STEREOCHEMICAL CRITERIA FOR POLYPEPTIDE AND PROTEIN CHAIN CONFORMATIONS

II. Allowed Conformations for a Pair of Peptide Units

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ABSTRACT The conformation of a polypeptide or protein chain may be specified by stating the orientations of the two linked peptide residues at each alpha carbon atom in the chain, namely the two dihedral angles ϕ, ϕ' about the single bonds N—αC and αC—C′ from a defined standard conformation. By using certain criteria of minimum contact distances between the various atoms, the allowed anges of (ϕ, ϕ') have been worked out for three values of the angle N-αC-C′ (τ), namely 105, 110, and 115° for non-glycyl, and 110 and 115° for glycyl residues. The theory is compared with all the available crystallographic data (up to early 1965) on simple (di- and tri-) peptides, cyclic peptides, polypeptide and protein structures, and the observed data fully support the conclusions from theory. The effect of the gamma carbon atom, in its three possible positions, is also discussed, and is found to alter the outer limits of the allowed region of (ϕ, ϕ') only slightly. The paper contains exhaustive references to the published data on these structures, using x-ray diffraction.

INTRODUCTION

The conformation of the backbone of a polypeptide or protein chain can be completely specified by giving the relative orientations of the two linked peptide groups at each α-carbon atom. When the relative orientations of the peptide group are the same at every α-carbon atom, then the chain takes up a regular helical folding. In Part I (Ramakrishnan, 1964) methods were described of calculating the parameters of such a helix, mainly the number of residues per turn, n, and the unit translation of the residue along the helix, h, from a knowledge of the geometry of the peptide group and the relative orientations of the linked peptide groups. However, not all possible orientations will be stereochemically allowed, because of the short contacts between the atoms of the adjacent residues. A study of this type has been made using certain definite stereochemical criteria and the preliminary results corresponding to the angle N-αC-C′ (τ) = 100° have already been published (Sasisekharan, 1962;

Ramachandran, Ramakrishnan, and Sasisekharan, 1963*a* and *b*). This paper describes the fuller details of the results for this value of the angle, τ, and also for two other values of τ, namely 105 and 115°. In addition, a complete survey of the structures of di- and tripeptides, cyclic peptides, and polypeptides has also been made to check the predicted ranges of allowed conformations. These results are discussed in addition to a study of the conformations which occur in myoglobin.

It may be mentioned that although studies of this type have been reported so far in the literature (Huggins, 1943; Bragg, Kendrew, and Perutz, 1950; Pauling, Corey, and Branson, 1951; Low and Baybutt, 1952; Low and Grenville-Wells 1953; Donohue, 1953; Lindley and Rollett, 1955; Shimanouchi and Mizushima, 1955; Scheraga, 1960), they have not been definitive. Mizushima and Shimanouchi (1961) have taken into account the restrictions or barriers due to the rotation about the two single bonds meeting at the α-carbon atoms and have preferred nine conformations for the polypeptide chain. More recently, De Santis *et al.* (1965) have considered this problem from potential energy considerations, and Nemethy and Scheraga (1965) have obtained interesting results regarding extended chains and the formation of closed loops containing S-S bridges.

The notation used here for representing the relative orientation of the linked peptide groups is the same as that described in Part I (Ramakrishnan, 1964). The rotations of the two peptide groups are denoted by two dihedral angles (ϕ, ϕ') respectively, these being measured from an initial standard conformation $\phi = \phi' = 0°$, in which the planes of the two peptide groups lie in the plane N-αC-C′ containing the two axes of rotation, and the NH groups of the two residues are pointing towards each other.[1]

DETERMINATION OF ALLOWED CONFORMATIONS

In order to determine the allowed conformations, the contact distances between the atoms in the adjacent residues have to be examined using criteria for minimum van der Waals contact distances. This is best done by first working out the positions of

[1] At a recent conference of some of the representative workers in this field held in Bethesda, it was decided to denote the two dihedral angles about the bonds N—C_α and C_α—C′ by ϕ and ψ respectively, the sense of rotation being the same as that adopted here. The fully extended chain, with N—H and C′=O *trans* with respect to one another, is to be taken as the standard conformation with $\phi = \psi = 0$.

It is readily seen that the new ϕ is the same as the old ϕ, but that $\psi = 180° + \phi'$ (and $\phi' = 180° + \psi$). All the data reported here are thus readily converted into the (ϕ, ψ) coordinates. The diagrams in Figs. 2, 3, and 6 have to be shifted up by 180°, or half the total length, along the vertical direction. In particular, the right- and left-handed alpha helices will have (ϕ, ψ) equal to (133°, 123°) and (227°, 237°). As before, a helix with ($-\phi$, $-\psi$) will be inverse to one with (ϕ, ψ); *i.e.*, it will be of opposite sense, but having the same number of turns per unit.

This paper was finalized well before this meeting, and so the older conventions are adopted here. However, it is proposed to use the new notation in the following papers in this series.

the atoms in the two residues for various values of (ϕ, ϕ'). Actually, the coordinates of the atoms in the two residues were calculated with respect to a suitably chosen fixed coordinate system, from which the various contact distances were calculated for the various values of (ϕ, ϕ'). The following fixed system of coordinates was found to be convenient for this purpose.

The α-carbon atom C_2 at which the two residues [C_1—$C_1'O_1$—N_1H_1—C_2] and [C_2—$C_2'O_2$—N_2H_2—C_3] are linked together is taken to be the origin of coordinates of a system of axes $OXYZ$ defined as follows. The plane $N_1C_2C_2'$, which remains unchanged whatever be the rotation of the two groups, is taken as the XY plane with the Z axis upwards. The direction C_1C_2, which lies in the XY plane for $\phi = 0°$, is taken as the Y axis. The Z axis is the third perpendicular direction, such that the X, Y, and Z axes form a right-handed system of coordinates. This is shown in Fig. 1.

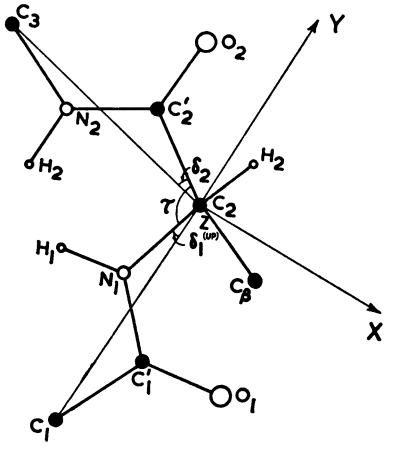

FIGURE 1 The two peptide groups in the initial conformation $\phi = \phi' = 0°$. The coordinate axes X, Y, and Z used in the present study are also marked.

Throughout this study, the peptide group has been taken to be planar with NH and CO groups in the *trans* configuration and having the dimensions given by Corey and Pauling (1953).

If x, y, z and x', y', z' are the coordinates of an atom before and after rotation, the relations connecting these are given by (Whittaker, 1952)

$$\begin{aligned} x' &= (a^2 + b^2 - c^2 - d^2)x + 2(bc - ad)y + 2(bd + ac)z \\ y' &= 2(bc + ad)x + (a^2 - b^2 + c^2 - d^2)y + 2(cd - ab)z \\ z' &= 2(bd - ac)x + 2(cd + ab)y + (a^2 - b^2 - c^2 + d^2)z \end{aligned} \tag{1a}$$

where

$$a = \cos(\omega/2), \quad b = L\sin(\omega/2), \quad c = M\sin(\omega/2), \quad d = N\sin(\omega/2) \tag{1b}$$

ω being the angle of rotation and L, M, N, the direction cosines of the axis of rotation with respect to the chosen system of coordinates. The sign of ω is taken

to be positive, when the rotation is counterclockwise viewing along the axis towards the origin.

The angles of rotation and direction cosines of the axes of rotation corresponding to the two peptide groups about the axes N_1—C_2 and C_2—C_2' are given in Table I.

The coordinates of the atoms C_1, C_1', O_1, and H_1 were evaluated at intervals of 10° of ϕ and the coordinates of the atoms O_2, N_2, H_2, and C_3 were evaluated at intervals of 10° for ϕ', using the relations (1) for three values of the angle τ, namely, 105, 110, and 115°. In fact, all the calculations corresponding to $\tau = 110$ and 115° were carried out using a desk calculator, while those corresponding to $\tau = 105°$ were carried out much later using an Elliott-803 electronic computer.

The next step towards working out the allowed conformations is to choose a set of permitted minimum contact distances between the different types of atoms. Only those conformations (ϕ, ϕ') which do not have any of the contact distances less than these minimum values now become allowed. Two such sets are given in Table II, termed "normally allowed" and "outer limit" contact distances. These are

TABLE I

ANGLES OF ROTATION AND THE DIRECTION COSINES OF THE AXES OF ROTATION USED IN THE RELATIONS (1)

Axis of rotation	Angle of rotation	Direction cosines of the axes of rotation		
		L	M	N
N_1—C_2	ϕ	$-\sin \delta_1$	$-\cos \delta_1$	0
C_2—C_2'	ϕ'	$-\sin \delta_1'$	$\cos \delta_1'$	0

where $\delta_1 = C_1\hat{C}_2N_1$, $\delta' = 180° - (\tau + \delta_1)$ and $\tau = N_1\hat{C}_2C_2'$

TABLE II

MINIMUM CONTACT DISTANCES ASSUMED

Contact	Normally allowed	Outer limit
	A	*A*
C ··· C	3.20	3.00
C' ··· C'	2.95	2.90
C ··· O	2.80	2.70
C ··· N	2.90	2.80
C ··· H	2.40	2.20
O ··· O	2.70	2.60
O ··· N	2.70	2.60
O ··· H	2.40	2.20
N ··· N	2.70	2.60
N ··· H	2.40	2.20
H ··· H	2.00	1.90

essentially the same as those given by Ramachandran, Ramakrishnan, and Sasisekharan (1963) arrived at from a study of the contact distances observed in structures of various organic compounds. Contact distances in between the two limits have also been observed in actual crystals, but not as frequently as the normally allowed values.

