STEREOCHEMICAL CRITERIA FOR POLYPEPTIDE AND PROTEIN CHAIN CONFORMATIONS

III. HELICAL AND HYDROGEN-BONDED POLYPEPTIDE CHAINS

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ABSTRACT The previous study, for a pair of peptide units, of the conformations which are allowed on the basis of stereochemical criteria of van der Waals contacts has been extended to the analysis of possible conformations of helical polypeptide chains. Computer methods have been developed which select conformations on the basis of both satisfactory interatomic contacts as well as the formation of good intramolecular hydrogen bonds. Such programs have been used to map the allowed dihedral angle pairs (ϕ, ψ) for helical polypeptide chains. This survey has been made for values of the N—C^{α}—C′ angle (τ) of 105°, 110°, and 115°, from which the significant influence of this angle in determining allowed helical conformations can be demonstrated. Calculations have also been carried out using potential energy functions for the interaction between nonbonded atoms. The potential energy contour maps obtained in this manner are basically similar to the conformational maps calculated by the first method.

1. INTRODUCTION

In the earlier papers (Sasisekharan, 1962; Ramachandran, Ramakrishnan, and Sasisekharan, 1963*a, b;* Ramakrishnan, 1964; Ramakrishnan and Ramachandran, 1965, Part II), the allowed conformations of a system of two linked peptide units were studied in detail on the basis of stereochemical criteria involving limited contact distances between atoms of different types. The conformations were described by a pair of dihedral angles (ϕ, ϕ'), which defined the angles of rotation of the two planar peptide units about the single bonds N—C^{α} and C^{α}—C′. However, following the standard nomenclature and conventions recently proposed by Edsall et al. (1966), we shall denote these dihedral angles by (ϕ, ψ). It is obvious that the backbone conformation of an arbitrarily folded polypeptide chain can be repre-

sented by specifying the pairs (ϕ_i, ψ_i) at each of the alpha carbon atoms in the chain. Also, if (ϕ_i, ψ_i) is the same for all the residues, then the chain assumes a helical conformation. The helical conformation defined by a pair of dihedral angles ϕ and ψ may still not represent a stereochemically allowed structure, even though the local conformation at every C^α atom is permissible for a pair of residues as determined in the previous studies. The reason for this is that there may still be short contacts between atoms belonging to two nonadjacent residues even in the absence of such short contacts between atoms of adjacent residues. Further, certain helical conformations may be particularly stabilized through the formation of intrachain hydrogen bonds. Questions such as these have been studied and a brief preliminary report has been published (Ramachandran, Ramakrishnan, and Venkatachalam, 1965). The present paper deals with a more detailed account of these studies. Most of the data included in this paper (sections 1 to 6) were obtained early in 1965 and were presented at the Gordon Conference on Proteins, New Hampton, 27 June to 2 July 1965 and the International Organization of Pure and Applied Biophysics Symposium on Some Biological Systems at the Molecular Level held in Naples, 8 to 11 September 1965.

2. CONFORMATIONAL MAPS FOR TWO PEPTIDE UNITS

The allowed (both partially and fully allowed) conformations for two peptide units are shown in the ϕ-ψ plane, according to the new conventions, in Figs. 1 and 2, corresponding to $\tau = 110°$. (τ is the N—C^α—C′ angle.) Fig. 1 corresponds to a glycyl α-carbon atom and the observed conformations in simple peptides and cyclic peptides involving glycyl residues are also shown in this figure. This map differs from the earlier one in Part II in that it is now plotted in the ϕ-ψ system and not in the ϕ-ϕ' system, which was used earlier. This has resulted in the upper and lower halves of the plane (from 0° to 360°) being interchanged. Further, in the region between the two outer limits, near about $\psi = 180°$, there are only two short contacts, both of which are not too serious. Along the line $\psi = 180°$, for example, the distance $N_1 \cdots N_2$ is 2.58 A which is only 0.02 A less than the outer limit, and the distance $N_1 \cdots H_2$ is 2.13 A which is only 0.07 A less than the corresponding outer limit. If one disregards these two short contacts, then the region bounded by the long, thin, dashed lines may be considered to be allowed. Also, for $\tau = 115°$, both these contact distances are larger and become allowed right through the value of $\psi = 180°$. (See Fig. 3*b* of Part II)

It will be seen that most of the observed conformations occur within the region bounded by the dashed lines. In fact, it is found that when the observed conformation has a ψ-value between 160° and 200°, the corresponding value of τ is also larger than 110°. (See Table VII, Part II, where it is found that, when $\phi' \approx 0°$, τ is appreciably larger than 110°.)

Fig. 1 differs from the corresponding Fig. 3 of Part II in another respect. There

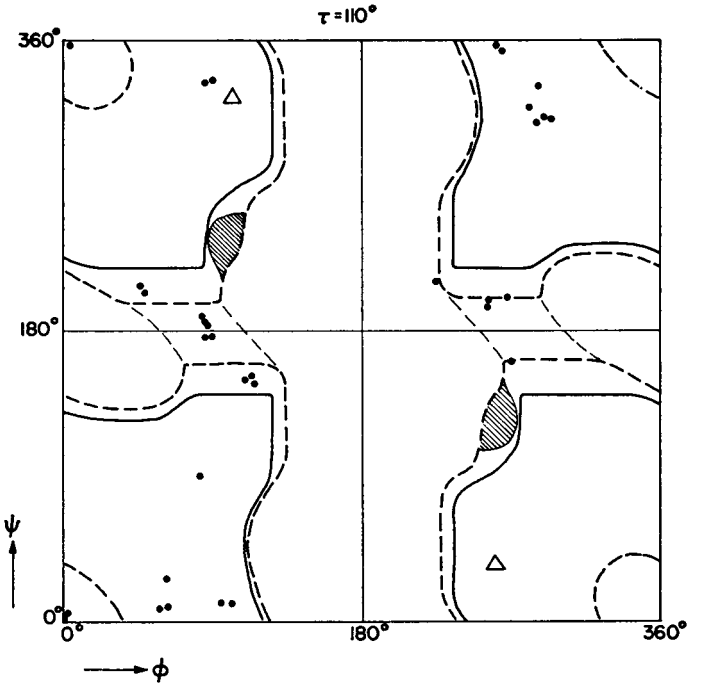


FIGURE 1 Conformational map for a pair of peptide units with a glycyl α-carbon atom, for $\tau = 110°$. All conformations observed in glycyl residues in simple di-, tri-, and tetrapeptides and in cyclic peptides are marked: Δ, polyglycine II; ——, fully allowed; - - -, outer limit; 1—3 hydrogen-bonded regions are shaded.

is also a slight extension of the outer-limit–allowed domain in the region around $\phi = 100°$, $\psi = 240°$ and also around $\phi = 260°$, $\psi = 120°$, which are shaded. For these conformations, a hydrogen bond is possible between the NH group of the second unit and the carbonyl oxygen of the first unit (see section 5 below). When this happens, the O···H contact is not really a short contact and so this prohibition must be disregarded. When this is done, we obtain the results shown in Fig. 1. Fig. 2 is the conformational map corresponding to $\tau = 110°$, for an L-alanyl α-carbon atom in the ϕ-ψ plane. This again differs from the corresponding map in Part II in that there is a bridging of the two regions above and below $\psi = 180°$ across the line $\psi = 180°$, for the same reason as for a glycyl α-carbon atom. The conformations observed in the nonhelical regions of the myoglobin chain are also marked in this figure. It will be noticed that most of them lie within the allowed regions. A few occur slightly outside, with the exception of two, marked G, G, which are well outside. It could be verified that these last two refer to glycyl residues, in which case they are in the allowed region of Fig. 1. These data on myoglobin are reproduced here by the kind permission of Dr. H. C. Watson of the Medical Research

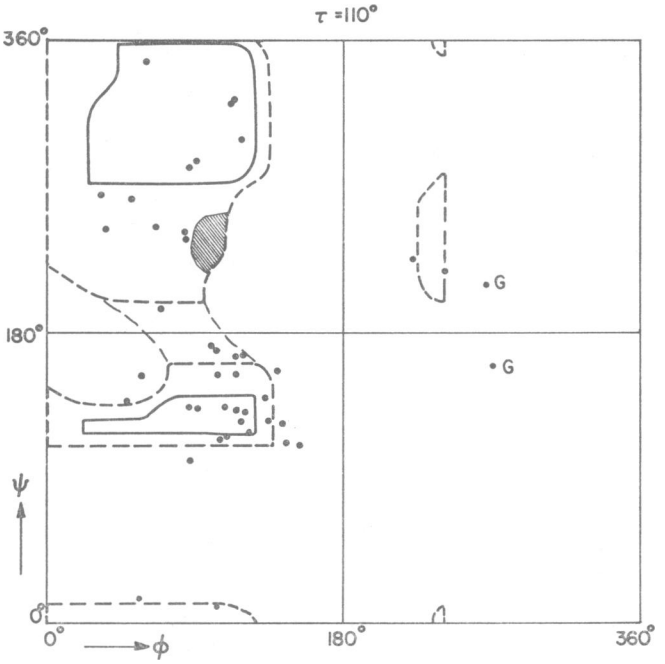


FIGURE 2 Conformational map for a pair of peptide units with an alanyl α-carbon atom for $\tau = 110°$. All observed conformations in the nonhelical region of the myoglobin chain are marked. The two conformations marked *G* which lie well outside the outer limit, refer to glycyl residues in the chain.

