POTENTIAL FUNCTIONS FOR HYDROGEN BOND INTERACTIONS

IV. Minimum Energy Conformation of the α-Helical Structure of Poly-L-Alanine

BY G. N. RAMACHANDRAN,[a, b] R. CHANDRASEKHARAN[a]

AND

R. CHIDAMBARAM[a, c]

Received September 3, 1971

ABSTRACT

Making use of the empirical potential functions for *peptide* NH . . O bonds, developed in this laboratory, the relative stabilities of the right- and left-handed α-helical structures of poly-L-alanine have been investigated, by calculating their conformational energies (V). The value of V_{min} of the right-handed helix (α_R) is about $-10{\cdot}4$ kcal/mole, and that of the left-handed helix (α_M) is about $-9{\cdot}6$ kcal/mole, showing that the former is lower in energy by $0{\cdot}8$ kcal/mole. The helical parameters of the stable conformation of α_R are $n \sim 3{\cdot}6$ and $h \sim 1{\cdot}5$ Å. The hydrogen bond of length 2·85 Å and nonlinearity of about 10° adds about 4·0 kcal/mole to the stabilising energy of the helix in the minimum enregy region. The energy minimum is not sharply defined, but occurs over a long valley, suggesting that a distribution of conformations (ϕ, ψ) of nearly the same energy may occur for the individual residues in a helix. The experimental data of α-helical fibres of poly-L-alanine are in good agreement with the theoretical results for α_R. In the case of proteins, the mean values of (ϕ, ψ) for different helices are distributed, but they invariably occur within the contour for $V = V_{min} + 2$ kcal/mole for α_R.

INTRODUCTION

THE α-helix, first proposed by Pauling and Corey[1] in 1951, is considered to be one of the stablest conformations of the polypeptide chain. Although this particular stability of the α-helix was at first attributed to the regular hydrogen bonds which occur in this helix, it has later been estabilished that

[a, b] Indian Institute of Science, Bangalore 12, India. (address to which reprint requests should be sent).

[a] Department of Biophysics, University of Chicago, Chicago, Illinois 60637, U.S.A.

[a, a] Nuclear Physics Division, Bhabha Atomic Research Centre, Trombay, Bombay-85, India.

its stability is also due to other interactions such as the van der Waals forces between atoms in neighbouring peptide units adjacent to one another in neighbouring turns of the helix.[2-4] Calculations of the van der Waals, electrostatic and other interactions between the various atoms in the polypeptide chain, excluding, however, the hydrogen bond interactions, do show a deep minimum in the region actually observed in the (ϕ, ψ)-map for the hydrogen-bonded conformations of the α-helical type. It was therefore considered worthwhile to work out in detail the variation of the energy of the helix for different values of ϕ and ψ, taking into consideration also the hydrogen bond energy.

A good potential function for the NH ... O bonds has been proposed recently by the authors.[5] However, the type of hydrogen bond considered therein was of an average type—that is, one in which both NH and CO groups may be either charged, or uncharged, *i.e.*, those of the type N^+H ... O^-C, N^+H ... OC, NH ... O^-C and NH ... OC. For our example of the backbone hydrogen bonds in a polypeptide chain, we have to consider the particular case of the hydrogen bond between peptide units, which corresponds to the last of the four types mentioned above. This type of hydrogen bond need not necessarily have the same minimum energy and the same variation with hydrogen bond length and angle as an average bond. Therefore, as a preliminary to the investigation of the minimum energy conformation of the α-helix, a study was made of the distribution of observed hydrogen bonds in peptides, and a potential function was derived for the peptide hydrogen bond, following the procedure of Ref. 5. A brief account of this study forms the previous part of this series.[6]

Using this particular potential function, the variation of the total energy of the α-helical structulre of poly-L-alanine with ϕ and ψ was studied and the results are presented here. It is interesting that the actual values of the helical parameters n and h of the minimum energy conformation agree remarkably well with the experimental values.[7] However, the energy minimum occurs over a long valley, in which ϕ and ψ vary over a range of about three degrees, the variation being in a co-ordinated way (with ϕ increasing the same amount as ψ decreases so that the valley is at about 135° to the ϕ-axis, *see* Fig. 1).

Method of Calculation

The procedure adopted for calculating the energy of a helical structure was fairly straightforward. The helix consisting of twelve peptide units

with L-Ala side-chains was generated corresponding to various assumed values of ϕ, ψ, ω and τ, using the standard *trans* planar peptide unit[8]. The methyl hydrogens were fixed in the staggered conformation (*i.e.*, $\chi = 60°$). The angle $\tau(=N - C^{\alpha} - C')$ was kept at the standard value of 110° in the initial stage of the calculations. The helical parameters n and h were determined from the elements of the transformation matrix which generates the helix.[8]