In order that the results can be applied to actual polypeptide and protein structures, the contact distances of the side chain atoms with those of the backbone atoms must also be considered. However, in this study, only the contact distances of the backbone atoms with the β-carbon atom were considered (see Appendix for the effect of C_γ).

The position of the β-carbon atom was fixed (using a stereographic projection technique) in such a way that the distance C_α—C_β is 1.54 A and that the angles N_1-C_2-C_β and C_2'-C_2-C_β had tetrahedral values (119°30′). Of the two possible positions of C_β, the one corresponding to the L-type of residues has been used in all these studies and hence the results pertain to L-amino acid residues. However, for D-amino acids, the corresponding allowed conformations will be $(-\phi, -\phi')$, where (ϕ, ϕ') refers to L-amino acids.

Since the positions of the atoms N_1, C_2' (which lie on the axis of rotation) and C_β do not change whatever the angle of rotation of the two residues is, only the following contact distances were calculated initially: (*a*) from C_β and C_2' to C_1, C_1', O_1 and H_1 for values of ϕ from 0 to 360° at intervals of 10°; and (*b*) from C_β and N_1, to O_2, N_2, H_2, and C_3 for values of ϕ' for 0 to 360° at intervals of 10°. These calculations were carried out corresponding to all three values of τ, namely, 105, 110, and 115°. When the ranges of ϕ and ϕ' allowed by each contact, using the minimum contact distances given in Table III, are combined, the resulting ranges given in the first half (*a*) of Table III are obtained.

Within the permitted ranges of ϕ and ϕ' given in Table III, the contact distances between the hydrogen atom, H, attached to the α-carbon atom and the atoms of the backbone were also examined and it was found that for all the three values of τ, no further restrictions arise due to these contact distances.

When the remaining contact distances *between* the atoms occurring in the *backbones* of the *two* residues (the positions of which change during the rotations ϕ or ϕ') were examined in the permitted range given in Table III, contact distances less than the minimum allowed values were found to occur for some values of (ϕ, ϕ') and thus some more conformations became disallowed on account of these short contacts. The resulting diagrams showing the regions of stereochemically allowed conformations are shown in Figs. 2*a*, *b*, and *c* corresponding to the three values of τ. In all these figures, the normally allowed regions are shown by continuous lines, while the boundaries of the region allowed by the outer limit values are shown by broken lines.

In order that the results can be applied to the conformations occurring with glycyl

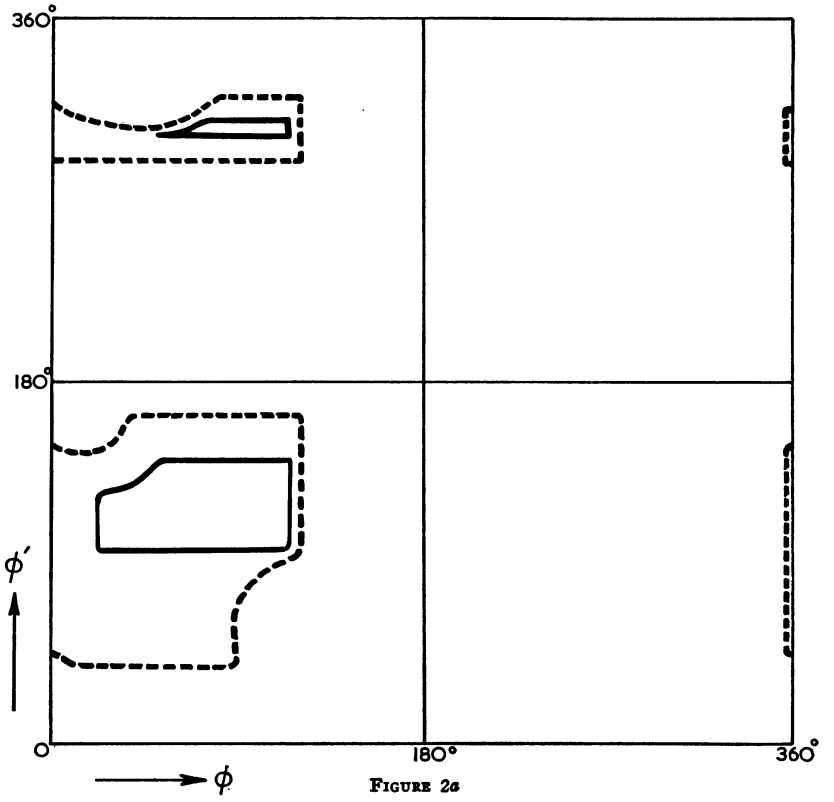

FIGURE 2a

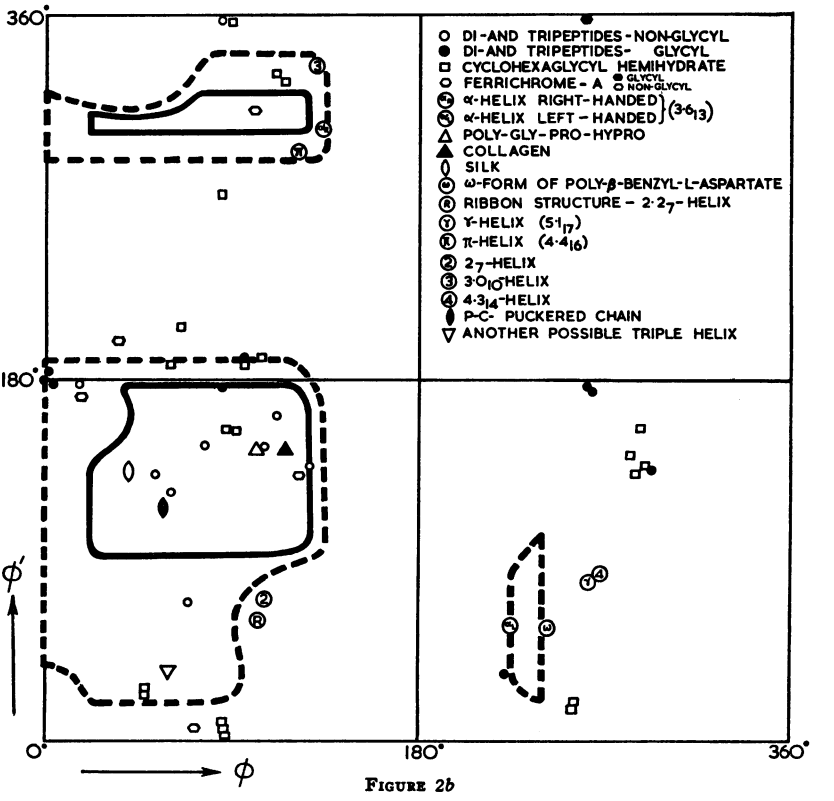

FIGURE 2b

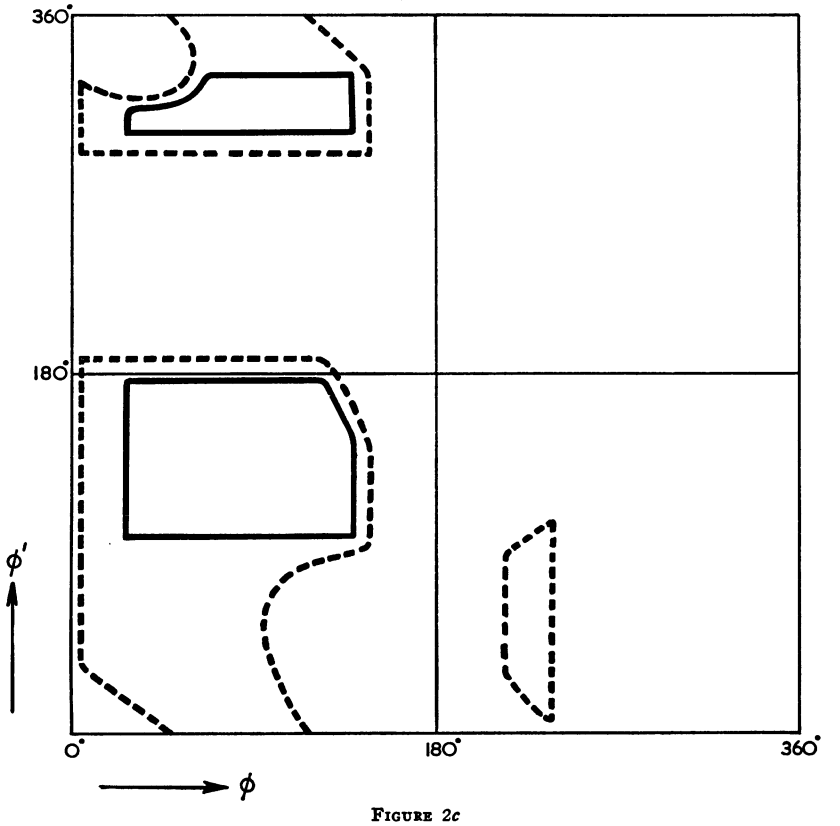

FIGURE 2c

FIGURE 2 The normally allowed (———) and the outer limit (– – – –) regions of (ϕ, ϕ') for (*a*) $\tau = 105°$, (*b*) $\tau = 110°$, (*c*) $\tau = 115°$. The conformations of known simple peptides, polypeptides, and proteins are also marked in Fig. 2*b*.

residues (*i.e.* where there is no β-carbon atom), the contract distances between the hydrogen atoms attached to the α-carbon atom and the various backbone atoms were evaluated for $\tau = 110$ and 115°. The two hydrogen atoms were fixed at tetrahedral directions to N_1—C_2 and C_2—C_2' and at a distance of 1.0A from C_2. It was found that both for $\tau = 110$ and 115°, for all values of ϕ and ϕ', there were no short contacts between the hydrogen atoms attached to the α-carbon atom and the backbone atoms. As a result, the permitted ranges of ϕ and ϕ' are larger for glycyl residues than for non-glycyl residues. The permitted ranges are given in the second half (*b*) of Table III. The regions of allowed conformations for glycyl residues when all the contact distances were taken into account are shown in Figs. 3*a* and *b*, where shaded regions are those allowed by only the outer limits, but not the normally allowed contact distances.

