformation matrix $\mathbf{T}_i$ which transforms a vector presented in the co-ordinate system associated with the virtual bond i to the co-ordinate system of virtual bond $i - 1$ was formed following the procedure of Ramachandran *et al* (1966). To obtain the required transformation matrix, the Y-axis of the co-ordinate system associated with the virtual bond is taken in the direction of the virtual bond and X-axis is placed in the plane of the peptide unit. The Z-axis

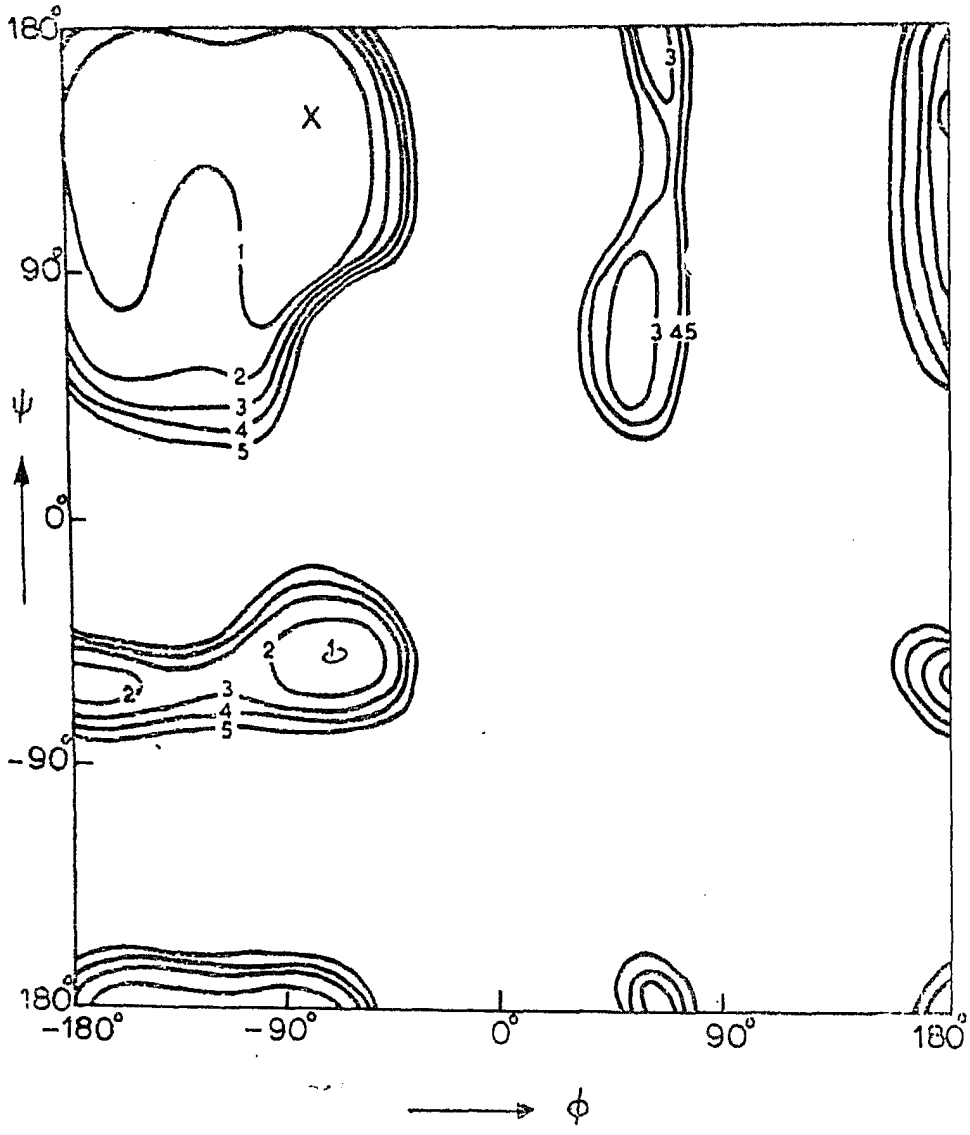

Figure 2. Conformational energy map for the L-alanyl residue calculated with set 1 parameters and with charges assigned by Brant *et al* (1967). Contours are shown at intervals of 1K cal. mole^{-1} relative to the lowest minima marked X.