The energy of a conformation was evaluated as the sum of contributions from non-bonded, electrostatic and hydrogen bond interactions and potential energy changes in bond angle (τ) and dihedral angles (ϕ, ψ, ω and χ). The non-bonded energy was computed using the "6-exp" Buckingham potential with the set of constants in Table XI of Ref. 8. The "6–12" Lennard-Jones potential was also used in the initial calculations. This yielded results not significantly different from the "6-exp" function. In view of this, only the results derived using the Buckingham potential are presented here. The electrostatic interactions were estimated[8] taking the monopole charges on the four atoms C', O, N and H of the peptide unit to be $+0{\cdot}4$, $-0{\cdot}4$, $-0{\cdot}3$ and $+0{\cdot}3$ (electron unit) respectively and an effectively dielectric constant of $4{\cdot}0$. The formulae for the variation of energy with changes in bond angle and dihedral angles were also taken into account following the procedure of Ramachandran and Sasisekharan,[8] except that K_{τ} was taken to be 80 kcal/mole, instead of 40 kcal/mole, in the formula $V_{\tau} = K_{\tau}(\triangle \tau)^2$ following Bixon and Lifson[9] and Ramachandran and coworkers.[10] The hydrogen bond function used was that given in Eqn. (1) of Ref. 6.

The calculations were carried out over a wide range of the conformational parameters of the right as well as the left-handed α-helical forms of poly-L-alanine (denoted by the symbols α_P and α_M, following Ref. 8). Unless otherwise specified, the peptide unit was taken to be planar ($\omega = 180°$) and the angle τ was chosen to be 110°. We follow the latest nomenclature[11] for the description of the polypeptide chain. The dihedral angles ϕ and ψ were varied at a coarse interval of 2° initially to determine the distribution of energy. In the later stages, this search was continued at closer intervals of 1°, and then of $0{\cdot}5°$, in the regions around the local minima, as required.

RESULTS FOR THE RIGHT-HANDED α-HELIX

It is of interest to mention first what happened when the average NH..O hydrogen bond potential function given in Ref. 5 was used in the present calculation. The minimum energy conformation disagreed with the experi-

mental observation in two respects, namely, (*a*) the helical parameters n and h were in the ranges of 3·65 to 3·70 and 1·42 to 1·48 Å respectively, these values being significantly higher and lower, respectively, than the corresponding experimental values[7,12] (namely, 3·615 and 1·495 Å), and (*b*) the conformational angles ϕ and ψ were both about 6° away from the observed values of (− 58°, − 48°[7]).

On a careful examination of the results, it became clear that these discrepancies arose essentially because of the fact that the hydrogen bond of the lowest energy in the potential function used corresponded to a length of 2·8 Å. It was also observed that if the hydrogen bond function has its minimum at a larger value, then the agreement between theory and observation would be expected to improve. Therefore, the *peptide* hydrogen bond function mentioned above, which is also theoretically expected to be the correct one for the α-helix, was employed and the results are described below.

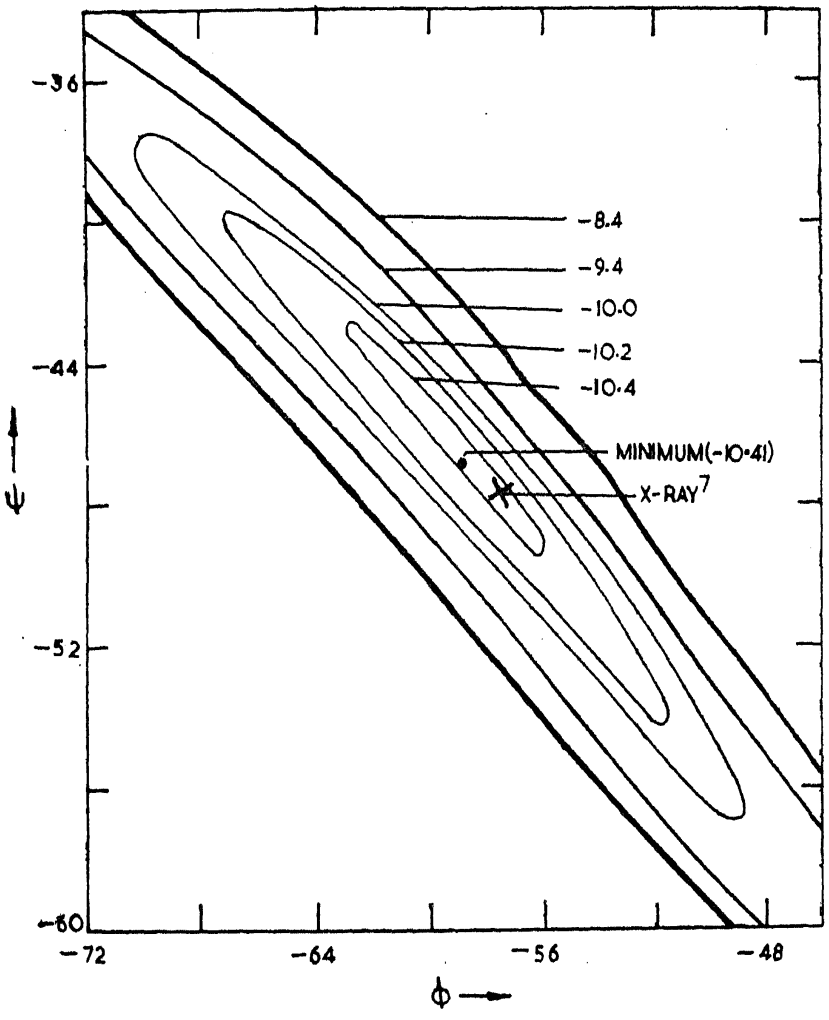

FIG. 1. Variation of energy in the (ϕ, ψ)-plane for $\tau = 110°$ and $\omega = 180°$, of the right-handed α-helical structure of poly-L-alanine. The innermost contour corresponds to − 10·4 kcal/mole and the outermost contour corresponds to a value 2·0 kcal/mole above this minimum energy.