It may be mentioned that for glycyl residues the contact distances between any

TABLE III

RANGES OF ϕ AND ϕ' ALLOWED BY THE CONTACT DISTANCES BETWEEN THE ATOMS N_1, C_2', C_β AND THE REMAINING BACKBONE ATOMS IN THE TWO RESIDUES FOR NON-GLYCYL RESIDUES AND BETWEEN THE ATOMS N_1, C, H_2', AND THE REMAINING BACKBONE ATOMS FOR GLYCYL RESIDUES

	Normally allowed range		Outer limit range	
	ϕ	ϕ'	ϕ	ϕ'
(*a*) Non-glycyl residues				
105°	22 to 115°	95 to 141° and 301 to 309°	0 to 120° and 357 to 360°	38 to 163° and 289 to 322°
110°	22 to 127°	92 to 179° and 300 to 320°	0 to 135° and 225 to 241°	20 to 190° and 289 to 338°
115°	27 to 139°	98 to 177° and 301 to 330°	4 to 147° and 213 to 237°	0 to 188° and 290 to 360°
(*b*) Glycyl residues				
110°	0 to 127° and 233 to 360°	40 to 320°	0 to 135° and 225 to 360°	22 to 338°
115°	0 to 139° and 221 to 360°	30 to 330°	0 to 147° and 213 to 360°	No restriction

two atoms of the backbone in the adjacent residues for any conformation (ϕ, ϕ') is the same as for the inverse conformation ($-\phi$, $-\phi'$) [as can be seen from the relations (1), where, if x', y', and z' are the coordinates of a backbone atom for ϕ (or ϕ') then the coordinates of the same atom for $-\phi$ (or $-\phi'$) are x', y', and $-z'$].

The results shown in Figs. 2 and 3 will be useful in investigating the conformation at an α-carbon atom when the two dihedral angles ϕ and ϕ' are known. For polypeptide and protein chains, the parameters that are obtainable from x-ray diffraction studies are the number of residues per turn, n, and the unit translation, h, (Ramachandran, 1960) along the axis of the helix. Hence, the stereochemically allowed combinations of n and h were evaluated using the detailed results of the calculations described in Part I (Ramakrishnan, 1964) and are represented graphically in Figs. 4*a*, *b*, and *c* for the three values of τ. In these figures also, the regions of (n, h) allowed by the normally allowed contact distances are shown by continuous line boundaries, while those allowed by outer limit values are shown by broken lines. The origins in these figures correspond to $n = \pm 2$, and $h = 0$ A. The positive and

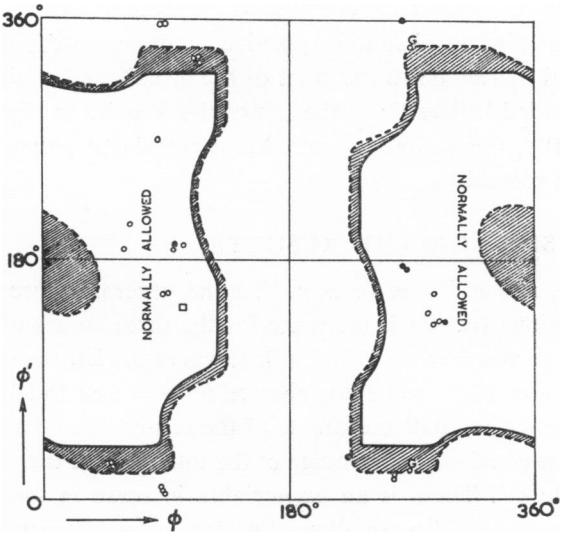

FIGURE 3*a*

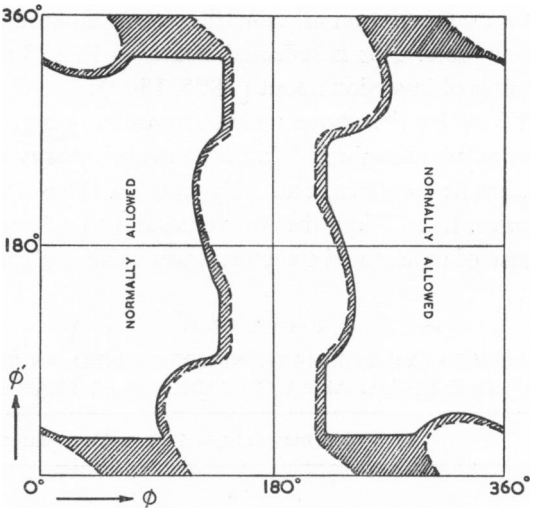

FIGURE 3*b*

FIGURE 3 Allowed regions of (ϕ, ϕ') for (*a*) $\tau = 110°$, (*b*) $\tau = 115°$, for glycyl residues. The shaded regions represent conformations which are allowed only by the outer limit contact distances. The glycyl conformations observed in simple peptides are also marked in Fig. 3*a*: ● = Di- and Tripeptides; ○ = Cyclic peptides; □ = Polyglycine II.

negative directions of the X axes correspond respectively to positive and negative values of n, but h is always taken to be positive. In other words, plots corresponding to all right-handed helices lie to the right of the Y axis and similarly points representing all left-handed helices lie to the left of the Y axis. In Fig. 4*b*, which corresponds to $\tau = 110°$, the various observed and postulated polypeptide and protein structures are also plotted.

DISCUSSION OF THE RESULTS

From Figs. 2*a*, *b*, and *c*, it can be seen that the general nature of the regions of allowed conformations (ϕ, ϕ') is the same for the three values of τ. An interesting feature is that the region around (220°, 60°), where the left-handed α-helix occurs, is not allowed for $\tau = 105°$, while it is allowed for $\tau = 110$ and 115°.

In order to have a quantitative estimate of the results, the area under the allowed regions may be expressed as a percentage of the total area of the plane ϕ—ϕ'. These are given in Table IV. There is an appreciable increase in the range of allowed regions with increasing τ. Also the allowed region is much larger for glycyl residues than when a β-carbon atom is present (about 6 times for normally allowed and 2.5 times for outer limit for $\tau = 110°$). Thus there is more freedom of rotation about the single bonds when the amino acid residue at the α-carbon atom is glycine than when it contains a β-carbon atom.

Since the α-carbon atom is not an asymmetric carbon atom in the case of glycine, no D or L isomer is possible. This is reflected clearly in Figs. 3*a* and *b*, which have the symmetry of a center of inversion about (180°, 180°).

In order to verify how far the above results from theory agree with observation, the parameters ϕ and ϕ' were evaluated from the available crystal structure data on di- and tripeptides, cyclic peptides, and polypeptides. These were calculated on an IBM 1620 computer. In addition, the two values of ϕ' (ϕ_1' and ϕ_2'), which alone are relevant in the case of amino acid structures, were also evaluated.

TABLE IV

AREA OF ALLOWED CONFORMATIONS EXPRESSED AS PERCENTAGE OF THE TOTAL AREA OF THE ($\phi - \phi'$) PLANE

τ	Normally allowed	Outer limit
	per cent	*per cent*
(*a*) Non-glycyl residues		
105°	3.5	12.7
110°	7.7	20.3
115°	8.8	24.2
(*b*) Glycyl residues		
110°	45.1	57.4
115°	56.7	66.8