Council Laboratory of Molecular Biology, Cambridge, England, who calculated these from the unrefined 2 A map coordinates of myoglobin, and they greatly support the criteria adopted in drawing the limits of the allowed region in Fig. 1.

It will be noted that the hydrogen-bonded region between neighboring units (the 1—3 hydrogen-bonded region[1]) occurs in this case also, but only in the left half of the plane (shown shaded). The corresponding hydrogen bond in the right lower half, leading to a left-handed helix is disallowed when there are beta carbon atoms.

More recently, the chain structure of lysozyme has been determined (Blake et al., 1965) and the dihedral angles (ϕ, ψ) in this structure have been plotted in the conformational map by Dr. Phillips (personal communication). While there is a small concentration around the conformations of the α-helix and the 3_{10}-helix (see below), the nonhelical conformations for nonglycyl residues are found to be distributed over the allowed regions of the conformational map (Fig. 2).

3. MATHEMATICS OF PEPTIDE ROTATIONS

In describing the peptide conformations, we shall follow the conventions and nomen-

[1] We follow the notation of Edsal et al. (1966). See section 3 for more details.

clature of Edsall et al., 1966. Specifically, the rigidly connected sequence of atoms —C^{α}(HR)—C'O—NH—is termed the peptide unit while the term "amino acid residue" will be used to denote the group—NH—C^{α}(HR)—C'O—. The direction of progress along the chain is from the amino end to the carboxyl end. However, the atoms are sequentially numbered using subscripts which denote the *peptide unit* to which they belong.[2] The rotations ϕ and ψ are used with subscripts identifying the C^{α} atom through which the axes of rotation N—C^{α} and C^{α}—C' pass.

As already mentioned, an arbitrary conformation of a polypeptide chain may be specified by a sequence of dihedral angels (ϕ_i, ψ_i). Before considering the helical chains, in which all the (ϕ_i, ψ_i) are the same, we shall consider the general case and indicate how the coordinates of the atoms and the other stereochemical parameters of the chain may be calculated from a knowledge of the data (ϕ_i, ψ_i). The procedure follows the methods proposed by Ramakrishnan (1964) and Nemethy and Scheraga (1965), but differs from them in details. The general approach derives from Eyring (1932).

We shall first consider the calculation of the coordinates of the atoms of a sequence of peptide units whose conformation is specified by a sequence of parameters (ϕ_i, ψ_i). Associate with every peptide unit i a rectangular coordinate system S_i fixed in it with the origin at the C^{α}_i atom. The y axis is taken along the direction C^{α}_i C^{α}_{i+1} and the x axis is chosen to lie in the plane of the peptide unit such that the coordinate system xyz is right-handed (Fig. 3). The system S_i rotates along with the ith peptide unit. Thus, any general conformation of the peptide chain may be represented by a sequence of such coordinate systems S_i. These coordinate systems are related to each other through affine transformations consisting of rotations and translations. We may begin the chain with the atom C^{α}_1 and, without loss of generality, the first peptide unit may always be kept fixed, and the coordinates of all the atoms may be conveniently referred to this fixed system S_1. The object of the analysis is to find the transformation $T_{i,1}$ which, when acting upon the position vector $\mathbf{r}_1$ of an atom in the first peptide unit, will yield the position vector $\mathbf{r}_i$ of the corresponding atom of the ith peptide unit, both $\mathbf{r}_1$ and $\mathbf{r}_i$ being referred to the same system S_1. Hence

$$\mathbf{r}_i = T_{i,1}\mathbf{r}_1 \tag{1}$$

Equation (1) permits of another useful interpretation. If $\mathbf{r}^{(i)}$ represents the position vector of an atom in the ith unit in the system S_i, then $T_{i,1}\mathbf{r}^{(i)}$ may be looked upon as the position vector of this *same* atom of the ith unit, but now referred to the system S_1. Therefore one can write

$$\mathbf{r}^{(1)} = T_{i,1}\mathbf{r}^{(i)} \tag{1a}$$

[2] This departure from the conventions has been found to be very necessary for the *mathematics* and for the *programing* of the computations. Since the peptide chain unit is specifically referred to in all the succeeding discussions, there is likely to be no confusion arising from this. However, in describing the *results,* we shall follow the conventions of Edsall et al. (1966) and refer to amino acid residues.

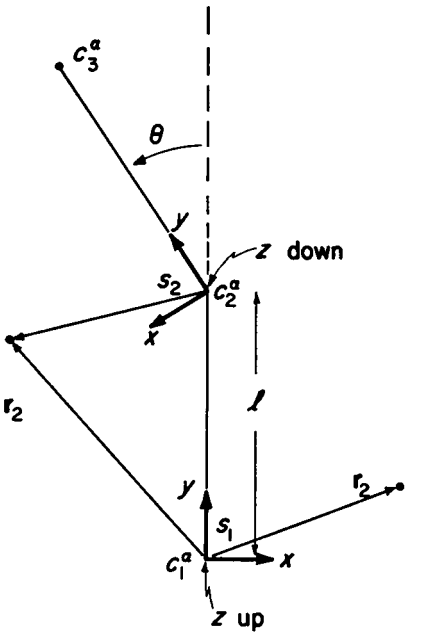


FIGURE 3 A schematic diagram of a system of two linked peptide units. The coordinate axes S_1 and S_2 are marked.

In this interpretation, $\mathbf{r}^{(i)}$ is evidently the position vector of the same point in the system S_1 and the transformation $T_{i,1}$ brings the system S_i into complete coincidence with the system S_1. The entire group of affine transformations, in fact, lends itself to such a double interpretation in general (see, for example, Birkhoff and MacLane, 1963).

For simplicity, consider only the first two units (Fig. 3) with $\phi = \psi = 0$. Let θ be the angle between $C_1^\alpha C_2^\alpha$ and $C_2^\alpha C_3^\alpha$ and l be the distance from C_1^α to C_2^α. The system S_1 can be brought into coincidence with S_2 by the following sequence of operations: (*a*) translate S_1 along its positive y direction through a distance l, thereby bringing the origin of S_1 into coincidence with that of S_2; (*b*) rotate the new system S_1 about its z axis in a clockwise sense through an angle θ in order to bring its y axis into alignment with that of S_2; (*c*) rotate the resulting system S_1 about the y axis through an angle of 180°. This brings the system S_1 into perfect coincidence with the position of the system S_2 for $\phi = \psi = 0$. For any general conformation of the system S_2, specified by a pair of angles (ϕ, ψ), the above three operations should then be followed by (*d*) a clockwise rotation of the system S_1 through an angle ϕ about the line $N_1 C_2^\alpha$ and (*e*) a clockwise rotation of S_1 through an angle ψ about $C_2^\alpha C_2'$, in order to make it coincide with the system S_2. Evidently this scheme of transformation consisting of the five operations should be exactly reversed in order to bring the system S_2 into coincidence with the original, fixed system S_1. The rotational parts of the above scheme may be represented by suitable orthogonal matrices (Jeffreys and Jeffreys,

1950). The clockwise rotation of a position vector of a point through an angle α about an axis of direction cosines λ, μ, ν passing through the origin of the coordinate system (in which the rotation is described) is represented by the orthogonal matrix

$$M^{\alpha}_{\lambda,\mu,\nu} = \begin{bmatrix} \cos\alpha + \lambda^2(1 - \cos\alpha) & \lambda\mu(1 - \cos\alpha) - \nu\sin\alpha & \lambda\nu(1 - \cos\alpha) + \mu\sin\alpha \\ \lambda\mu(1 - \cos\alpha) + \nu\sin\alpha & \cos\alpha + \mu^2(1 - \cos\alpha) & \mu\nu(1 - \cos\alpha) - \lambda\sin\alpha \\ \lambda\nu(1 - \cos\alpha) - \mu\sin\alpha & \mu\nu(1 - \cos\alpha) + \lambda\sin\alpha & \cos\alpha + \nu^2(1 - \cos\alpha) \end{bmatrix} \tag{2}$$