The variation of the energy per residue of the α-helix with the parameters ϕ and ψ is shown in Fig. 1. It will be seen that there is a deep minimum, which is however in the form of a long valley over which there is negligible variation of energy for small variations in ϕ and ψ. The minimum energy has a value of $-10{\cdot}41$ kcal/mole, of which the hydrogen bond contribution is about $-4{\cdot}0$ kcal/mole. Contours are drawn in Fig. 1 corresponding to changes in energy from $-10{\cdot}4$ kcal/mole to $-8{\cdot}4$ kcal/mole (nearly 2 kcal/mole above the minimum). In an actual α-helical structure which is not completely regular, it can be expected that the local conformation may correspond to a range of (ϕ, ψ) having V up to this value above the minimum. Therefore, the contour line for $-8{\cdot}4$ kcal/mole is shown by a thick line in this figure. Table I shows the variation of n and h with ϕ and ψ for values of ϕ and ψ occurring within the narrow valley covering a region up to 0·2 kcal/mole above the minimum energy at intervals of one degree for ϕ and ψ. It will be seen that both n and h are very nearly the same over this range and that their values are close to the observed values[7, 12] of $n = 3{\cdot}615$ and $h = 1{\cdot}495$ Å, particularly for the lowest energy values between $-10{\cdot}41$ and $-10{\cdot}30$ kcal/mole. In fact, it is well known[8] that the map showing the variation of n and h in the (ϕ, ψ)-plane has contour lines of both n and h almost parallel to each other in this range and that both of them remain constant in a line making an angle of approximately 135° to the ϕ-axis. Hence, it is that the n and h values are very nearly the same for almost all the conformations within the narrow valley in Fig. 1.

It should be deduced from this that, while the values of the helical parameters n and h may be very nearly the same, it is not essential that the values of ϕ and ψ themselves should be the same for every residue of a stable helical structure. Moreover, from considerations of the type mentioned earlier, namely, that in a complicated structure like a protein, in which the environments of the residues in a helical segment are not identical for every residue, the values of ϕ and ψ may vary somewhat from those corresponding to the minimum energy conformation. In fact, the observed values of (ϕ, ψ) in the crystal structures of myoglobin and lysozyme are plotted in Fig. 2 *a* along with the contour for $V = V_{min.} + 2{\cdot}0$ kcal/mole. It will be seen that the observed local conformations (ϕ, ψ) occur in a much wider region than that enclosed by the contour. This may be due to several reasons:

(*a*) The angles ϕ and ψ as calculated from the cyrstal structure data are expected to be accurate only to 10° or 15°.

(*b*) The helices are not completely regular and in some cases they are appreciably distorted.

However, if the *average* values of (ϕ, ψ) for each individual helical segment are calculated, these are found to occur mostly within the 2·0 kcal/mole contour for a large number of proteins (Fig. 2 *b*). The most conspicuous exception is L6 at (— 61°, — 30°) in lysozyme. This contains some hydrogen bonds corresponding to the 3_{10}-helix, whose allowed region occurs well

TABLE I

*Low energy conformations of the right-handed α-helical structure of poly-*L-*alanine having* V *less than* 0·2 *kcal/mole above the minimum energy* (— 10·41 *kcal/mole*)* ($\tau = 110°$, $\omega = 180)°$

Dihedral angles		Helical parameters		Hydrogen bond		Tilt of peptide unit	Energy V
ϕ(°)	ψ(°)	n	h (Å)	Length R (Å)	Angle θ(°)	δ(°)	kcal/mole
−61	−45	3·62	1·48	2·87	16	10	−10·40
−60	−46	3·62	1·48	2·86	15	9	−10·41
−59	−47	3·63	1·48	2·85	14	8	−10·40
−62	−44	3·61	1·48	2·88	18	11	−10·38
−59	−46	3·60	1·50	2·91	16	9	−10·36
−58	−48	3·63	1·48	2·84	13	8	−10·38
−57	−47	3·60	1·50	2·90	14	8	−10·37
−57	−49	3·63	1·48	2·83	11	7	−10·36
−57	−48	3·61	1·50	2·89	13	7	−10·37
−56	−49	3·61	1·50	2·89	12	6	−10·36
−64	−42	3·60	1·48	2·91	20	12	−10·30
−63	−43	3·61	1·48	2·90	19	11	−10·35
−61	−44	3·59	1·50	2·93	18	10	−10·31
−60	−45	3·60	1·50	2·92	17	9	−10·34
−56	−50	3·64	1·48	2·83	10	6	−10·34
−55	−50	3·62	1·50	2·88	11	6	−10·33
−55	−51	3·64	1·48	2·82	9	5	−10·30
−54	−51	3·62	1·50	2·88	10	5	−10·30
−65	−42	3·63	1·46	2·87	20	13	−10·27
−64	−43	3·63	1·46	2·85	19	12	−10·29
−63	−44	3·63	1·46	2·84	17	11	−10·29
−62	−45	3·64	1·46	2·82	16	10	−10·28
−62	−43	3·59	1·50	2·92	19	11	−10·26
−61	−46	3·64	1·46	2·81	15	10	−10·27
−54	−52	3·65	1·48	2·82	8	5	−10·27
−53	−52	3·63	1·50	2·88	9	4	−10·26
−66	−41	3·62	1·47	2·88	21	13	−10·23
−65	−41	3·60	1·48	2·93	21	13	−10·24
−60	−47	3·65	1·46	2·80	14	9	−10·25
−59	−48	3·65	1·46	2·79	12	8	−10·22
−53	−53	3·65	1·48	2·82	7	4	−10·22
−52	−53	3·63	1·50	2·88	8	3	−10·21