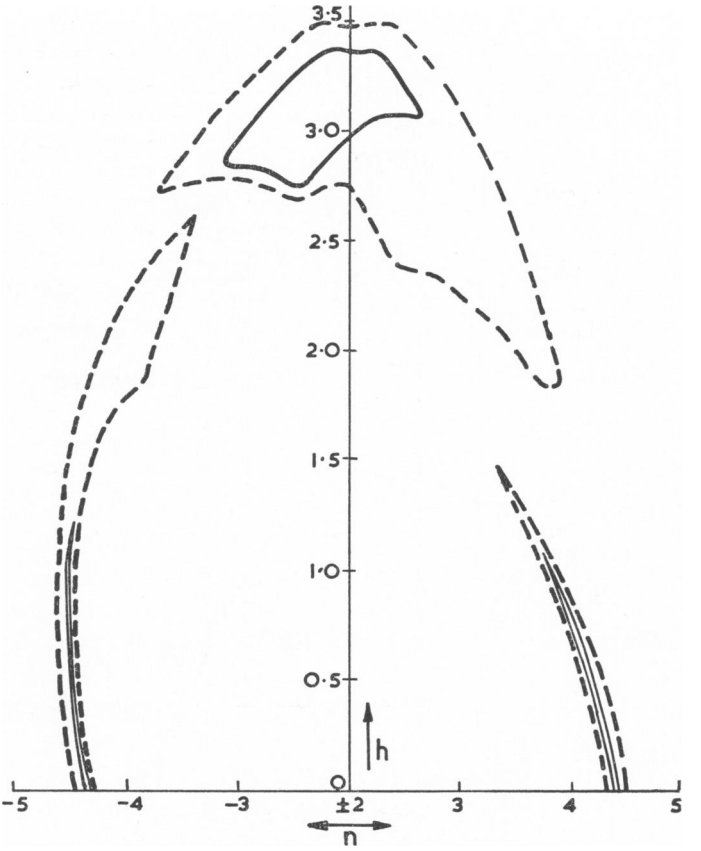

FIGURE 4*a*

Amino Acids. The two values ϕ_1', ϕ_2' for the various known crystal structures of amino acids are given in Table V along with the values of the angle N-αC-C′ (τ). In general, ϕ' lies between ± 30° and 180° ± 30°, exceptions being L-arginine HCl (Mazumdar, 1964), DL-aspartic acid (Dawson, 1953), L-glutamic acid (Hirokawa, 1955), L-tyrosine HCl and L-tyrosine HBr (Srinivasan, 1959*a* and *b*).

Di- and Tripeptides. In this case, for the N-terminal residues, ϕ is the only relevant parameter, since the positions of the hydrogen atoms attached to N_0 are not usually available. For the C-terminal residues, there is one value of ϕ and two values of ϕ' corresponding to the two oxygen atoms of the $C\begin{smallmatrix}O_0\\O_0^-\end{smallmatrix}$ group. For the other residues (middle), there is one value of ϕ and one value of ϕ'. These are shown in Fig. 5, where the α-carbon atom under consideration is C_1. The results are given in Table VI along with the value of the angle N-αC-C′ (τ).

Considering the N-terminal residues, for which only ϕ' is relevant, the observed

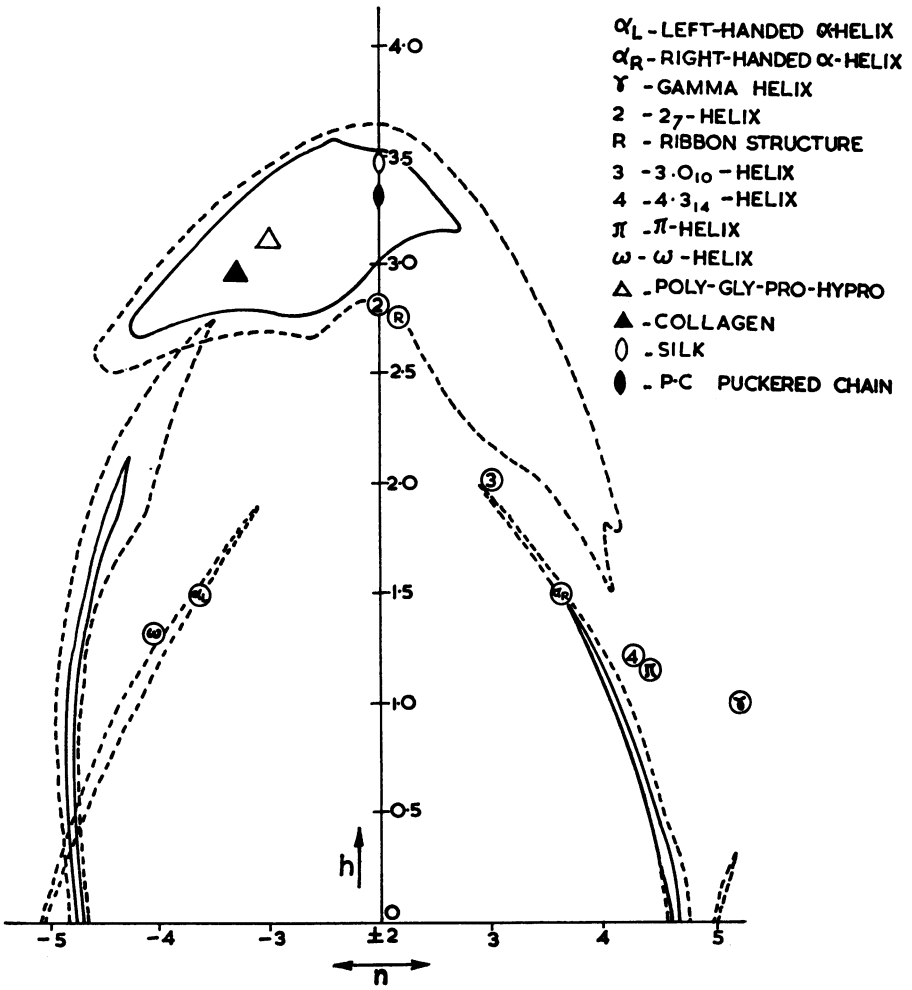

FIGURE 4*b*

values of ϕ' lie well within the allowed region mentioned in Table III both in the case of glycyl and non-glycyl residues. In addition, ϕ', in general, lies between $180° \pm 30°$ agreeing well with the trend observed in amino acid structures.

In the case of C-terminal and middle residues, the conformations are plotted in Fig. 2*b*, which also contains the allowed regions of (ϕ, ϕ') for $\tau = 110°$. For better comparison the conformations at the α-carbon atoms corresponding to glycyl residues are also plotted in Fig. 3*a* which gives the allowed regions of (ϕ, ϕ') for such residues for $\tau = 110°$.

Both for glycyl and non-glycyl residues, almost all the conformations lie within the allowed region. In the case of non-glycyl residues it is very interesting to note

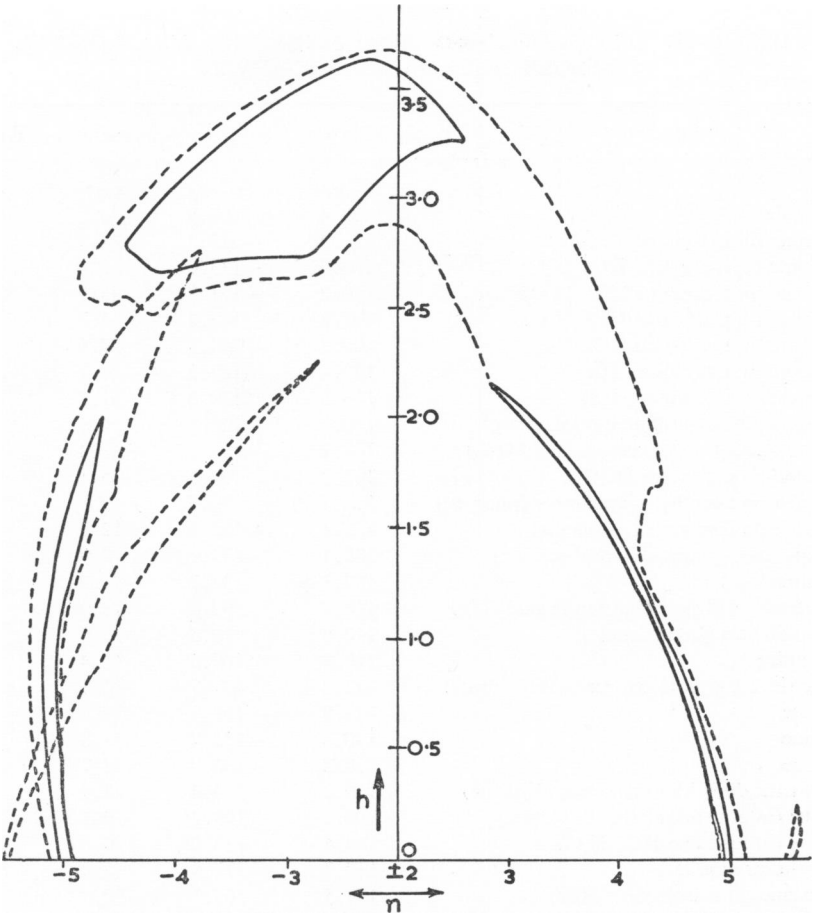

FIGURE 4*c*

FIGURE 4 The normally allowed (———) and the other limit (– – – –) regions of (n, h) for (a) $\tau = 105°$, (b) $\tau = 110°$, (c) $\tau = 115°$. The known polypeptide and protein structures are also marked in Fig. 4*b*—(αL) = left-handed α-helix; (αR) = right-handed α-helix; (γ) = gamma helix; (2) = 2_7-helix; (R) = ribbon structure; (3) = 3.0_{10}-helix; (4) = 4.3_{14}-helix; (π) = π-helix; (ω) = ω-helix; Δ = poly-gly, -pro, -hypro; ▲ = collagen; ○ = silk; ● = P-C puckered chain.

that six out of nine conformations listed in Table VI lie well within the normally allowed region and two more lie within the outer limit but outside the normally allowed region. The one conformation that lies outside the allowed region is that of the cysteine residue of the compound glutathione. But the value of τ in this case is 115° and so the conformation comes into the allowed region corresponding to $\tau = 115°$ (Fig. 2*c*). In addition, the value of ϕ' in many cases lies close to 150°, which agrees well with the trend observed in amino acids.