Again, in the reverse interpretation, this represents also an anticlockwise rotation of the *coordinate axes* through an angle α about the same axis with direction cosines λ, μ, ν passing through its origin. Thus the matrix in equation (2) may well be employed to perform the desired operations in the reverse order from (*e*) to (*b*). The equation (1*a*) for the case of two peptide units, with the local conformation given by (ϕ, ψ), then becomes

$$\mathbf{r}^{(1)} = T_{2,1}\mathbf{r}^{(2)} = \mathbf{L} + [M_{0,0,1}M_{0,1,0}M^{\phi}_{l_1,m_1,n_1}M^{\psi}_{l_2,m_2,n_2}]\mathbf{r}^{(2)} \tag{3}$$

where the translation vector **L** has components 0, l, 0, and l_1, m_1, n_1 and l_2, m_2, n_2 are the direction cosines of the axes of rotations $N_1\,C^{\alpha}_2$ and $C^{\alpha}_2\,C'_2$ respectively corresponding to $\phi = \psi = 0$. Each matrix in the product in equation (3) is then evaluated using the expression (2) by substituting the appropriate angle of rotation and the direction cosines. Equation (3) may be written as

$$\mathbf{r}^{(1)} = \mathbf{L} + R_{2,1}\mathbf{r}^{(2)} \tag{4}$$

where

$$R_{2,1} = \begin{bmatrix} -\cos\theta & -\sin\theta & 0 \\ -\sin\theta & \cos\theta & 0 \\ 0 & 0 & -1 \end{bmatrix} [M^{\phi}_{l_1,m_1,n_1}][M^{\psi}_{l_2,m_2,n_2}]$$

The angle θ depends on the angle τ and the definition of the standard conformation $\phi = \psi = 0$. With the coordinates for the peptide unit as determined from the usual Pauling-Corey parameters (Corey and Pauling, 1953), it is found that $\theta = 144°\,36' - \tau$.

Considering two adjacent peptide units $i - 1$ and i, the transformation $T_{i,i-1}$ which brings the system S_i into coincidence with S_{i-1} is of the same form as $T_{2,1}$:

$$\mathbf{r}^{(i-1)} = \mathbf{L} + R_{i,i-1}\mathbf{r}^{(i)} \tag{5}$$

where the matrix $R_{i,i-1}$ similarly would involve the rotational parameters (ϕ_i, ψ_i). If there are N peptide units and all the (ϕ_i, ψ_i), for $i = 1$ to N, are known, then $T_{i,i-1}$ may be evaluated up to $T_{N,N-1}$. Then the required transformation $T_{N,1}$ which brings the system S_N to S_1 may be written as

$$T_{N,1} = T_{N,N-1}T_{N-1,N-2}\cdots T_{2,1} = \prod_{i=2}^{N} T_{i,i-1} \tag{6}$$

Since each term of the continued product represents a rotation and a translation, $T_{N,1}$ also has a rotational part P_N and a translational part $\mathbf{D}_N$ such that

$$\mathbf{r}^{(1)} = \mathbf{D}_N + [P_N]\mathbf{r}^{(N)} \tag{7}$$

where

$$P_N = \prod_{i=2}^{N} R_{i,i-1} \tag{8}$$

and

$$\mathbf{D}_N = \sum_{i=2}^{N} P_{i-1}\mathbf{L}$$

and P_1 is a unit matrix. Equations (7) and (8) can then be used to calculate the coordinates of the atoms of the Nth unit when all parameters (ϕ_i, ψ_i) are known up to $i = N$. These equations have been used in developing programs for calculating the coordinates of a polypeptide chain, for the CDC 3600 and IBM 7090 digital computer systems.

The procedure outlined here has to be modified slightly when computing the positions of the H^{α} atom and the other side group atoms attached to any C^{α} atom in the chain. This is because these atoms of the ith unit say, do not take part in the rotation ψ_i and hence for calculating the coordinates of these atoms alone, the last matrix $M^{\psi_i}_{i,m_i,n_i}$ has to be dropped from expression (3). The rest of the procedure remains unaltered.

4. DETAILS OF THE CALCULATIONS

The mathematics of peptide rotations developed in the last section is applicable to any general conformation of a polypeptide chain and, in particular, it may be used for the study of helical conformations. The procedure that we have followed in the present case is well illustrated in the flow chart given in Fig. 4. Broadly, the scheme is one of computing the coordinates of the atoms in a helical chain whose conformation is specified by a pair of dihedral angles (ϕ, ψ). With these as coordinates, a search is made for the occurrence of (*a*) short contacts between atoms belonging to different peptide units and (*b*) hydrogen bonding between the carbonyl oxygen of one unit and the nitrogen of another.

(*a*) *Search for Short Contacts.* The distances between atoms belonging to different peptide units are evaluated to check for the presence of any short contacts between atoms. Since the conformation being studied here is helical, only contacts between atoms of the *first unit* and those of all other units need to be considered. Let us denote by $i \cdots j$ the contacts between atoms of the ith unit and those of the jth unit. In this notation all the contacts of the type $1 \cdots j$ are computed here. These distances are then checked to see whether they are permissible, making use of a set of contact-distance criteria. The set of criteria used in this study is identical with that used in Part II, Table II, except for the interaction $C' \cdots C'$ for which the normally

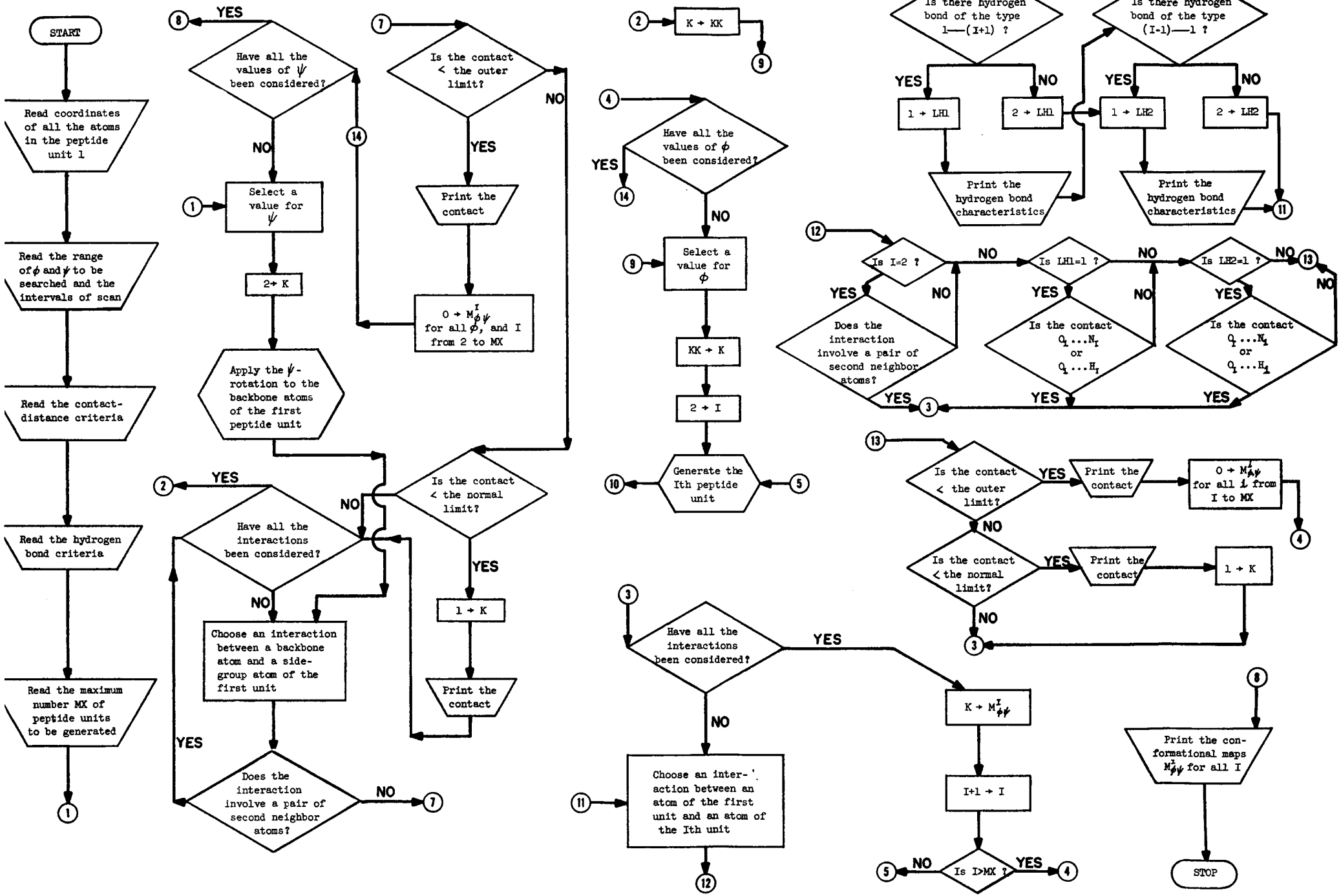


FIGURE 4 Flow chart of the digital computer program for working out the possible helical conformations.

allowed and outer limit distances are taken to be 3.0 and 2.9 A, respectively, as representative of better mean values of the distances usually observed in amino acids and peptides. If any $1 \cdots j$ contact is found to be less than the outer limit for that interaction, then the resulting conformation is rejected as untenable. In the present case, a helical chain with six peptide units was considered and the contacts of the type $1 \cdots 2$, $1 \cdots 3$, $1 \cdots 4$, $1 \cdots 5$, and $1 \cdots 6$ were evaluated in the order given. $1 \cdots 2$ contacts had to be considered because of the slight difference between the contact-distance criteria used here and those in Part II. While considering $1 \cdots 2$ contacts, care was taken to omit the second-neighbor interactions of the type $N_1 \cdots C_2'$, $N_1 \cdots C_2^\beta$, and $N_1 \cdots H_2^\alpha$.