*The conformations are collected in three groups (*a*) V < − 10·4, (*b*) − 10·4 < V < − 10·3 and (*c*) −10·3 < V < − 10·2, but in each group they are listed according to increasing values of (ϕ, ψ) at intervals of 1°.

above that of the α-helix. The helix M7 with seven residues in myoglobin with a mean value of (— 57°, — 38°), which is also somewhat outside the contour marked in Fig. 2 *b* is described as a distorted helix by the original author.[13]

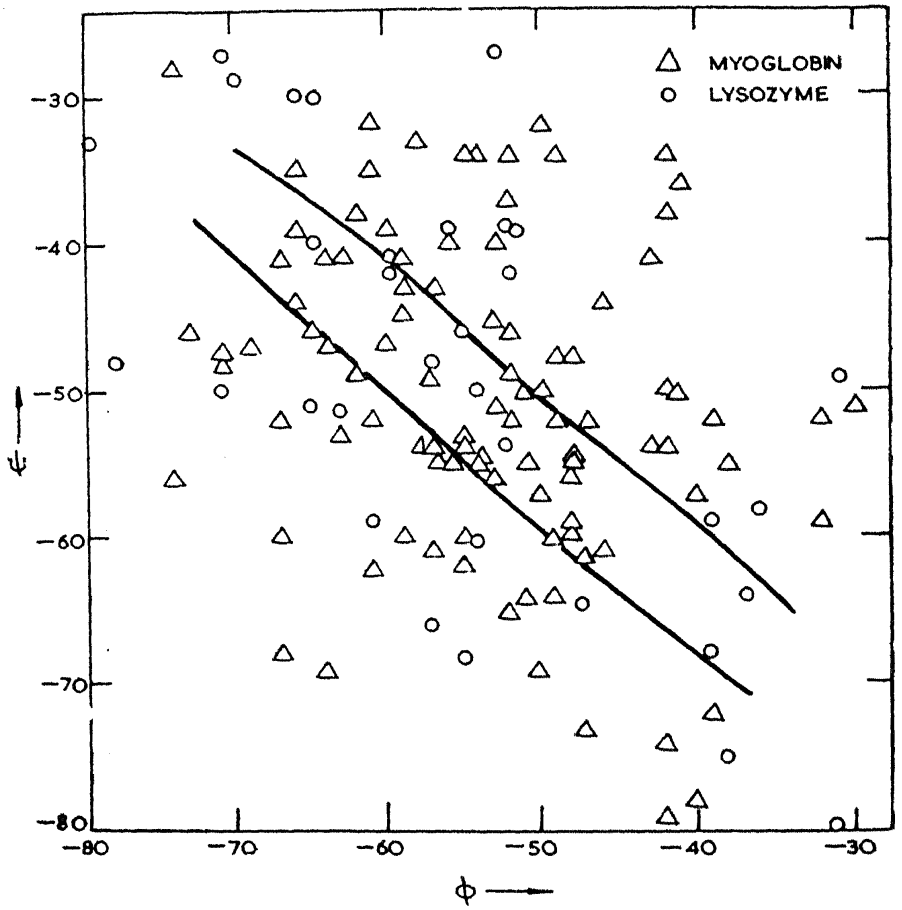

FIG. 2 *a*. Distribution of (ϕ, ψ) for the various amino-acid residues of myoglobin and lysozyme occurring in the α-helical region. The contour for V_{min} + 2·0 kcal/mole is also shown. (△ = myoglobin, O = lysozyme.)

STRUCTURAL FEATURES OF THE α-HELIX

The right-handed α-helical structure with $n \sim 3{\cdot}6$ and $h \sim 1{\cdot}5$ Å consists of regular NH $\cdots$O bonds formed between every carbonyl oxygen and an amino nitrogen three units ahead of it. In the minimum energy region ($\sim$ — 10·4 kcal/mole) the hydrogen bond length varies from 2·85 to 2·90 Å and the bond is invariably not straight. The non-linearity of the bond is in the range of 10° to 20°.

Another interesting feature of the low energy conformations is related to the orientation of the peptide unit with respect to the helical axis. The peptide unit is somewhat inclined to the axis of the helix in such a manner

that the NH group is pointed towards the interior and the carbonyl oxygen is directed away from the centre of the helix. The tilt angle δ is about 5° to 10°. Both of the above features agree with observation[7], which gives R = 2·86 Å, θ = 10°, δ = 6°, as calculated by us from the data in Ref. 7. The fact that the stability of the Pauling α-helix increases when the peptide units are tilted so that the NH groups point inwards was suggested by Ramachandran[14] and by Sasisekharan[15] as early as 1962. It is seen from Table I that, as the non-linearity of the hydrogen bond increases, this angle also increases.