TABLE V

OBSERVED VALUES OF ϕ' IN AMINO ACIDS. THE VALUE OF THE ANGLE N-αC-C' (τ) IS ALSO GIVEN

Amino acid	τ	ϕ_1'	ϕ_2'	Reference
	degrees	*degrees*	*degrees*	
DL-Alanine	108.4	162.9	−15.3	1
L-Arginine (in L-arginine dihydrate)	110.9	−169.3	12.1	2
*L-Arginine (in L-arginine HBr, H_2O)	109.5	−173.6	2.8	3
L-Arginine (in L-arginine HBr, H_2O)	110.2	−151.2	23.9	3
*L-Arginine (in L-arginine HCl, H_2O)	107.8	−174.2	1.7	3
L-Arginine (in L-arginine HCl, H_2O)	109.5	−154.3	22.6	3
*L-Arginine (in L-arginine HCl)	109.4	129.2	−45.7	3
L-Arginine (in L-arginine HCl)	108.0	139.6	−41.7	3
Asparagine (in asparagine monohydrate)	110.1	−168.8	6.1	4
DL-Aspartic acid (in DL-aspartic acid HCl)	109.7	−137.8	42.4	5
L-Cysteine (in L-cysteine HCl)	105.2	169.1	−5.1	6
L-Cysteine (in S-methyl-L-cysteine sulphoxide)	111.1	176.7	−9.8	7
L-Cysteine (in hexagonal L-cysteine)	108.4	−165.8	12.3	8
L-Cystine (in L-cystine dihydrobromide)	108.1	−179.2	0.9	9
L-Glutamic acid	108.3	134.2	−31.9	10
DL-Glutamic acid (in DL-glutamic acid HCl)	110.7	−162.4	18.0	11
L-Glutamine (in glutathione)	110.6	170.5	−12.5	12
L-Glutamine	110.8	160.5	−11.4	13
Glycine (in bis glycino copper monohydrate)	111.3	171.7	−6.1	14
α-Glycine	111.8	−160.9	18.4	15
β-Glycine	110.8	−152.7	23.8	16
γ-Glycine	110.8	−165.0	11.7	17
Histidine (in dihistidino zinc pentahydrate)	110.8	176.4	−3.9	18
Histidine (in dihistidino zinc dihydrate)	109.0	−168.2	9.1	19
Histidine (in histidine HCl, H_2O)	109.4	180.0	0.0	20
Hydroxy-L-proline II	110.7	−177.0	2.0	21
D-Isoleucine (in D-isoleucine HBr)	108.5	165.7	−20.6	22
L-Leucine (in L-leucine HBr)	110.1	160.4	−12.3	23
L-lysine (in L-lysine HCl)	109.7	160.1	−17.2	24
α-Methionine	112.1	146.3	−30.4	25
β-Methionine	110.0	150.6	−31.6	25
DL-Norleucine	109.0	144.6	−24.4	26
Proline (in copper proline dihydrate)	109.7	−169.0	11.0	27
DL-Serine	110.1	−176.3	1.2	28
L-Threonine	110.4	153.9	−28.8	29
Tryptophan	111.9	175.8	21.4	30
L-Tyrosine (in L-tyrosine HCl)	110.1	142.1	−25.4	31
L-Tyrosine (in L-tyrosine HBr)	108.4	143.9	−30.4	32
L-Valine (in L-valine HBr)	108.1	168.4	−14.7	33
L-Valine (in L-valine HCl)	105.9	172.2	−8.9	34
L-Valine (in L-valine HCl, H_2O)	108.2	−173.1	5.3	35

*In the structures of L-arginine HBr, L-arginine HCl, H_2O, and L-arginine HCl, there are two molecules per asymmetric unit.

References: (1) Donohue (1951); (2) Karle and Karle (1964); (3) Work done in Madras, Mazumdar (1964); (4) Kartha and De Vries (1961); (5) Dr. B. Dawson, private communication; (6) Work done in

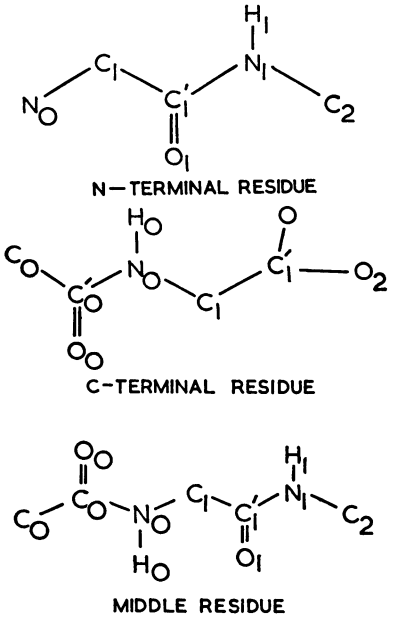

FIGURE 5 The environment around the α-carbon atom C_1 in the case of N-terminal, C-terminal, and middle residues of peptides.

Considering the glycyl residues, almost all of them lie within the allowed region. Also in the case of C-terminal glycyl residues of the compounds Cys-Gly NaI and Gly-Phe-Gly and in the case of middle and C-terminal residues in Gly-Gly-Gly copper chloride, the observed conformations are such that they are allowed only for glycyl residues.

Cyclic Peptides. Recently, the crystal structures of two cyclic hexapeptides, cyclohexaglycyl hemihydrate (Karle and Karle, 1963), and Ferrichrome A (Zalkin, Foster, and Templeton, 1964) have been reported. Of these, the former contains four molecules per asymmetric unit and offers twenty four glycyl conformations for investigation. The latter hexapeptide (Ferrichrome A) with the sequence Gly-Ser-Ser-Orn-Orn-Orn (Orn standing for ornithine) gives six conformations, one glycyl and five non-glycyl. The parameters (ϕ, ϕ') for these two hexapeptides are given in

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Madras; (7) Hine (1962); (8) Oughton and Harrison (1959); (9) Peterson *et al.* (1960); (10) Hirokawa (1955); (11) Dawson (1953); (12) Wright (1958); (13) Cochran and Penfold (1952); (14) Freeman *et al.* (1964*b*); (15) Marsh (1958); (16) Itaka (1960); (17) Itaka (1961); (18) Harding and Cole (1963); (19) Kretsinger and Cotton (1963); (20) Donohue and Caron (1964); (21) Donohue and Trueblood (1952); (22) Trommel and Bijvoet (1954); (23) Subramanian (1965); (24) Wright and Marsh (1962); (25) Mathieson (1952); (26) Mathieson (1953); (27) Mathieson and Welsh (1952); (28) Shoemaker *et al.* (1953); (29) Shoemaker *et al.* (1951); (30) Work done in Madras; (31) Srinivasan (1959*a*); (32) Srinivasan (1959*b*); (33) Parthasarathy (1962); (34) Work done in Madras; (35) Work done in Madras.

TABLE VI

THE CONFORMATIONS (ϕ, ϕ') OBSERVED IN DI- AND TRIPEPTIDES. THE ANGLE N-αC-C′ AT THE CORRESPONDING α-CARBON ATOMS ARE ALSO LISTED

Peptides		τ	ϕ	ϕ_1'	ϕ_2'	Reference*
		degrees	*degrees*	*degrees*	*degrees*	
N-acetyl glycine		110.4	1.5	−176.4	3.3	1
(*a*) Dipeptides						
β-Gly-Gly	N-term-Gly	110.0	—	−155.4	—	2
	C-term-Gly	110.7	−0.8	−180.0	4.7	2
Gly-L-Asp	N-term-Gly	111.2	—	−171.4	—	3
	C-term-Asp	109.5	69.0	−116.7	69.2	3
Gly-L-Tyr	N-term-Gly	112.1	—	173.6	—	4
	C-term-Tyr	111.4	76.6	146.6	−30.9	4
Gly-L-Tyr	N-term-Gly	112.1	—	157.4	—	5
	C-term-Tyr	112.0	106.3	146.7	−23.7	5
N-N′ diglycyl	N-term-Gly	109.0	—	−137.1	—	6
cystine	C-term-Cys	109.5	16.5	178.8	−3.7	6
Cys-Gly sodium	N-term-Cys	111.5	—	171.7	—	7
iodide	C-term-Gly	125.7	−136.8	−147.6	33.1	7
Glutathione	Middle-Cys	115.0	89.3	−2.8	—	8
(γ-Glu-Cys-Gly)	C-term-Gly	109.5	95.5	−169.1	10.0	8
p-tosyl-L-Pro-L-	N-term-Pro	106.4	—	163.8	—	9
hypro	C-term-Hypro	117.8	128.5	136.6	−43.6	9
L-Thr-L-Phe	N-term-Thr	106.4	—	140.9	—	10
	C-term-Phe	107.8	61.4	123.5	−67.0	10
L-Leu-Gly	N-term-Leu	109.1	—	−144.0	—	11
	C-term-Gly	112.3	86.4	176.6	2.9	11
(*b*) Tripeptides						
	N-term-Gly	107.9	—	169.4	—	12
Gly-Gly-Gly-$CuCl_2$	Middle-Gly	111.1	−65.6	133.4	—	12
	C-term-Gly	110.7	−94.0	173.3	−4.7	12
	N-term-Gly	109.9	—	126.8	—	13
Gly-L-Phe-Gly	Middle-Phe	107.0	52.9	132.7	—	13
	C-term-Gly	114.7	−96.5	175.4	−0.9	13
	N-term-Leu	107.9	—	152.7	—	14
L-Leu-L-Pro-Gly	Middle-Pro	111.3	112.2	162.5	—	14
	C-term-Gly	114.5	3.7	178.1	0.5	14

*(1) Donohue and Marsh (1962); (2) Hughes and Moore (1949); (3) Pasternak *et al.* (1954); (4) Smith and Wiebenga (1953); (5) Pasternak (1956); (6) Yakel and Hughes (1954); (7) Dyer (1951); (8) Wright (1958); (9) Fridrichsons and Mathieson (1962); (10) Work done in Madras; (11) Work done in Madras; (12) Freeman *et al.* (1964*a*); (13) Marsh and Glusker (1961); (14) Leung and Marsh (1958).