is chosen in the direction required for a right handed co-ordinate system. Intervals of 10° in ϕ and ψ were used for these calculations. It is to be noted that the earlier investigators have used an interval of 30° for ϕ, and ψ, which is too large and hence limits the number of conformations considered to compute the averaged matrix $\langle \mathbf{T} \rangle$. In the energy calculations, the methyl group is treated as a single unit and the charge on the carbon atom of the methyl group has been chosen as the net charge of the carbon and the hydrogens attached to it.

The conformational maps obtained with set 1 and set 2 parameters for the alanyl and glycyl residues and with set 3 parameters for N-methylalanyl and N-methylglycyl residues are shown in figures 2 to 6.

4. Results and discussion

Table 1A shows that the charges obtained on H, N, C′ and O in the present study are very nearly the same in polyglycine and polyalanine and are comparable with those assigned by the earlier investigators. Table 1B also shows that the charges on N, C′, O, C^N, H^1, H^2 and H^3 are very nearly the same in poly (N-methylglycine) and poly (N-methylalanine). It is also seen that the charges on C^α, C^β and other side group atoms are significant, for all the polypeptide chains, except for polyglycine.

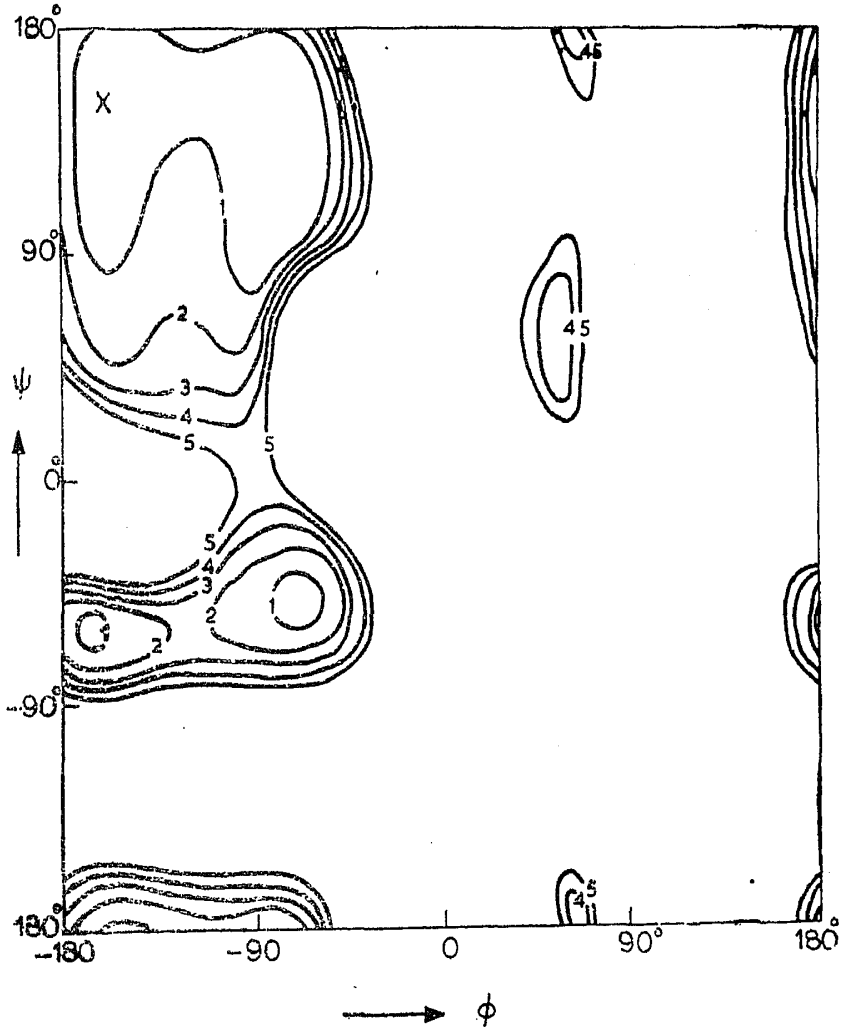

Figure 3. Conformational energy map for the L-alanyl residue calculated with set 2 parameters and with charges assigned in the present study. Contours are shown at intervals of 1K cal. mole^{-1} relative to the lowest minima marked X.

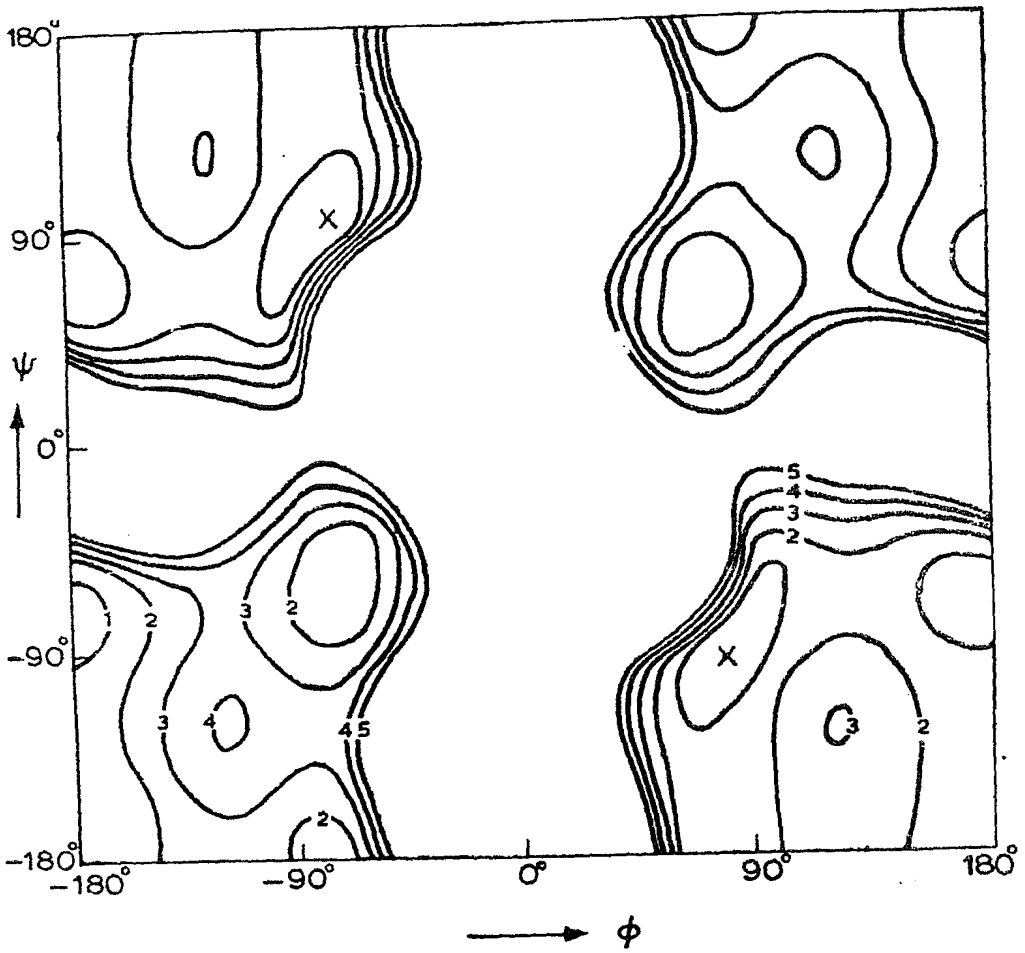

Figure 4. Conformational energy map for the glycyl residue calculated with set 2 parameters and with charges assigned in the present study. Contours are shown at intervals of 1K cal. mole^{-1} relative to the lowest minima marked X.