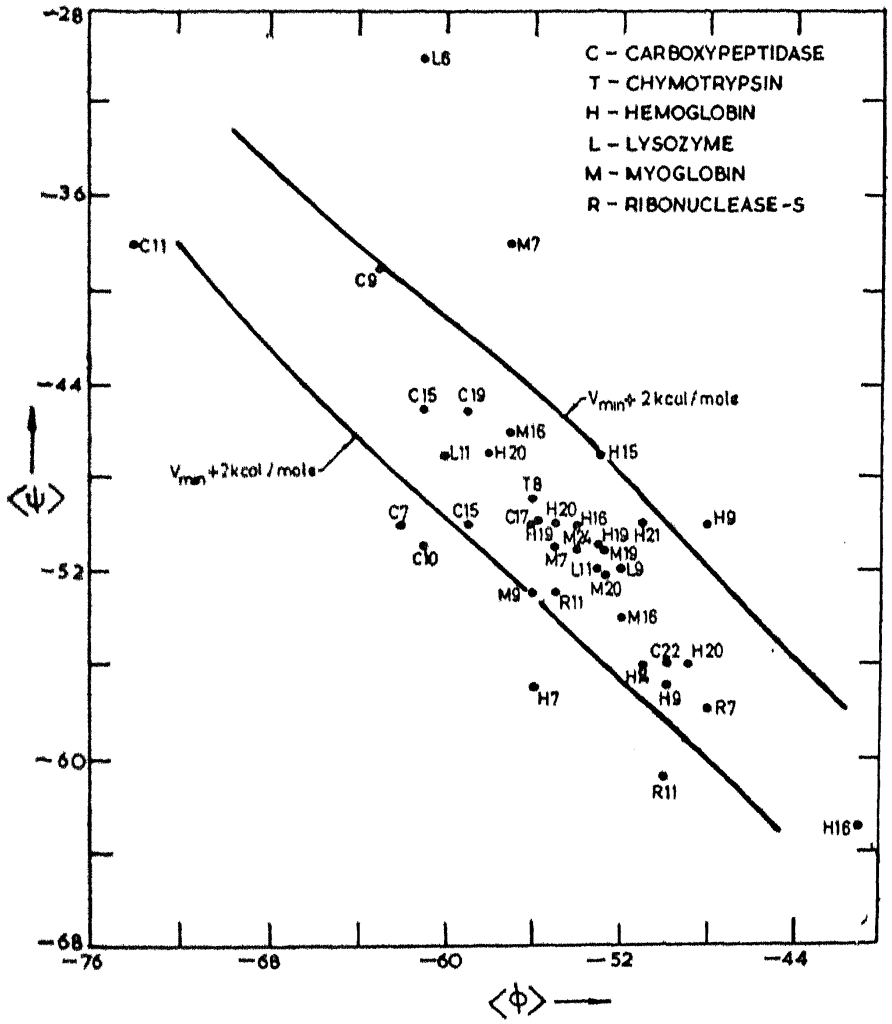

FIG. 2 *b*. Mean values of (ϕ, ψ) for the different helical segments in the protein crystal structures Carboxypetpidase,[17] Chymotrypsin,[18] Hemoglobin,[10] Lysozyme[8,20] Myoglobin,[13] and Ribonuclease.[21] The symbols give the letter indicating the protein and the number of residues in the helical segment (*eg.* R11 is a segment in ribonuclease S with 11 residues). Note that most of the points lie within the contour for V_{min} + 2·0 kcal/mole.

The fact that the peptide unit is tilted such that the NH group is pointed inwards is significant in relation to the stability of the α-helix in solution

in a polar solvent. Even if the solvent contains an acceptor atom for hydrogen bonds (say an oxygen atom in C=O), since this oxygen cannot approach closer than 2·6 Å from the acceptor oxygen in the helix itself, the angle θ for the disturbing solvent acceptor atom cannot in general be less than about 40° which greatly reduces the perturbing influence of the polar solvent in disturbing the α-helix. This fact, together with the common occurrence of hydrophobic side groups in the exposed regions of a helix, is responsible for the large stability of the α-helices occurring in proteins in biological systems.

Effect of Varying τ, ω and χ on V_{min} of the α_P-helix

The results mentioned above were obtained from the first phase of the calculations in which only two parameters (namely, ϕ and ψ) were varied, while the other three prarameters τ, ω and χ were kept constant at their standard values. To study the influence of these latter parameters on the conformation of this helical structure, the region of low energy conformations was explored in greater detail in the second phase of the calculations. This was actually done in two stages:

(*a*) The parameter χ alone was varied in the range 50° to 70° at intervals of 5°, using, however, the standard values of $\tau = 110°$ and $\omega = 180°$, and

(*b*) the parameters τ and ω were varied in the range $\tau = 110° \pm 2°$ (1°) and $\omega = 180° \pm 3°$ (1°), keeping χ at 60°. The salient features arising from the results of these calculations may be summarised as follows.