Table VII[2] and are plotted in Fig. 2*b*. The glycyl conformations are also plotted in Fig. 3*a*.

In the case of cyclohexaglycyl hemihydrate, a majority of the conformations lie within the allowed regions. However, a few of them (1, 5, 7, 10, 12, 13, 15) are clustered around 0° (or 360°) of ϕ'. Although these conformations are not allowed for $\tau = 110°$, they do become allowed for higher values of τ (see Fig. 3*b*). It can be

TABLE VII

OBSERVED CONFORMATIONS (ϕ, ϕ') IN CYCLOHEXAGLYCYL HEMIHYDRATE AND IN FERRICHROME A

	Residue	τ	ϕ	ϕ'
		degrees	*degrees*	*degrees*
Cyclohexaglycyl hemihydrate*				
	Gly (1)	112.8	85.6	9.9
	Gly (2)	111.2	111.6	−29.2
	Gly (3)	111.7	85.4	−88.9
	Gly (4)	112.6	112.7	−29.3
	Gly (5)	110.8	86.6	6.9
	Gly (6)	113.0	115.6	−33.0
	Gly (7)	112.1	86.8	5.8
	Gly (8)	111.5	114.5	−32.6
	Gly (9)	110.2	49.4	24.3
	Gly (10)	116.2	89.7	−3.8
	Gly (11)	110.0	48.2	26.3
	Gly (12)	115.4	86.9	−1.6
	Gly (13)	114.9	−103.1	16.9
	Gly (14)	102.4	87.3	155.1
	Gly (15)	113.7	−102.9	18.1
	Gly (16)	105.3	−71.8	155.9
	Gly (17)	105.3	−71.8	154.9
	Gly (18)	109.5	−76.4	141.0
	Gly (19)	109.7	−72.3	133.3
	Gly (20)	105.3	−68.2	135.3
	Gly (21)	110.2	65.0	−153.3
	Gly (22)	108.3	60.1	−171.8
	Gly (23)	110.0	95.7	−171.1
	Gly (24)	109.5	104.1	−169.2
Ferrichrome A‡				
	Gly	114.7	−98.1	−1.2
	Ser 1	114.2	123.7	132.3
	Ser 2	104.0	17.3	171.5
	Orn 1	113.5	71.6	6.6
	Orn 2	113.2	101.4	−47.2
	Orn 3	106.1	34.8	−160.7

*Karle and Karle (1963); marked by the symbol, □, in Fig. 2*b*.
‡Zalkin *et al.* (1963); marked by the open hexagon in Fig. 2*b*.

[2] The coordinates of the atoms in the cyclic hexapeptide Ferrichrome A has kindly been furnished by Dr. Zalkin to us prior to publication.

seen from Table VII that for all these conformations, the value of τ is systematically greater than 110° and thus these conformations also become allowed, under the short contact criteria adopted in this paper.

In the case of the cyclic peptide Ferrichrome A, the conformations corresponding to Ser 1, Ser 2, and Orn 1 lie well within the allowed region. Though the conformation corresponding to Orn 2 apparently lies outside the allowed region, it is allowed on account of the higher value of τ (114°) at the α-carbon atom. The corresponding Orn 3 has a short contact $C_\beta \ldots N_2$ according to the present study, but in the actual structure this has a value of 2.96 A, which is greater than the minimum value given in Table II. Thus this conformation is also allowed. The conformation corresponding to the glycyl residue has a value of 115° for τ and hence becomes allowed (Fig. 3*b*). An interesting feature of this conformation is that it is allowed only for a glycyl residue. The value of ϕ' for this conformation lies close to 360° which is also the case with many conformations in the previous cyclic peptide.

Peptides in the Non-Helical Regions of Myoglobin. The structure of the crystalline protein myoglobin contains long segments of α-helices linked by non-helical peptide segments (Kendrew *et al.* 1960, 1961). The conformations at the α-carbon atoms in these non-helical regions have been plotted by Dr. H. C. Watson of Medical Research Council, Cambridge, England, who has kindly made the plot available to the authors prior to publication. Most of the conformations lie within the allowed regions for $\tau = 110°$, with a clustering of conformations around that of the right-handed α-helix. A few are allowed only for $\tau > 110°$. In fact, two conformations lie far outside the allowed limits, namely those of the residues EF3 and HC2. On examination, it is found that the short contact responsible for disallowing those two involve the β-carbon atom, so that they will become allowed if they were glycyl residues. When this fact was communicated to Dr. Watson, it was learned that these were in fact glycyl residues, as expected. The others that lie just outside the allowed regions have ϕ' close to 0° (or 360°) and hence are likely to have a value of τ greater than 110°, in which case they also come within the allowed region.[3]

Polypeptides and Proteins. The conformations of the various structures proposed for polypeptides and fibrous proteins have been calculated from the reported coordinates of the atoms and are given in Table VIII along with the data on the number of residues per turn, n, and the unit translation, h, along the axis of the axis of the helix. These are plotted in Fig. 2*b* and also in Fig. 4*b*, which gives the allowed combinations of (n, h) for $\tau = 110°$.

All the structures except four (γ-helix, 4.3_{14}-helix, 2_7-helix, and 2.2_7-helix) lie within the allowed regions, some well within the normally allowed regions and some others only within the outer limit region. These structures are discussed below.

[3] The $\phi - \phi'$ diagram for myoglobin will be published shortly by Dr. Kendrew and Dr. Watson (private communication).

TABLE VIII

OBSERVED AND POSTULATED CONFORMATIONS (ϕ, ϕ') FOR POLYPEPTIDE AND PROTEIN STRUCTURES. THE PARAMETERS n AND h ARE ALSO LISTED

Polypeptide or protein	τ	ϕ	ϕ'	n	h	Reference*
	degrees	*degrees*	*degrees*		*A*	
α-helix (3.6_{13})	109.5	133.0	−57.2	3.615	1.495	1
γ-helix (5.1_{17})	110.1	−96.3	78.0	5.143	0.98	2
2_7a-helix	111.3	105.1	69.5	2.000	2.80	1
2.2_7-helix (ribbon structure)	111.6	101.9	59.2	2.169	2.75	3
3.0_{10}-helix	111.6	130.7	−25.7	3.000	2.00	3
4.3_{14}-helix	100.5	−91.9	91.7	4.337	1.20	3
π-helix (4.4_{16})	114.9	122.9	−69.7	4.40	1.15	4
Polyglycine II	109.1	102.0	145.8	−3.00	3.10	5
Poly-L-proline II	110.0	102.8	145.9	−3.00	3.12	6
Poly-L-proline II	108.8	103.7	145.1	−3.00	3.12	7
Poly-L-hydroxyproline A	105.5	103.1	147.6	−3.00	3.05	8
ω-form of poly-β-benzyl-L-aspartate	109.9	−115.6	55.4	−4.00	1.325	9
Collagen type helix	110	116	145	−3.28	2.95	10
Silk	110	40	135	2.00	3.45	11
P-C puckered chain	110	57	118	2.00	3.3	12

*(1) Bamford *et al.* (1956); (2) Pauling and Corey (1951); (3) Donohue (1953); (4) Low and Grenville-Wells (1953); (5) Crick and Rich (1955); (6) Sasisekharan (1959*a*); (7) Cowan and McGavin (1955); (8) Sasisekharan (1959*b*); (9) Bradbury *et al.* (1962); (10) Ramachandran (1963); (11) Marsh *et al.* (1955); (12) Pauling and Corey (1953).

α-helix. Both the right-handed α-helix (132°, -57°) and the left-handed α-helix (-132°, 57°) lie within the outer limit regions only. However, slight perturbations such as changing the value of τ would bring the right-handed helix into the normally allowed region but the left-hand α-helix is always outside the normal limits although it is just allowed by the outer limits. Also the right-handed α-helix can accommodate L-proline towards an end, since $\phi' \approx 120°$, while the left-handed α-helix cannot. Even in the former case, because the planes of the peptide groups are nearly vertical, short contacts arise between the imino carbon atom, C, and the atoms of the backbone of the residue on top of it, if it is in the middle. It has been found that the two α-helical portions adjoining the proline ring must be inclined by at least 35° in order that these short contacts may be relieved. Even in this case, there arise two short contacts $C_2' \ldots C_1' = 2.61$ A and $C_2' \ldots O_1 = 2.45$ A between atoms in the adjacent residues and these can be relieved only by distorting the planarity of the peptide group.

γ-helix. The conformation of the right-handed γ-helix (−96°, 78°) lies well outside the allowed region and hence is not likely to be observed.