(*b*) *Search for Hydrogen Bonds.* There are two types of hydrogen bonding that have to be searched for. The first type is the one linking the oxygen of the first to the nitrogen of the *j*th unit and the second type is that which links the oxygen of the *j*th unit with the nitrogen of the first unit. These are respectively termed 1—$(j + 1)$ and $(j - 1)$—1 hydrogen bonding, following the conventions of Edsall et al. (1966).[3]

The criteria used for deciding whether a hydrogen bond is formed are that (*a*) the distance N···O between the relevant pair of atoms N and O should lie between 2.6 and 3.2 A and (*b*) that the angle between the directions N—H and N···O (referred to as hydrogen bond angle in this paper) is less than 30°. The mean value observed for the distance N(H)···O for a hydrogen bond between a peptide NH and a carbonyl oxygen is about 2.9 A (see for example, Pimentel and McClellan, 1960) and a variation of ±0.3 A was allowed to take care of all practical cases. As a matter of computational detail, it may be mentioned that the search for the hydrogen bond always preceded the search for any short contacts, since when a good hydrogen bond is observed the corresponding O···H and O···N distances are to be ignored while studying the short contacts. (See flow chart)

The standard Pauling-Corey parameters were used for computing the coordinates of the peptide unit. The angle τ between the bonds N—C^α and C^α—C′ was, however, varied between 105° and 115°. The β-carbon atom and the H^α atom were fixed in the L-configuration such that the length C^α—C^β was 1.54 A and the length C^α—H^α was 1.00 A and the angle between C^α–C^β and C^α–H^α had a value of 109° 28′. The plane of the atoms C^α, C^β, and H^α was taken to be perpendicular to the plane of the atoms C^α, N, and C′. These conditions automatically made the four atoms C′, N, C^β, and H^α lie at the four corners of a tetrahedron with C^α at the centre when τ was 109° 28′.

At first, the conformational map was worked out by varying the dihedral angles ϕ and ψ at intervals of 5°. Such 5° maps were obtained for τ = 105°, 108°, 110°,

[3] The slight confusion in the notation is unfortunately unavoidable, as all the mathematics has to be carried out in terms of the rigid peptide units.

112°, and 115°. Further, in the regions where hydrogen bonding was expected, a 2° scan of the conformations (ϕ, ψ) was made for τ = 105°, 110°, and 115°.

5. DISCUSSION OF THE RESULTS

The short contacts due to a chain taking up a helical conformation all occur near the right- and left-handed α-helical regions. This is just as it should be, since it is around these regions that the unit height (h) is small enough to make it possible for the atoms of different peptide units to come close to each other. Again it is near these regions that hydrogen-bonded conformations may be expected.

Figs. 5 to 7 show the allowed regions near the conformations corresponding to the right- and left-handed α-helices for τ = 105°, 110°, and 115°. We shall first discuss

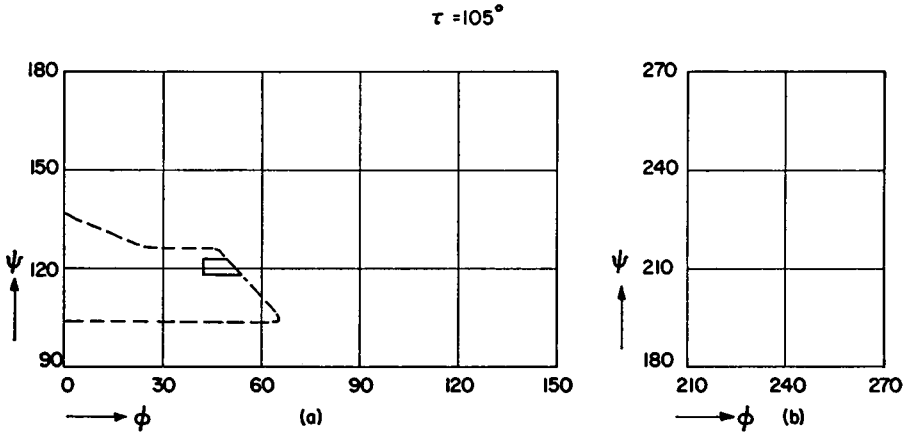


FIGURE 5 Conformational map for a perfect helix of poly-L-alanine for τ = 105°; ——, fully allowed; - - -, outer limit. (a) Right-handed helices; (b) left-handed helices. Left-handed helical conformations are completely disallowed. There is no allowed hydrogen bonded conformation.

Fig. 6 for the normal value of 110°. The main effect of a helical coiling is that there is a region of disallowed conformations, going right across the middle of Fig. 6*a*, arising from short contacts between nonadjacent units. A similar cutting off of the upper end of the allowed region in the dipeptide map is also seen in Fig. 5*b*. The short contacts which make these regions disallowed are listed in Table I and it will be noticed that most of them involve either the O_1 or the C_1^β atom. Similar data are available for other values of τ (see section 6).

Fig. 6 also contains the allowed hydrogen bonded regions marked on it as shaded regions. These involve hydrogen bonds between either N_3H_3 or N_4H_4 with O_1. These are marked respectively as 1—4 and 1—5 in Fig. 6, following the new conventions (Edsall et al., 1966). All these, as well as the allowed 1—3 hydrogen bonded conformations are marked out in Figs. 8*a*, *b*, and *c*. Table II lists these

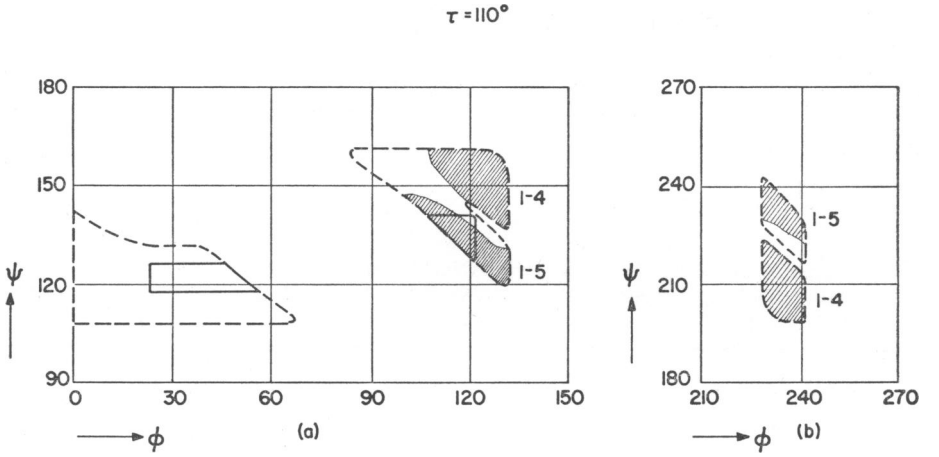


FIGURE 6 Conformational map for a perfect helix of poly-L-alanine for $\tau = 110°$; ——, fully allowed; - - -, outer limit. (*a*) Right-handed helices; (*b*) left-handed helices. Hydrogen-bonded conformations are shown shaded. 1—4 (3_{10}-helix type); 1—5 (α-helix type).

TABLE I

SHORT-CONTACTS WHICH DISALLOW HELICAL CONFORMATIONS FOR $\tau = 110°$*

ϕ	ψ	Interaction	Contact A
Right-handed helical conformations			
50	130	$C_1^\beta \ldots C_5'$	2.47
60	120	$C_1^\beta \ldots C_5'$	2.99
	130	$C_1^\alpha \ldots C_5'$	2.09
70	110	$C_1^\beta \ldots O_5$	2.33
	120	$C_1^\alpha \ldots C_5'$	2.57
	130	$C_1^\alpha \ldots C_5'$	1.18
	140	$C_1^\alpha \ldots C_5'$	2.05
80	110	$C_1^\alpha \ldots O_5$	2.00
	120	$C_1^\alpha \ldots C_5'$	1.49
	130	$C_1^\alpha \ldots C_5'$	1.83
	140	$C_1^\alpha \ldots C_5^\beta$	1.97
	150	$C_1' \ldots C_5^\beta$	2.78
	160	$O_1 \ldots C_5^\beta$	2.50
90	110	$C_1^\alpha \ldots C_5'$	1.84
	120	$C_1^\alpha \ldots C_5'$	1.79
	130	$C_1^\alpha \ldots N_5$	2.78
	140	$C_1^\alpha \ldots C_5^\beta$	2.85
	150	$O_1 \ldots C_5^\beta$	2.14
100	110	$C_1^\alpha \ldots C_5'$	1.92
	120	$C_1^\alpha \ldots N_5$	2.50
	130	$C_1^\alpha \ldots C_5^\beta$	2.66
	140	$O_1 \ldots H_4$	1.90
110	110	$C_1^\alpha \ldots N_5$	2.36
	120	$C_1^\alpha \ldots C_5^\beta$	2.60
	130	$O_1 \ldots N_4$	2.46
120	110	$C_1^\alpha \ldots C_5^\beta$	2.69
	120	$O_1 \ldots N_4$	2.40
130	110	$O_1 \ldots N_4$	2.45
Left-handed helical conformations			
230	250	$O_1 \ldots N_4$	2.45
	260	$C_1' \ldots H_5$	2.10
	270	$C_1^\alpha \ldots N_5$	2.64
240	240	$O_1 \ldots N_4$	2.40
	250	$C_1' \ldots H_5$	1.91
	260	$C_1^\alpha \ldots N_5$	2.40
	270	$C_1^\alpha \ldots C_5'$	2.55
	280	$C_1^\alpha \ldots C_5'$	2.84