The effect of the change in χ is shown in Table II for all examples with $V_{min} \leq -10{\cdot}2$ kcal/mole. It will be seen from this that the energy is a minimum for some value of χ between 55° and 60° (for $\phi = -56°$, $\psi = -50°$, the actual values are $-10{\cdot}36$ kcal/mole for 55° and $-10{\cdot}34$ kcal/mole for 60°, although they appear as $-10{\cdot}4$ kcal/mole and $-10{\cdot}3$ kcal/mole for these two values, because of rounding off errors, in Table II). In view of this, it was not considered worthwhile to make detailed calculations for χ other than the standard value of 60°.

The variation of V with τ and ω was investigated in detail for $\chi = 60°$. The minimum energy values alone are summarised in Table III in which, for each combination of τ and ω, the data for (ϕ, ψ) are given for which

V_{min} varies by less than 0·01 kcal/mole from the minimum. It will be seen from this table that the minimum energy conformation for each τ is close to $\phi = -58°$, $\psi = -48°$, which are the experimentally determined values for the fibre structureo fpoly-L-alanine. Since the variations in the values of the minimum energy for τ varying from 110° to 112° are less than 0·1 kcal/mole and since we have not included the effect of neighbouring helices in the crystal structure in making these calculations, it is not possible to state which is the absolute minimum energy conformation according to theory, except to indicate that τ and ω are not significantly different from the standard values of 110° and 180° respectively, again as found by Arnott and Dover[7] from their refinement of X-ray data.

TABLE II

*Low energy conformations of the right-handed α-helical structure of poly-L-alanine showing the effect of rotating the methyl hydrogens** ($\tau = 110°$ *and* $\omega = 180)°$

ϕ(°)	ψ(°)	n	h(Å)	R(Å)	θ(°)	δ(°)	V in kcal/mole for values of χ equal to				
							50°	55°	60°	65°	70°
−62	−44	3·61	1·48	2·88	18	11	−10·2	−10·4	−10·4	−10·3	−10·0
−60	−46	3·62	1·48	2·86	15	9	−10·2	−10·4	−10·4	−10·3	−10·0
−58	−48	3·63	1·48	2·84	13	8	−10·3	−10·4	−10·4	−10·2	− 9·9
−56	−50	3·64	1·48	2·83	10	6	−10·2	−10·4	−10·3	−10·2	− 9·8
−64	−42	3·60	1·48	2·91	20	12	−10·1	−10·3	−10·3	−10·2	− 9·9
−54	−52	3·65	1·48	2·82	8	5	−10·2	−10·3	−10·3	−10·1	− 9·7
−58	−46	3·58	1·52	2·96	16	8	−10·2	−10·2	−10·2	− 9·9	− 9·5
−56	−48	3·59	1·52	2·95	14	7	−10·2	−10·3	−10·2	− 9·9	− 9·5
−52	−54	3·66	1·48	2·82	6	3	−10·1	−10·2	−10·2	−10·0	− 9·6
−66	−40	3·60	1·49	2·95	23	14	− 9·9	−10·1	−10·2	−10·1	− 9·8

*Only those conformations with V_{min} less than − 10·2 kcal/mole are listed.

Left-handed α-helical form of poly-L-alaline.—In so far as the backbone conformation alone is concerned, the inverse local conformation of the right-handed structure of given n and h generates the corresponding left

TABLE III

Minimum energy conformations of the right-handed α-helical structure of poly-L-alanine with τ and ω varied from the standard values at 1° intervals

τ(°)	Δω*(°)	φ(°)	ψ(°)	n	h(Å)	R(Å)	θ(°)	δ(°)	V kcal/mole
109	−2	−66	−38	3·50	1·45	2 82	26	14	− 9·28
	−1	−67	−38	3·53	1·46	2·85	26	15	− 9·72
	0	−64	−41	3·56	1·47	2·87	23	12	− 9·98
		−63	−42	3·56	1·47	2·85	21	12	− 9·98
	+1	−62	−44	3·60	1·47	2·85	19	11	−10·17
		−61	−45	3·61	1·47	2·84	17	10	−10·17
		−60	−45	3·59	1·49	2·89	18	9	−10·17
		−59	−46	3·59	1·49	2·88	17	9	−10·17
		−58	−47	3·60	1·49	2·87	15	8	−10·17
	+2	−59	−47	3·63	1·49	2·90	15	8	−10·30
		−58	−48	3·63	1·49	2·89	14	7	−10·30
		−57	−49	3·64	1·49	2·88	12	7	−10·30
	+3	−58	−49	3·67	1·50	2·91	12	7	−10·31
		−57	−50	3·68	1·50	2·91	11	6	−10·31
110	−3	−67	−37	3·51	1·46	2·87	27	15	− 9·76
	−2	−63	−41	3·54	1·47	2·87	22	12	−10·00
	−1	−62	−43	3·58	1·48	2·87	19	11	−10·25
		−61	−44	3·58	1·48	2·85	18	10	−10·25
	0	−60	−46	3·62	1·48	2·86	15	9	−10·40
	+1	−59	−48	3·66	1·48	2·87	12	8	−10·47
		−58	−49	3·67	1·48	2·86	11	7	−10·47
	+2	−58	−50	3·71	1·49	2·89	10	7	−10·44
	+3	−58	−51	3·75	1·49	2·93	9	6	−10·31
		−57	−52	3·75	1·49	2·93	7	6	−10·31
111	−3	−65	−40	3·56	1·46	2·87	22	13	−10·19
		−64	−41	3·57	1·46	2·85	21	12	−10·19
	−2	−60	−45	3·59	1·48	2·87	16	10	−10·38
	−1	−59	−47	3·64	1·49	2·88	13	8	−10·49
		−58	−48	3·64	1·48	2·87	12	8	−10·49
	0	−58	−49	3·68	1·49	2·90	10	7	−10·50
		−57	−50	3·68	1·49	2·89	9	7	−10·50
	+1	−57	−51	3·72	1·49	2·93	8	6	−10·42
	+2	−59	−51	3·78	1·48	2·93	9	7	−10·27
		−58	−52	3·79	1·48	2·92	8	6	−10·27
	+3	−58	−57	3·83	1·48	2·97	8	6	−10·04
112	−3	−59	−46	3·61	1·49	2·89	14	9	−10·38
		−58	−47	3·61	1·49	2·88	12	8	−10·38
	−2	−57	−49	3·65	1·49	2·90	10	7	−10·45
	−1	−57	−50	3·69	1·50	2·93	8	7	−10·41
		−56	−51	3·70	1·50	2·92	7	6	−10·41
	0	−58	−51	3·76	1·48	2·92	8	7	−10·30
		−57	−52	3·76	1·48	2·91	7	6	−10·30
	+1	−58	−52	3·80	1·49	2·96	8	6	−10·11
		−57	−53	3·80	1·49	2·96	7	6	−10·11
	+2	−58	−53	3·84	1·49	3·01	8	6	− 9·80
	+3	−59	−54	3·91	1·47	3·03	11	6	− 9·47