2_7a-helix. This type of folding originally proposed by Huggins (1943) and designated as 2_7-helix by Bragg *et al.* (1950) has the conformation (105°, 70°) and lies apparently outside the allowed region. But the short contact in this case (as well as in the case of the 2.2_7-helix to be discussed next) is $O_1 \cdots H_2$ (1.93A). Since this distance forms part of a possible intrachain hydrogen bond with $N_2 \cdots O_1$ = 2.72 A and $H_2\hat{N}_2O_1 = 28°30'$, this is really not a short contact.

2.2_7-helix (ribbon structure). This structure proposed by Donohue (1953) has the conformation (102°, 59°) and this also apparently lies outside the allowed region. As in the previous case, the short contact $O_1 \cdots H_2$ (1.88 A) forms part of a possible intrachain hydrogen bond and so this conformation becomes allowed. In fact a similar type of folding has been observed by Kakudo *et al.* (1963) in a tetrapeptide.

3.0_{10}-helix. This structure proposed by Bragg *et al.* (1950) and Donohue (1953) has the conformation (131°, −26°) for the right-handed helix and lies close to the α-helix conformation, within the outer limit region. This structure is also quite likely to occur.

4.3_{14}-helix. This structure, also proposed by Donohue (1953), has the conformation (−92°, 92°) for the right-handed helix and lies close to the γ-helix, outside the allowed regions and hence is not likely to occur.

4.4_{16}-helix (π). The two slightly different forms of π-helices proposed by Low and Grenville-Wells (1953), one with $n = 4.3$ and $h = 1.14$ A and the other with $n = 4.4$ and $h = 1.15$ A, have the conformations (124°, −69°) and (123°, −70°) lying close to the conformation of α-helix and are quite likely to occur.

Poly-Gly, -Pro, -Hypro. The conformations of these helices (left-handed) lie close to (100°, 150°) well within the normally allowed region. In the case of poly-L-proline and poly-L-hydroxyproline, the value of ϕ is necessarily close to 120° for the imino residues to be accommodated. Also, the right-handed counterparts of these cannot accommodate imino acid residues, since the value of ϕ is close to −120° in these cases.

ω-Form of Poly-β-Benzyl-L-Aspartate. The structure of the ω-form of poly-β-benzyl-L-aspartate has been determined by Bradbury *et al.* (1962) to be a left-handed helix with a fourfold screw axis and with a folding similar to the 4_{13}-helix proposed by Bragg *et al.* (1950). The conformation of this helix is (−116°, 55°) and this lies just outside the allowed region. However, by making the peptide groups non-planar, the short contact that disallows this conformation, namely $C_\beta \cdots C_1'$, can be made to have a value of 3.13 A, which is allowed by the outer limit. Thus this structure, in the form proposed, also becomes allowed.

Silk. Silk has the conformation close to (40°, 135°) which lies well within the allowed region. The structure is slightly buckled with $h = 3.45$ A, smaller than that of a fully extended chain ($h = 3.63$ A). This is due to the presence of side chains like alanine, etc. In fact, the conformations of other β-forms of polypeptides

like polyglycine, β-poly-L-alanine, β-poly-γ-methyl-L-glutamate have conformations close to silk and are also slightly bucked.

P-C Puckered Chain. This chain conformation proposed by Pauling and Corey (1953) for the β-form of proteins has the conformation (57°, 118°), close to that of silk. But this is much more buckled and the value of h in these cases is 3.3 A, less than that for silk (3.45 A). This is fully allowed, but this chain cannot accommodate proline and this has led to the suggestion of an alternative puckered chain (Pauling and Corey, 1953; Ramachandran, 1962; Sasisekharan, 1962).

Collagen. The polypeptide chains of the collagen helix ($n = -3.28$ and $h = 2.95$ A) has the conformation (116°, 145°). Because $\phi \approx 120°$, imino acid residues can be readily accommodated. The value of ϕ' close to 180° is also a natural occurrence in the case of amino acids. Consequently, the fact that this conformation provides enough sites for imino acids and that it has a natural value of ϕ' suggest that, even without internal hydrogen bonding, the collagen chain conformation is likely to be quite stable.

The conformation of collagen lies within the normally allowed region. Another interesting feature is that if ϕ' is kept close to 120°, the value of h does not vary from 2.9 A by more than 5 per cent within the allowed region, thus explaining the relative inextensibility of collagen.

CONCLUDING REMARKS

Thus the conformations observed in simple and cyclic peptides are seen to confirm the predictions of the theory, thereby proving the validity of the stereochemical criteria assumed in this study.

Finally, the results obtained in our study agree well with those obtained by others from considerations of potential energy. Thus, Mizushima and Shimanouchi (1961), from a study of the internal rotation of simple molecules have preferred nine orientations. In our notation, these correspond to $\phi = 0$, 120, and 240°, $\phi' = 60$, 180, and 300°. These are given in Table IX, along with the values of the unit twist t and the unit translation h. An examination of these in the light of the present study shows that all except *d, e,* and *i* lie within the allowed region. Even among these conformations, *i* becomes allowed because of the formation of a hydrogen bond of the type N_2-H_2 . . . O_1, which offsets the short contact, thus leaving the conformation *d* and *e* alone to be really disallowed.

Recently, De Santis *et al.* (1963) have attempted a study of the stability of helical conformations of linear polypeptide chains from potential energy considerations. Although there is a broad similarity between their potential energy contours and the boundaries of our diagrams, there are some differences, for example a deep potential minimum corresponding to about (120°, 300°) and another one at about (160°, 60°). Neither of these are particularly populated in Fig. 2*b*, showing that

TABLE IX

PREFERRED ORIENTATIONS OF POLYPEPTIDE CHAIN IN OUR NOTATION (*ACCORDING TO MIZUSHIMA AND SHIMANOUCHI*)

Conformation	Twist angles		Unit twist	No. of residues per turn	Residue height
	ϕ	ϕ'	t	n	h
	degrees		*degrees*		*A*
a	0	180	180	2.00	3.63
b	0	−60	−96	−3.75	2.45
c	0	60	96	3.75	2.45
d	240	180	84	4.28	2.72
e	240	−60	176	2.05	2.63
f	240	60	−90	−4.00	1.16
g	120	180	−84	−4.28	2.72
h	120	−60	90	4.00	1.16
i	120	60	−176	−2.05	2.63

for a single pair of residues these are not specially favored. The potential energy data that are fed in such studies may have to be further revised.

APPENDIX

While evaluating the contact distances for non-glycyl residues, only the β-carbon atom of the side chain was taken into account. It has been possible to extend the study to working out the effect of the γ-carbon atom on the conformation of the two residues. This was made possible since it has been found in Madras, from an analysis of the observed orientation of the side chains in the structures of various amino acids and peptides, that there are three preferred positions for the γ-carbon atom.

The position of C_γ may be represented by a parameter ψ which measures the rotation of C_γ about the C_α—C_β bond from a standard initial conformation ($\psi = 0°$) in which the atom C_γ lies in the plane N-C_α-C_β and with atoms N and C_γ being in the *cis* configuration with respect to the C_α—C_β bond. Viewing from C_β to C_α, ψ is taken to be positive for an counterclockwise rotation. With this convention, the three positions of C_γ are found to lie close to values of 60, 180, and 300° for ψ, and these are indicated by indices 1, 2, and 3 respectively.

The contact distances between the γ-carbon atom in these three positions and the backbone atoms were calculated in the range of (ϕ, ϕ') allowed by the β-carbon atom (given in Table III) for $\tau = 110°$. The alterations in the outer limit boundaries thus worked out are shown in Fig. 6, where the shifts in the boundary due to the γ-carbon atom in the three positions are shown by arrows.

In all the three cases, there are some additional restrictions in the allowed ranges of either ϕ or ϕ', or both, as can be seen from Fig. 6. For the first position of C_γ ($\psi = 60°$), there are restrictions on both ϕ and ϕ', while for the second position ($\psi = 180°$), the restrictions are on ϕ' only and for the third position ($\psi = 300°$), on ϕ only.

Fig. 6 also contains the conformations of the right-handed and left-handed α-helices marked in it. It is interesting to note that for the first position C_γ ($\psi = 60°$) the con-

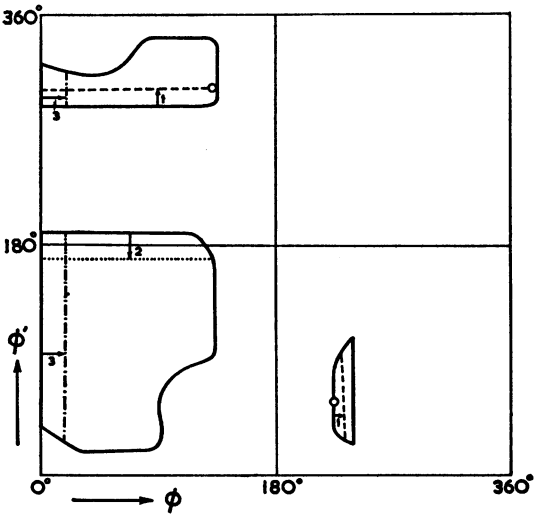

FIGURE 6 The shifts in the boundaries (indicated by →) of the allowed regions (outer limit) taking into account the restrictions due to γ-carbon atom in the three positions: ——— C_β alone; ---- with C_γ in position 1; ..... C_γ in position 2; and -.-.-.-. C_γ in position 3. The conformations of the right- and left-handed α-helices are also marked.

formation of the right-handed α-helix lies just on the boundary of the outer limit region, while the conformation of the left-handed α-helix goes outside the outer limit. In the other two cases, both are only within the outer limits and there is no obvious case for either the right-handed or the left-handed α-helix to be more likely. In the analysis made on amino acid side group conformations (Lakshminarayanan, unpublished), both L-glutamic acid and L-glutamic acid HCl have the γ-carbon atom in position 1, which may explain why poly-L-glutamic acid has a hight-handed helix. However, the three positions are found to occur fairly evenly distributed in different amino acids, and this explanation will not hold good for the very common occurrence of the right-handed alpha helix in preference to the left-handed one in various polypeptides.