* The lower suffixes all refer to the number of the unit in which the atom occurs.

helical conformations which are stabilized by hydrogen bonds, along with the bonding characteristics, as well as the number of residues per turn n and the unit translation h of the helices. The data are listed in Table II at intervals of 5° for ϕ and for the 1—3, 1—4, and 1—5 hydrogen bonds for $\tau = 110°$. For 115° only the 1—6 hydrogen bond (π-helix type) is listed in Table III, but at intervals of 2° for ϕ and ψ.

TABLE II

HYDROGEN BOND LENGTHS AND ANGLES IN POLYPEPTIDE HELICES FOR $\tau = 110°$. THE CORRESPONDING NUMBER OF RESIDUES PER TURN n AND THE UNIT TRANSLATION h OF THE HELICES ARE ALSO INCLUDED.

ϕ	ψ	Length	Angle NH∧NO	Angle CO∧ON	n	h
		A				A
a) 1—3 Hydrogen bond						
90	225	3.10	28	103	2.42	2.58
	230	3.09	27	101	2.36	2.64
	235	3.09	26	99	2.30	2.69
	240	3.09	27	98	2.24	2.73
	245	3.11	29	96	2.19	2.78
95	220	2.98	28	102	2.43	2.53
	225	2.96	26	100	2.36	2.58
	230	2.95	24	98	2.30	2.63
	235	2.95	24	97	2.24	2.68
	240	2.96	25	95	2.19	2.72
	245	2.97	27	93	2.13	2.76
	250	3.00	30	91	2.08	2.80
100	225	2.82	23	97	2.30	2.58
	230	2.81	22	95	2.25	2.62
	235	2.81	22	94	2.19	2.67
	240	2.82	24	92	2.14	2.71
	245	2.84	26	90	2.09	2.75
	250	2.87	30	89	2.04	2.79
105	230	2.67	19	92	2.19	2.61
	235	2.67	20	90	2.14	2.66
	240	2.69	22	89	2.09	2.69
	245	2.71	26	87	2.04	2.73
	250	2.74	30	86	−2.01	2.76
b) 1—4 Hydrogen bond						
			Right-handed helices			
115	150	3.05	29	70	3.34	1.70
	155	3.00	20	66	3.23	1.78
	160	2.99	13	62	3.12	1.86
120	150	2.90	23	62	3.24	1.77
	155	2.88	14	58	3.13	1.85
	160	2.91	10	55	3.03	1.92
125	145	2.82	26	58	3.26	1.76
	150	2.80	16	54	3.15	1.83
	155	2.81	9	51	3.05	1.90
	160	2.86	10	49	2.95	1.97
130	140	2.76	29	54	3.27	1.75
	145	2.72	19	50	3.16	1.83
	150	2.73	9	47	3.06	1.89
	155	2.77	6	45	2.96	1.95
			Left-handed helices			
230	205	2.77	6	45	−2.96	1.95
	210	2.73	9	47	−3.06	1.89
	215	2.72	19	50	−3.16	1.83
	220	2.76	29	54	−3.27	1.75
235	200	2.86	10	49	−2.95	1.97
	205	2.81	9	51	−3.05	1.90
	210	2.80	16	54	−3.15	1.83
	215	2.82	26	58	−3.26	1.76
240	200	2.91	10	55	−3.03	1.92
	205	2.88	14	58	−3.13	1.85
	210	2.90	23	62	−3.24	1.77
c) 1—5 Hydrogen bond						
			Right-handed helices			
105	140	2.66	27	48	3.79	1.30
	145	2.91	29	43	3.66	1.43
110	140	2.79	23	38	3.68	1.41
	145	3.11	29	37	3.55	1.52
115	135	2.69	17	32	3.70	1.40
	140	3.00	23	32	3.58	1.51
120	130	2.64	11	27	3.73	1.39
	135	2.92	17	26	3.60	1.50
125	125	2.62	7	20	3.75	1.40
	130	2.88	11	21	3.62	1.50
130	120	2.63	7	14	3.77	1.41
	125	2.88	6	16	3.64	1.50
	130	3.19	17	23	3.51	1.59
			Left-handed helices			
230	230	3.19	17	23	−3.51	1.59
	235	2.88	6	16	−3.64	1.50
	240	2.63	7	14	−3.77	1.41
235	230	2.88	11	21	3.62	1.50
	235	2.62	7	20	3.75	1.40
240	225	2.92	17	26	3.60	1.50
	230	2.64	11	27	3.73	1.39

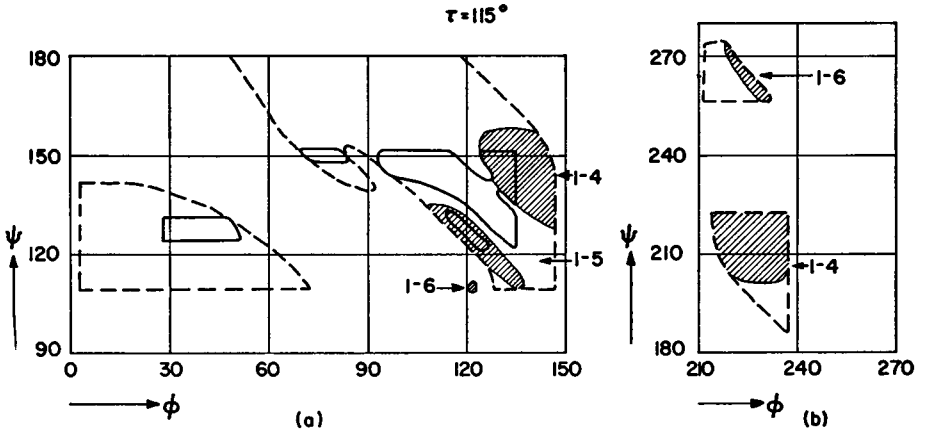


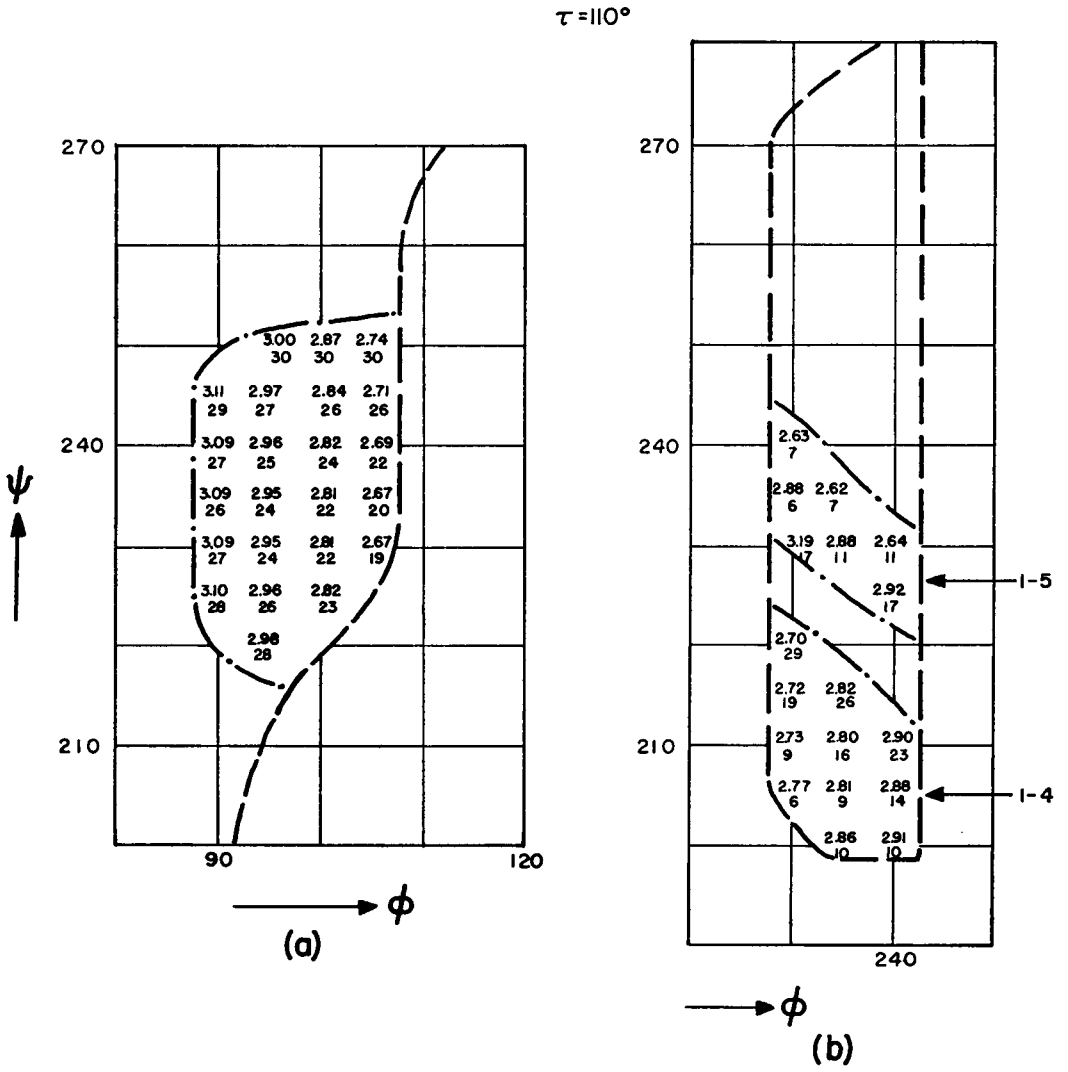
FIGURE 7 Conformational map for a perfect helix of poly-L-alanine for $\tau = 115°$; ——, fully allowed; - - -, outer limit. (*a*) Right-handed helices; (*b*) left-handed helices. Hydrogen-bonded conformations are shown shaded. 1—4 and 1—5 same as in Fig. 6; 1—6 (π-helix type).