*Δω is the deviation of ω from the standard value of 180° for the *trans* peptide units.

handed structure of the same energy with helical parameters $-n$ and h. In the presence of the side group in the same asymmetric-L-configuration in both structures, the energy values will not be the same for the two. In order to compare the relative stabilities of the right- and left-handed α-helical structures of poly-L-alanine, the energy values were computed for the left-handed structure for different values of ϕ and ψ, keeping the other three parameters τ, ω and χ equal to their respective standard values. The results are summarised below.

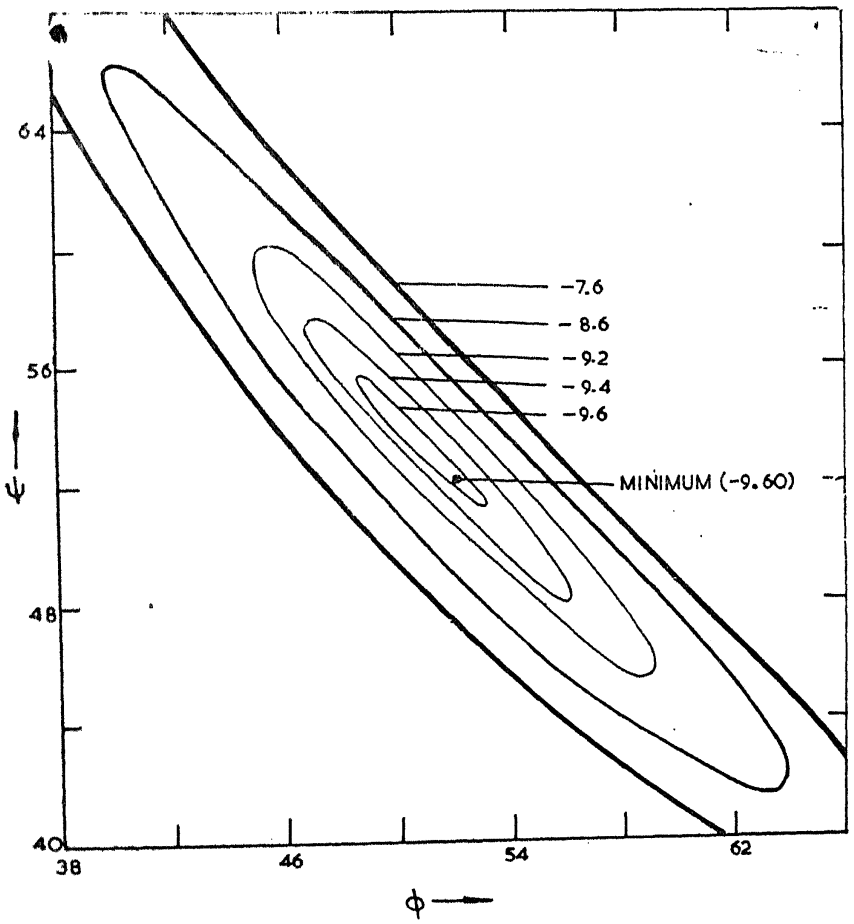

FIG. 3. Variation of energy of the left-handed α-helical structure of poly-L-alanine in the (ϕ, ψ)-plane for $\tau = 110°$ and $\omega = 180°$. The innermost contour corresponds to $-9{\cdot}6$ kcal/mole and the outermost contour is drawn for a value 2·0 kcal/mole above this minimum energy.