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REFERENCES

BAMFORD, C. H., ELLIOTT, A., and HANBY, W. E., 1956, Synthetic Polypeptides, New York, Academic Press, 260.

BRADBURY, E. M., BROWN, L., DOWNIE, A. R., ELLIOTT, A., FRASER, R. D. B., and HANBY, W. E., 1962, *J. Mol. Biol.*, **5**, 230.

BRAGG, W. L., KENDREW, J. C., and PERUTZ, M. F., 1950, *Proc. Roy. Soc., London, Series A,* **203,** 321.
COCHRAN, W., and PENFOLD, B. R., 1952, *Acta Cryst.,* **5,** 644.
COREY, R. B., and PAULING, L., 1953, *Proc. Roy. Soc., London, Series B,* **141,** 10.
COWAN, P. M., and MCGAVIN, S., 1955, *Nature,* **176,** 501.
CRICK, F. H. C., and RICH, A., 1955, *Nature,* **176,** 780.
DAWSON, B., 1953, *Acta Cryst.,* **6,** 81.
DE SANTIS, P., GIGLIO, E., LIQUORI, A. M., and RIPAMONTI, A., 1965, *Nature,* **206,** 456.
DONOHUE, J., 1951, *J. Am. Chem. Soc.,* **72,** 949.
DONOHUE, J., 1953, *Proc. Nat. Acad. Sc.,* **39,** 205.
DONOHUE, J., and CARON, A., 1964, *Acta Cryst.,* **17,** 1178.
DONOHUE, J., and MARSH, R. E., 1962, *Acta Cryst.,* **15,** 941.
DONOHUE, J., and TRUEBLOOD, K. N., 1952, *Acta Cryst.,* **5,** 419.
DYER, H. B., 1951, *Acta Cryst.,* **4,** 42.
FREEMAN, H. C., ROBINSON, G., and SCHOONE, J. C., 1964*a*, *Acta Cryst.,* **17,** 719.
FREEMAN, H. C., SNOW, M. R., NITTA, I., and TOMITA, K., 1964*b*, *Acta Cryst.,* **17,** 1463.
FRIDRICHSONS, J., and MATHIESON, A. MCL., 1962, *Acta Cryst.,* **15,** 569.
HARDING, M. M., and COLE, S. J., 1963, *Acta Cryst.,* **16,** 643.
HINE, R., 1962, *Acta Cryst.,* **15,** 635.
HIROKAWA, S., 1955, *Acta Cryst.,* **8,** 637.
HUGGINS, M. L., 1943, *Chem. Rev.,* **32,** 195.
HUGHES, E. W., and MOORE, W. J., 1949, *J. Am. Chem. Soc.,* **71,** 2618.
ITAKA, Y., 1960, *Acta Cryst.,* **13,** 35.
ITAKA, Y., 1961, *Acta Cryst.,* **14,** 1.
KAKUDO, M., SASADA, Y., KATSUBE, Y., SAKAKIBARA, S., and AKABORI, S., 1963, in Aspects of Protein Structure, (G. N. Ramachandran, editor), London, Academic Press, 69.
KARLE, I. L., and KARLE, J., 1963, *Acta Cryst.,* **16,** 969.
KARLE, I. L., and KARLE, J., 1964, *Acta Cryst.,* **17,** 835.
KARTHA, G., and DE VRIES, A., 1961, *Nature,* **192,** 862.
KENDREW, J. C., DICKERSON, R. E., STRANDBERG, B. E., HART, R. G., DAVIES, D. R., PHILLIPS, D. C., and SHORE, V. C., 1960, *Nature,* **185,** 422.
KENDREW, J. C., WATSON, H. C., STRANDBERG, B. E., DICKERSON, R. E., PHILLIPS, D. C., and SHORE, V. C., 1961, *Nature,* **190,** 666.
KRETSINGER, R. H., and COTTON, F. A., 1963, *Acta Cryst.,* **16,** 651.
LEUNG, Y. C., and MARSH, R. E., 1958, *Acta Cryst.,* **11,** 17.
LINDLEY, H., and ROLLETT, J. S., 1955, *Biochim. et Biophysica Acta,* **18,** 183.
LOW, B. W., and BAYBUTT, R. B., 1952, *J. Am. Chem. Soc.,* **74,** 5806.
LOW, B. W., and GRENVILLE-WELLS, H. J., 1953, *Proc. Nat. Acad. Sc.,* **39,** 785.
MARSH, R. E., 1958, *Acta Cryst.,* **11,** 654.
MARSH, R. E., and GLUSKER, J. P., 1961, *Acta Cryst.,* **14,** 1110.
MARSH, R. E., COREY, R. B., and PAULING, L., 1955, *Acta Cryst.,* **8,** 710.
MATHIESON, A. MCL., 1952, *Acta Cryst.,* **5,** 332.
MATHIESON, A. MCL., 1953, *Acta Cryst.,* **6,** 399.
MATHIESON, A. MCL., and WELSH, H. K., 1952, *Acta Cryst.,* **5,** 599.
MAZUMDAR, S. K., 1964, Ph.D. Thesis, Madras University.
MIZUSHIMA, S., and SHIMANOUCHI, T., 1961, *Advanc. Enzymol.,* **23,** 1.
NÉMETHY, G., and SCHERAGA, H. A., 1965, *Biopolymers,* **3,** 155.
OUGHTON, B. M., and HARRISON, P. M., 1959, *Acta Cryst.,* **12,** 396.
PARTHASARATHY, R., 1962, Ph.D. Thesis, Madras University.
PASTERNAK, R. A., 1956, *Acta Cryst.,* **9,** 341.
PASTERNAK, R. A., KATZ, L., and COREY, R. B., 1954, *Acta Cryst.,* **7,** 225.
PAULING, L., and COREY, R. B., 1951, *Proc. Nat. Acad. Sc.,* **37,** 235.
PAULING, L., and COREY, R. B., 1953, *Proc. Nat. Acad. Sc.,* **39,** 253.

PAULING, L., COREY, R. B., and BRANSON, H. R., 1951, *Proc. Nat. Acad. Sc.*, **37**, 205.
PETERSON, J., STEINRAUF, L. K., and JENSON, L. H., 1960, *Acta Cryst.*, **13**, 104.
RAMACHANDRAN, G. N., 1960, *Proc. Indian Acad. Sc.*, **A52**, 240.
RAMACHANDRAN, G. N., 1962, *in* Collagen, (N. Ramanathan, editor), New York, John Wiley & Sons, 1.
RAMACHANDRAN, G. N., 1963, *in* Aspects of Protein Structure, (G. N. Ramachandran, editor), London, Academic Press, 39.
RAMACHANDRAN, G. N., RAMAKRISHNAN, C., and SASISEKHARAN, V., 1963*a*, *in* Aspects of Protein Structure, (G. N. Ramachandran, editor), London, Academic Press, 121.
RAMACHANDRAN, G. N., RAMAKRISHNAN, C., and SASISEKHARAN, V., 1963*b*, *J. Mol. Biol.*, **7**, 95.
RAMAKRISHNAN, C., 1964, *Proc. Indian Acad. Sc.*, **A59**, 327.
SASISEKHARAN, V., 1959*a*, *Acta Cryst.*, **12**, 897.
SASISEKHARAN, V., 1959*b*, *Acta Cryst.*, **12**, 903.
SASISEKHARAN, V., 1962, *in* Collagen, (N. Ramanathan, editor), New York, John Wiley and Sons, 39.
SCHERAGA, H. A., 1960, *J. Am. Chem. Soc.*, **82**, 3847.
SHIMANOUCHI, T., and MIZUSHIMA, S., 1955, *J. Chem. Physics*, **23**, 707.
SHOEMAKER, D. P., DONOHUE, J., SCHOMAKER, V., and COREY, R. B., 1951, *J. Am. Chem. Soc.*, **72**, 2328.
SHOEMAKER, D. P., BARREAU, R. E., and DONOHUE, J., 1953, *Acta Cryst.*, **6**, 241.
SMITH, D. W., and WIEBENGA, E. H., 1953, *Acta Cryst.*, **6**, 531.
SRINIVASAN, R., 1959*a*, *Proc. Indian Acad. Sc.*, **A49**, 340.
SRINIVASAN, R., 1959*b*, *Proc. Indian Acad. Sc.*, **A50**, 19.
SUBRAMANIAN, E., 1965, Ph.D. Thesis, Madras University.
TROMMEL, J., and BIJVOET, J. M., 1954, *Acta Cryst.*, **7**, 703.
WHITTAKER, E. T., A Treatise on Analytical Dynamics of Particles and Rigid Bodies, Cambridge, Cambridge University Press, 1952, 8.
WRIGHT, D. A., and MARSH, R. E., 1962, *Acta Cryst.*, **15**, 54.
WRIGHT, W. B., 1958, *Acta Cryst.*, **11**, 632.
YAKEL, H. L., and HUGHES, E. W., 1954, *Acta Cryst.*, **7**, 291.
ZALKIN, A., FOSTER, J. D., and TEMPLETON, H., 1964, *Science*, **146**, 261.