A comparison of the energy contours in figures 2 and 3 reveals that variations in the peptide parameters and small variation in the charges on the atoms in peptide unit and the charge on C^{α} atom do affect the position of the energy minima and to a small extent the shapes of the energy contours. Similarly minor changes in the energy contours have also been observed in the conformational maps obtained for glycyl, N-methyl-L-alanyl and N-methylglycyl residues when different sets of parameters are used.

The characteristic ratio C_f (where f denotes free rotation) has been calculated by using eq. (2) giving equal statistical weights to all the conformational states. The values of C_f obtained with set 1, set 2 and set 3 parameters are respectively 1·93, 1·99 and 2·00. These results suggest that small variations in the peptide parameters may not affect the free rotational dimensions significantly.

A value of 10·1 has been obtained, at $T = 310°$ K, for the characteristic ratio C_∞ of an infinitely long poly L-alanine chain with Pauling-Corey parameters (set 1) and the charges assigned by Brant, Miller and Flory (1967). This value is slightly higher than the value of 9·3 reported by Brant and Flory (1965). This difference arises mainly because of the reason that Brant *et al* have used an interval of 30° in ϕ and ψ in computing the conformational energies, whereas the present

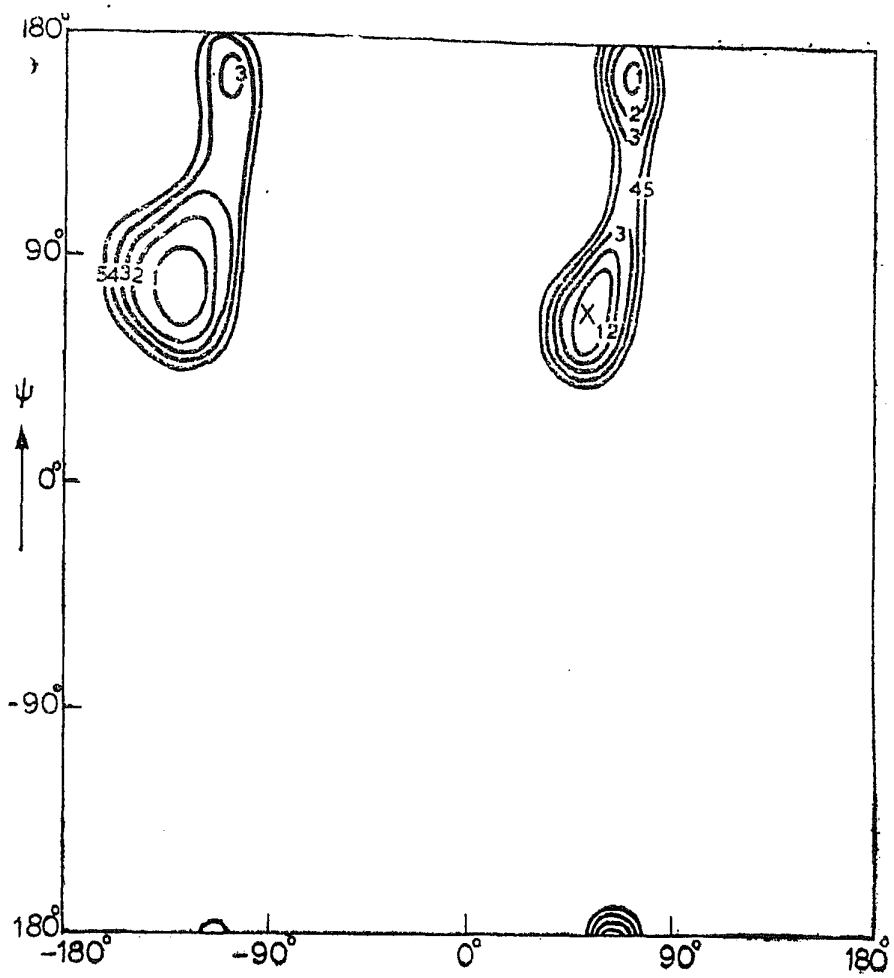

Figure 5. Conformational energy map for N-methyl-L-alanyl residue calculated with set 3 parameters and with charges assigned in the present study. Contours are shown at intervals of 1K cal. mole^{-1} relative to the lowest minima marked X.

Table 3. Calculated C_∞ values, with different sets of parameters at 298° K

Polypeptide	Set 1	Set 2	Set 3
Poly (L-alanine)	9·9 (10·4)*	8·1†	..
Polyglycine	2·1	2·2	..
Poly (N-methyl-L-alanine)	1·0	2·4	1·5
Poly (N-methylglycine)	2·8	2·5	2·2

* Values obtained with the charges assigned by Brant *et al.*

† This value remained practically the same (8·15) when the peptide dimensions reported by Ramachandran *et al* (1974) were used.

calculations have been made at intervals of 10° in order to include more number of conformations. The calculated value of 8·1 for C_∞ of poly L-alanine with set 2 parameters (table 3) is significantly different from the value of 9·9 obtained with set 1 parameters indicating that small differences in the peptide parameters do affect the calculated chain dimensions considerably. The value of 9·9 also differs from 10·4 obtained with set 1 parameters, and the charges of Brant *et al* indicating that the effect on C_∞ due to differences in the charges is very small (5%). On the other hand, it is seen from table 3 that the calculated C_∞ values of