TABLE III

DATA AT INTERVALS OF 2° FOR ϕ AND ψ FOR HYDROGEN BOND LENGTHS AND ANGLES FOR ALLOWED HELICES HAVING THE 1—6 HYDROGEN BOND, FOR $\tau = 115°$. THE π-HELIX BELONGS TO THIS CLASS.

ϕ	ψ	Length A	Angle NH ∧ NO	Angle CO ∧ ON	n	h A
a) *Right-handed helix*						
122	112	2.88	12	14	4.20	1.00
b) *Left-handed helices*						
218	272	3.11	30	22	−4.66	1.18
220	268	3.19	24	20	−4.57	1.22
	270	3.00	28	20	−4.65	1.16
222	266	3.09	22	18	−4.56	1.20
	268	2.90	25	17	−4.63	1.15
224	264	3.01	19	16	−4.54	1.18
	266	2.81	22	15	−4.61	1.13
226	260	3.15	16	17	−4.46	1.22
	262	2.93	17	14	−4.53	1.17
228	258	3.08	14	15	−4.44	1.21
	260	2.86	14	12	−4.51	1.16
230	256	3.02	12	14	−4.43	1.20
	258	2.79	11	10	−4.50	1.15
232	256	2.74	9	8	−4.48	1.14

Fig. 8*a* shows the conformations where a hydrogen bond may be formed between two adjacent peptide units. It turns out that they are only of the type 1—3 and not 3—1. The bond lengths and the bond angles are also marked in the conformational map. The hydrogen bond angle between the directions N—H and N···O is always poor and is greater than 20°. The number of residues per turn varies from 2.0 to 2.4. Moreover these 1—3 hydrogen bonds occur only in right-handed helices, with L-residues. The corresponding left-handed helical conformations are disallowed because of short contacts involving the C^{β} atom in the L-configuration. The conformations of the so called ribbon structures, the 2_7 helix (Bamford et al., 1956)



and the 2.2_7 helix proposed by Donohue (1953), lie within the outer limits in this region. In general, helices with this type of 1—3 hydrogen bonding may be called the n_7 helices.

From Figs. 8*b* and *c* it will be seen that the 1—4 hydrogen bonding is formed over a fairly wide region of allowed helical conformations. This entire region lies only within the outer limits. However, since very good hydrogen bonds with a bond angle of 10° or better may be formed near (125°, 155°) and (235°, 205°), these helices are likely to occur. The 1—4 hydrogen-bonded helices can be either

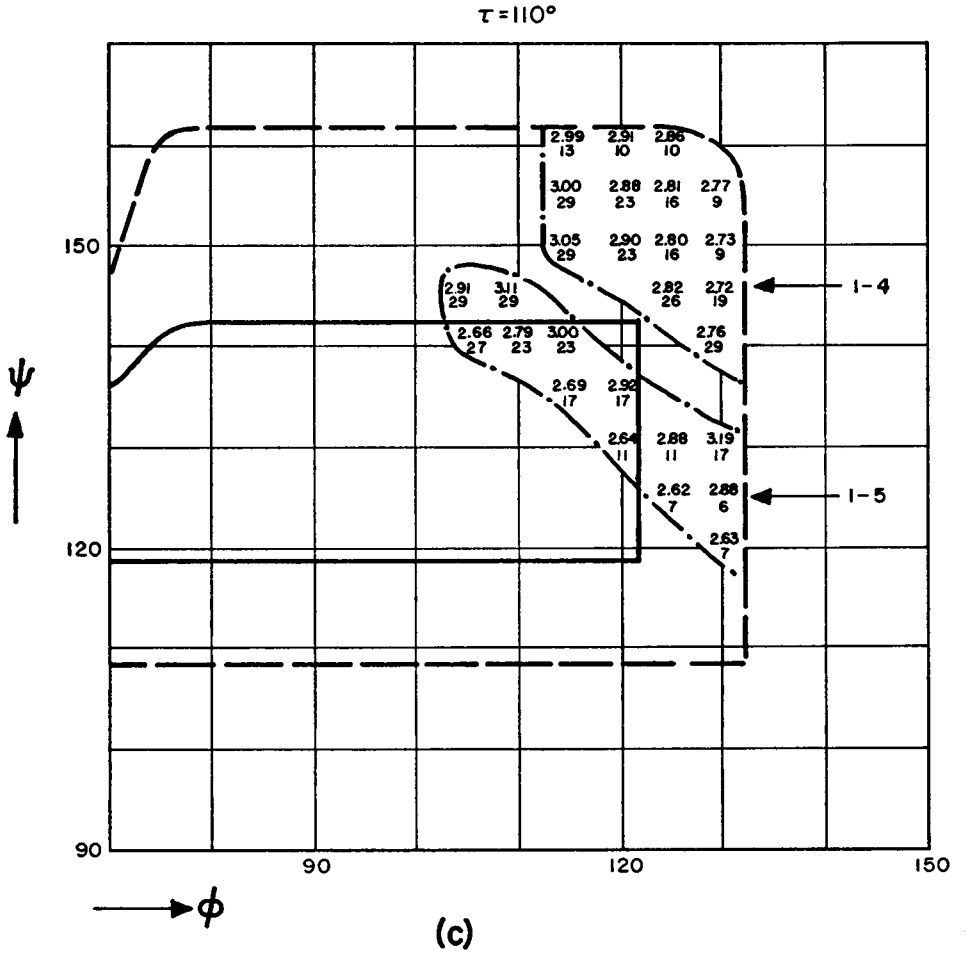


FIGURE 8 Conformational maps showing 1—3, 1—4, and 1—5 hydrogen-bonded conformations for $\tau = 110°$. Hydrogen bond lengths and angles are marked. The outer and the fully allowed limits are shown in these figures, with the usual notation, for a pair of alanyl residues. (*a*) 1—3 hydrogen bonded conformations; (*b*) other left-handed helices; (*c*) other right-handed helices.

right- or left-handed. The value of n varies from ($\pm$) 2.9 to 3.3 and the Bragg-Kendrew-Perutz 3.0_{10} helix is a possible structure in this region. More generally, this type of 1—4 hydrogen-bonded helices may be called the n_{10} helices.

Just as with the 1—4 type of helices, 1—5 hydrogen-bonded helices may also be formed over a *range* of conformations in both the right- and left-handed forms. Especially good hydrogen bonds are formed near (125°, 130°) and its inverse conformation (235°, 230°) for $\tau = 110°$. This conformation corresponds to $n = \pm 3.62$ and $h = 1.49$. These values are remarkably close to the values found in the α-helical form of poly-L-alanine, for which $n = 3.615 \pm 0.003$ and $h = 1.495 \pm 0.003$ (Elliott and Malcolm, 1958, for example). The conformation (125°, 130°) lies just outside the normal limits, but is well within the outer limits. The left-handed α-helix lies only within the outer limits and it appears for this reason that the right-handed α-helix is more stable when there is a β-carbon atom in the L-configuration, since it can occur within the normal limits. It is interesting to note that there is no disallowed region completely separating the 1—4 hydrogen bonded conformations from the 1—5 bonded conformations (Fig. 6), although there is a cleft going in from the right-hand side. The significance of this is that the 3_{10} helix may be gradually transformed into an α-helix (and vice versa) maintaining throughout a near-helical conformation of the chain. This further suggests that, in a case where one of the conformations should turn out to be impossible (say, as a result of side chain interactions), the chain may "slip" into the other conformation, which may be allowed. In fact, the 3_{10} helix would derive its importance mainly from its proximity to the more stable α-helix. These remarks apply equally well to the left-handed conformations. The relative stability of the right-and left-handed conformations is discussed in section 7.

The α-helix is thus seen to belong to a general class of helices characterized by 1—5 hydrogen bonding. As mentioned above, this class of helices may, in fact, be considered to be n_{13} helices where n is not restricted to a value of 3.6 but may vary from 3.6 to 3.8. The ω-helix with $n = 4.0$ proposed by Bragg et al. (1950) and subsequently found to occur in poly-β-benzyl-L-aspartate by Bradbury et al., (1962), is topologically similar to the α-helix, in that it is also an n_{13} helix. However, this conformation is strictly outside the allowed regions owing to short contacts between neighboring units. As reported in Part II, these short contacts may be relieved if the peptide group is allowed to deviate from planarity, as has been shown by Bradbury et al. (1962).

Quite strangely, no hydrogen bonding between a unit and the fifth one from it, i.e. of the type 1—6 (the so called π-helix), has been found within the allowed regions for $\tau = 110°$. This apparently eliminates the possibility of the π-helix, for this value of the angle τ. The few 1—6 hydrogen bonds that are found to be possible are around the disallowed region (138°, 96°) and, moreover, the hydrogen bond angle here is as large as 27°. Although hydrogen-bonded conformations of

the type 1—3, 1—4, 1—5 were found to be possible, no such conformations from a nitrogen of a residue to an oxygen of a succeeding residue, i.e. bonds of the type n—1, with n = 2, 3, 4, 5 etc., were found to be at all possible for a chain with β-carbon atoms (e.g., alanyl residues). This rules out the Pauling-Corey γ-helix (type 5—1) for a chain with β-carbon atoms.

6. EFFECT OF τ ON HELICAL CONFORMATIONS

It was chiefly the conspicuous absence of the π-helix anywhere near the allowed conformations for $\tau = 110°$, which prompted a study of the effect of changes in τ on the possible helical and hydrogen-bonded conformations. As already mentioned, the study was made for $\tau = 105°$, 108°, 110°, 112°, and 115°. The results are quite interesting and are discussed below.

Very strikingly, at $\tau = 105°$, no hydrogen-bonded helical conformation of any type is possible. Fig. 5 shows the conformational map for $\tau = 105°$, analogous to Fig. 6. As τ is increased to 108°, the 1—4 and 1—5 hydrogen-bonded conformations, both right- and left-handed types, begin to appear within the outer limits, but the hydrogen bond angle is larger than 24°. At $\tau = 110°$, as already described, both are possible and the bonds are good. At $\tau = 112°$, while 1—4 and 1—5 hydrogen-bonded conformations are still possible to a limited extent, there is a sign for the first time of the possibility of a short (2.76 A) 1—6 hydrogen-bonded left-handed conformation (bond angle $\approx$ 20°) at (230°, 255°), while the corresponding right-handed conformation is disallowed due to short contacts.

The allowed and hydrogen-bonded regions for $\tau = 115°$ are shown in Fig. 7. Although the 1—4 and 1—5 hydrogen bonds (both right- and left-handed) are possible, the hydrogen bonds are quite poor (N···O distance > 3.0 A), but the 1—6 hydrogen-bonded conformations are just possible. It was at first difficult to detect the presence of these in a survey made at intervals of 5° for ϕ and ψ, but a two-degree scan of conformations yielded the map shown in Fig. 7. The value of n varies from 4.3 to 4.7 within this very limited region (see Table III). Though the hydrogen bond is good, the region is extremely limited because the 1···5 contacts are very sensitive to changes in the dihedral angles ϕ and ψ.

The 1—3 type of hydrogen bonding (the ribbon structure) is possible for all the values of ψ considered, but the hydrogen bond angle is always large.

Thus, we have the important result that the α-helix is possible only for values of τ close to the undistorted tetrahedral angle at the α-carbon atom. At most, only deviations of up to about ±2° from the tetrahedral value may be allowed if the peptide unit itself is not distorted. When there is a β-carbon atom present, all the hydrogen bonds that were found to occur even for different values of τ are of the type 1—j and the geometry of the peptide unit is just not suited for the formation of j—1 hydrogen bonds when we have a C^β atom attached to the α-carbon atom.

7. USE OF POTENTIAL FUNCTIONS

Whereas the approach described in the previous sections and in the earlier studies is essentially equivalent to one of associating with every atom of the peptide unit a hard sphere and then studying the various conformations through disallowing "short contacts" between such spheres, a more sophisticated approach would be to study the relative stabilities of the different conformations through the calculation of the potential energy of interaction between the nonbonded atoms in each conformation. The principle of this method is that the conformation of a polypeptide chain is governed chiefly by van der Waals interactions between nonbonded atoms. Other types of forces such as electrostatic interactions and torsional potentials for rotation about the bonds N—C^{α} and C^{α}—C′ will also have to be taken into account. As a first approximation, we shall consider only the effects of the van der Waals interactions in this paper. The others will be considered in later parts of this series.

The basic problem in the present approach is thus to find a "reasonable" set of potential functions for the various nonbonded pairs in a polypeptide structure. De Santis et al. (1965) have in fact made such a study of the stability of helical conformations of polypeptide chains. They have adopted mostly the set of semiempirical potential functions proposed by Mason and Kreevoy (1955), with some modifications which were found to fit polymer structures. The potential energy diagram for a helical polypeptide chain obtained by them has contours broadly similar to the boundaries in our conformational maps, but there are some differences in detail regarding the positions of certain minima (see page 929 of Part II). Scott and Scheraga (1965) have recently proposed a method for computing the parameters in the potential functions for the interaction of a pair of nonbonded atoms, using a function of the form

$$V = ae^{-br} - c/r^{6} \tag{9}$$

They have estimated the value of c using the Slater-Kirkwood equation (see for example, Pitzer, 1959) and the values of atomic and group polarizabilities as given by Ketelaar (1958). They used values obtained through molecular beam-scattering studies of Amdur and coworkers (Amdur and Harkness, 1954; Amdur and Mason, 1955) for the parameter b. However, Brant and Flory (1965) have suggested that a constant value of $b = 4.6$ may be used for all the interactions. The parameter a is then calculated by requiring that V be a minimum at a distance equal to the sum of the van der Waals radii $r_p + r_q$ of the interacting atoms p and q. We have adopted the value of b suggested by Brant and Flory. Different sets of van der Waals radii have been used in the literature for r_p, and for these, we have also adopted the values suggested by Brant and Flory. Table IV lists the values of the parameters a, b, and c used in this study.

TABLE IV

PARAMETERS USED IN THE POTENTIAL FUNCTIONS FOR THE VARIOUS NONBONDED INTERACTIONS.*

Interaction	$a \times 10^{-4}$	c
H...H	0.829	46.8
C...H	7.79	165.8
N...H	5.34	156.0
O...H	3.83	124.1
CH_2...H	14.9	226.9
C...C	92.4	599.9
N...C	60.5	571.2
O...C	43.3	461.6
CH_2...C	187.0	822.8
N...N	40.4	546.9
O...N	294.0	446.1
CH_2...N	121.0	783.6
O...O	21.7	368.9
CH_2...O	86.0	633.9
CH_2...CH_2	382.0	1128.0

* The van der Waals interaction between a pair of nonbonded atoms is given by $V = ae^{-br} - cr^{-6}$. b is taken to be 4.6 for all interactions. V is minimized at the sum of the van der Waals radii R. The values above have been obtained by using the radii $R_H = 1.20$, $R_O = 1.50$, $R_N = 1.55$, $R_C = 1.7$ and $R_{CH_2} = 1.85$ A.

(*a*) *Potential Contours for a Pair of Peptide Units.* With these potential functions, the potential energy V of interaction between a pair of peptide units has been calculated over the whole range of ϕ and ψ (0° to 360°, at intervals of 10°) for an alanyl residue, and the contours of constant V are plotted in Fig. 9. It will be noticed that there is a very close resemblance between the contour for $V = 0$ (the dotted line) and the outer limit contour of Fig. 2. In particular, the bridging across $\psi = 180°$ in the left half of the plane is quite evident. The extension of the contour on the right-hand side up from about $\psi = 240°$ up to about $\psi = 360°$ should also be noticed. It may also be mentioned that the observed data for (ϕ, ψ) in myoglobin and lysozyme fit the contours in Fig. 9 very well. In lysozyme, in particular, the two deepest minima are the regions which are most populated. Such a good agreement is not obtained for the contours calculated from the potential used by Liquori. These contours, shown in Fig. 10, were obtained by using the potential energy functions adopted by De Santis et al. (1965). The difference in shape between the contours obtained from the Scheraga-Flory parameters and those of Liquori would appear to require further study.