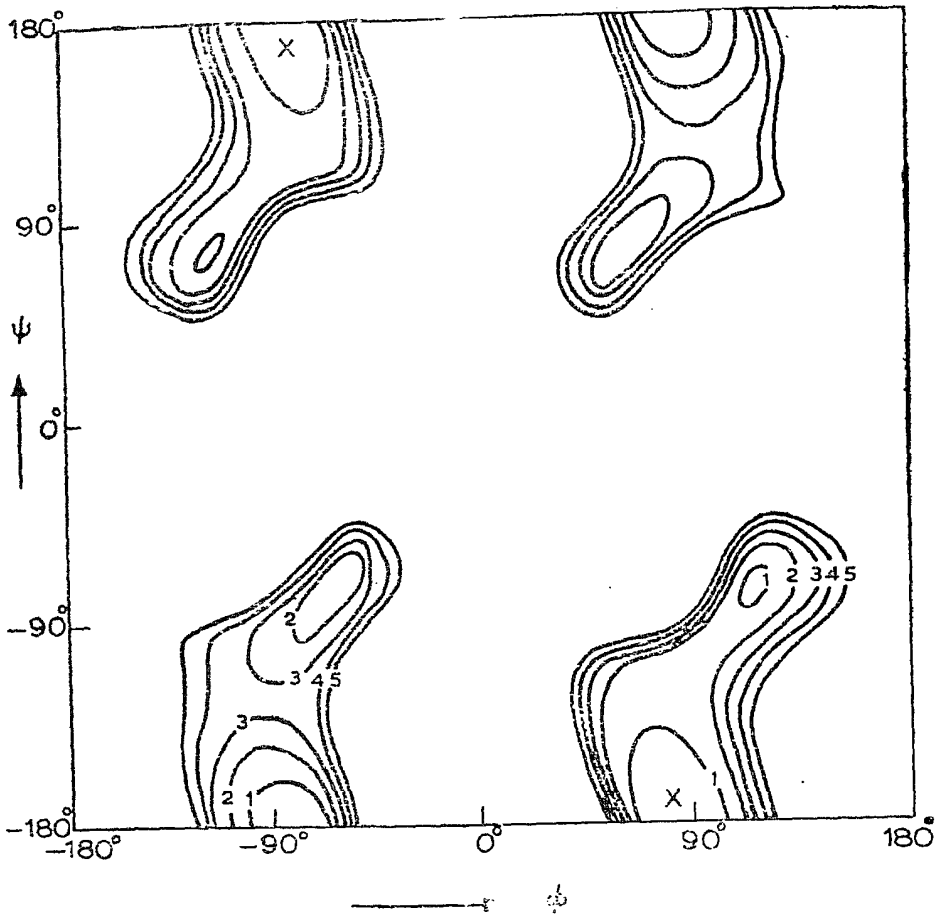

Figure 6. Conformational energy map for N-methylglycyl residue with set 3 parameters and with charges assigned in the present study. Contours are shown at intervals of 1K cal. mole^{-1} relative to the lowest minima marked X.

polyglycine, poly (N-methylalanine) and poly (N-methylglycine) are affected significantly due to the small differences in the input peptide parameters.

The computed value of 8·1 for C_∞ obtained for poly (L-alanine) with the average parameters (set 2) using the charges assigned in the present study is very close but slightly lower than the average experimental value of about 8·6 obtained for poly (α-L-amino acid) chains (Brant and Flory 1965; Fujita *et al* 1966; Terbujevich *et al* 1967). As pointed out by us (Srinivasan and Rao 1972), the angle N–C^α–C′ might fluctuate by about $\pm$ 3° in solution and these deviations from the average value of 110·8°, always lead to an increase in C_∞ for poly (L-alanine) type of polypeptides. This may explain the slightly lower value of the calculated C_∞ compared to the observed values for this type of polypeptide chains.

Values of 1·0 and 2·8 (table 3) respectively for poly (N-methylalanine) and poly (N-methylglycine) obtained with set 1 parameters are also different from 0·58 and 2·97 reported by Tanaka and Nakajima. It is also interesting to note (table 3) that the computed value of 2·2 for C_∞ of poly (N-methylglycine) with set 3 parameters is close to the experimental value of 1·8 $\pm$ 0·2 (Fessler and Ogston 1951). This suggests that poly (N-methylglycine) may prefer to have the peptide unit geometry close to set 3 in solution. This is understandable from the fact that set 3 parameters are derived from a crystal structure containing prolyl residue, which has close similarity to that of N-substituted aminoacids.