(*b*) *Potential Contours for Helical Chains.* With the Scheraga-Flory parameters, the potential contour map has been worked out for helical conformations of poly-L-alanine over the complete range of ϕ and ψ for $\tau = 110°$. This is shown in Fig. 11. It will be seen that this map is practically the same as that for a

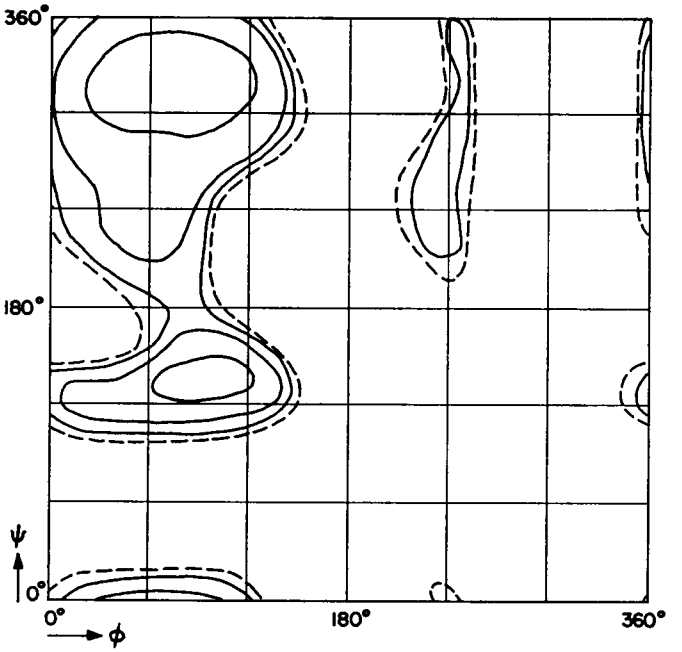


FIGURE 9 Potential energy distribution in the ϕ-ψ plane for a pair of peptide units with alanyl residues, with the potential energy parameters of Scheraga and Flory, for $\tau = 110°$. Contours are drawn at intervals of 1 kcal/mole going down from 0 kcal/mole. The zero contour is dashed.

pair of peptide units linked at an alanyl α-carbon atom in the region bounded by $\phi = 0°$ to 180°, $\psi = 240°$ to 360°. If the *n, h* data of Ramakrishnan (1964) are examined, it will be seen that, in this region, the unit height *h* is greater than 2 A, so that there is practically no interaction between a unit and units other than the first neighbor. The interactions between nonfirst neighbors occur between $\psi = 90°$ and 180° in the left half of the diagram and between 180° and 270° in the right half of the diagram. There are two completely forbidden regions (large positive *V*) going diagonally across the middle of these regions, which correspond to the line $h = 0$ and its vicinity. This is obvious, for when *h* is small the helix will coil back on itself. (Note the corresponding forbidden region in Fig. 6.)

However, near and fringing these forbidden regions, there are domains of large negative van der Waals energy, going down to about −7.0 kcal/mole on the left half and about −5.0 kcal/mole on the right half of the map. These are close to the conformations (ϕ, ψ) which lead to right- and left-handed α-helices, which are marked on the diagram. As mentioned in section 5, there are a number of conformations of the residues for which hydrogen-bonded helices of the type 1—*j* are possible. The van der Waals energies corresponding to these conformations are

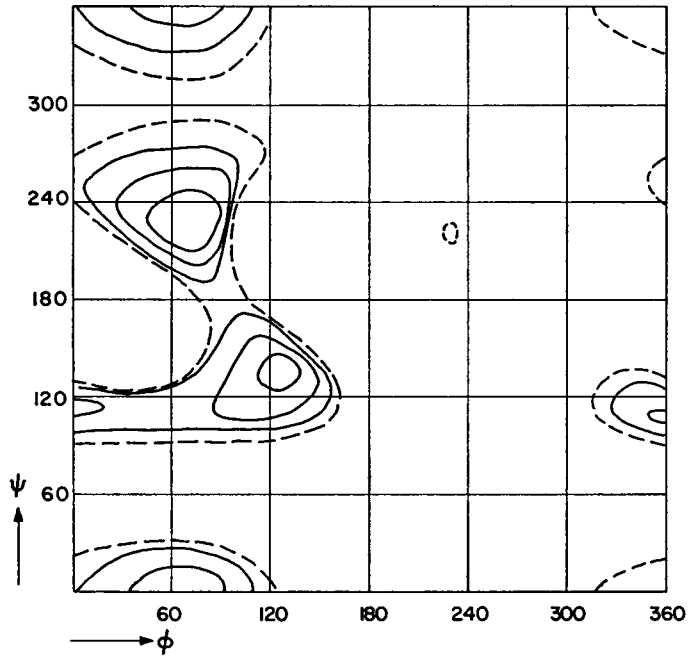


FIGURE 10 Potential energy distribution in the ϕ-ψ plane for a pair of peptide units with alanyl residues, with the potential energy parameters of Liquori, for $\tau = 110°$.

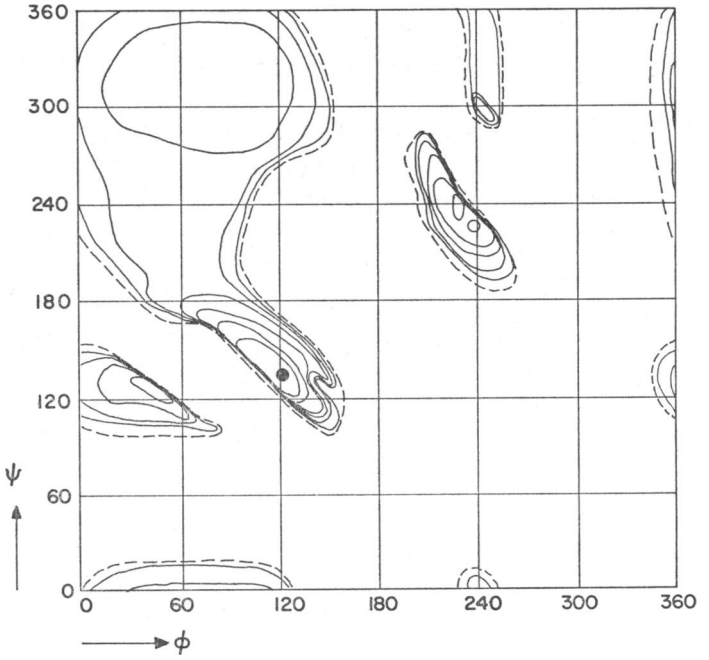


FIGURE 11 Potential energy distribution in the ϕ-ψ plane for a *helix* of poly-L-alanine for $\tau = 110°$. ●, right-handed α-helix; ○, left-handed α-helix.

TABLE V

VAN DER WAALS POTENTIAL ENERGIES OF VARIOUS STANDARD HELICAL POLYPEPTIDE CHAIN CONFORMATIONS

Type of hydrogen bond	Name of helix	ϕ	ψ	Energy *kcal/mole*
1—3	2.2_7-helix (n = 2.17, h = 2.75 A)			
	Right-handed	100°	240°	−2.0
	Left-handed	260°	120°	large
1—4	3_{10}-helix (n = 3.00, h = 1.80 A)			
	Right-handed	122°	158°	−4.0
	Left-handed	238°	202°	−3.2
1—5	Alpha helix (n = 3.60, h = 1.50 A)			
	Right-handed	122°	134°	−7.1
	Left-handed	238°	226°	−5.1

therefore tabulated in Table V. It will be seen from this that, of all the hydrogen-bonded conformations, the right-handed α-helix is by far the stablest, even with regard to van der Waals stabilizing energy. Although the n_{10}-helix is good for hydrogen bond formation, its van der Waals energy is not quite favorable, because it has several close (although not forbidding) short contacts. The possible effect of a distortion from planarity of the peptide unit in relieving these short contacts is to be investigated.

Although the n_7-helix has a sort of a hydrogen bond, the van der Waals energy contour map does not exhibit a minimum in this region, and so it does not appear likely that an extended helix of this type will be observed.

A large amount of numerical data not reported in this paper have been obtained during the course of this investigation. As these will be of value to workers in the field, it is proposed to collect them and deposit them for reference in a suitable place. The information regarding this will be reported in a succeeding part of this series of papers.

APPENDIX

After this paper had been completed, it was brought to our attention that accurate spectroscopic values are available for the C—H bond length and that this is very close to 1.1 A rather than 1.0 A (Herzberg, 1945). In view of this, the effect of the 1.1 A C—H

distance was determined on the allowed regions for a pair of peptide residues. No differences were, however, observed in the resulting maps analogous to Figs. 1 and 2, calculated at intervals of 5° for ϕ and ψ. It does not appear, therefore, that any of the physical conclusions reported in this paper require modification on this basis.

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