A comparison of figures 3 and 5, and 4 and 6 indicate that the replacement of the peptide hydrogen by a methyl group in both poly (L-alanine) and polyglycine highly restricts the freedom of rotation of the peptide units; however, this replacement leads to significantly low values of C_∞ for the former and slightly higher values for the latter. These results suggest that there is no correlation between values C_∞ and the number of allowed conformations, even among polypeptide chains. The values of 1–2·5 obtained for C_∞ of poly (N-methyl-L-alanine) are small compared to 8–10 obtained for poly (L-alanine). This is difficult to reconcile at first, since from the conformational maps, it is clear that peptide units in poly (N-methyl-L-alanine) are more restricted compared to those in poly (L-alanine). A very high value of C_∞ for poly (N-methyl-L-alanine) is expected, since it is generally believed that the restricted rotation of the chain units always lead to increased values of C_∞. However, this is not revealed in the calculations of C_∞. It is probable that though the peptide units are highly restricted in poly (N-methyl-L-alanine) the favoured conformations of chain units are such that, the molecule, as a whole, takes up a compact conformation compared to poly (L-alanine). This further supports the earlier conclusions of Yathindra and Rao (1972) that the ratio (C_∞/C_f) need not always provide information about the flexibility or freedom of rotation of chain units. This rather throws light on the compactness of the molecules as a whole. The results on polyglycine and poly-(N-methylglycine) also support this point.

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