Similar to the findings for the α_P-helix, the minimum energy conformations for the α_M-helix occur in a long valley with nearly constant values for n and h, close to $-3{\cdot}6$ and 1·5 Å respectively. The variation of the energy of the helix in the (ϕ, ψ)-plane is shown in Fig. 3. The low energy conformations and their characteristic parameters are listed in Table IV. The minimum energy in this region is about $-9{\cdot}6$ kcal/mole, which is 0·8 kcal/mole above that of the corresponding right-handed structure. We

believe that this difference of nearly 1 kcal/mole is significant and makes the α_P-structure more stable than α_M for poly-L-alanine. In the case of longer side-chains, it may be expected that there may be a drastic reversal in the difference between the right- and left-handed structures owing to side-chain-back-bone interactions, so that the latter may be more favourable in some cases.[16]

TABLE IV

Low energy conformations of the left-handed α-helical structure of poly-L-alanine having $\triangle V$ *less than* 0·3 *kcal/mole above the minimum energy* (− 9·6 *kcal/mole*). ($\tau = 110°$ *and* $\omega = 180°$)

ϕ(°)	ψ(°)	n	h(Å)	R(Å)	θ(°)	δ(°)	V kcal/mole
50	54	−3·61	1·52	2·94	9	2	−9·6
52	52	−3·60	1·52	2·94	10	4	−9·6
48	56	−3·62	1·53	2·95	8	1	−9·5
54	50	−3·60	1·52	2·94	12	5	−9·5
46	58	−3·63	1·53	2·96	8	−1	−9·3
48	58	−3·67	1·49	2·84	3	0	−9·3
50	56	−3·67	1·49	2·82	4	2	−9·3
52	54	−3·66	1·48	2·82	6	3	−9·3
54	52	−3·65	1·48	2·82	8	5	−9·3
56	50	−3·64	1·48	2·83	10	6	−9·3
58	46	−3·58	1·52	2·96	16	8	−9·3

Calculations made with values of τ different from the standard value of 110° resulted always in a destabilisation of the left-handed structure. No attempts were made to vary ω and to explore the minimum energy region in detail in this case.

ACKNOWLEDGMENT

This work was generously supported by N.I.H. Grants AM 11493 given to G.N.R. in Chicago and AM 15964 given to him in Bangalore. We would like to thank Drs. M. F. Perutz and M. Muirhead for making available to us the hemoglobin data prior to publication.

REFERENCES

1. Pauling, L. and Corey, R. B. *Proc. Nat. Acad. Sci.*, 1951, 37, 235.

2. Ramachandran, G. N., Venkatachalam, C. M. and Krimm, S. — *Biophys. J.*, 1966, **6** 849.
3. Liquori, A. M. .. *J. Polymer Sci. Pt. C*, 1966, **12**, 209.
4. Scott, R. A. and Scheraga, H. A. — *J. Chem. Phys.*, 1966, **45**, 2091.
5. Balasubramanian, R., Chidambaram, R. and Ramachandran, G. N. — *Biochem. Biophys. Acta*, 1970, **221**, 196.
6. Ramachandran, G. N., Chandrasekaran, R. and Chidambaram, R. — This Journal, Part III.
7. Arnott, S. and Dover, S. D. — *J. Mol. Biol.*, 1967, **30**, 209.
8. Ramachandran, G. N. and Sasisekharan, V. — *Advan. Protein Chem.*, 1968, **23**, 283.
9. Bixon, M. and Lifson, S. — *Tetrahedron*, 1967, **23**, 769.
10. Ramachandran, G. N., Lakshminarayanan, A. V., Balasubramanian, R. and Tegoni, G. — *Biochem. Biophys. Acta*, 1970, **221**, 165.
11. IUPAC-IUB Commission on Biochemical Nomenclature, *Biochemistry*, 1970, **9**, 3471.
12. Ramachandran, G. N. .. *Proc. Ind. Acad. Sci.*, 1960, **52 A**, 240.
13. Watson, H. C. .. *Progress in Stereochemistry*, 1969, **4**, 299.
14. Ramachandran, G. N. .. In *Collagen*, N. Ramanathan, Ed., Interscience, New York, 1962, p. 3.
15. Sasisekharan, V. .. In *Collagen*, N. Ramanathan, Ed., Interscience New York, 1962, p. 39.
16. Scott, R. A., Vanderkooi, G., Leach, S. J., Gibson, K. D., Ooi, T. and Neméthy, G. — In *Conformation of Biopolymers*, G. N. Ramachandran, Ed, Academic Press, London, Vol. I, 1967, p. 43.
17. Lipscomb, W. N., Reeke, G. N., Hartsuck, J. A., Quiocho, F. A. and Bethge, P. H. — *Phil. Trans. Roy. Soc. Lond.*, 1970, **257 B**, 177.
18. Birktoft, J. J., Matthews, B. W. and Blow, D. M. — *Biochem. Biophys. Res. Comm.*, 1969, **36**, 131.
19. Muirhead, M. and Perutz, M. F. — Personal communication.

20. Blake, C. C. F., Koenig, D. F., Mair, G. A., North, A. C. T., Phillips, D. C. and Sarma, V. R. *Proc. Roy. Soc. Lond.*, 1967, **167 B**, 365.

21. Wyckoff, H. W., Tsernoglou, D., Hanson, A. W., Knox, J. R., Lee, B. and Richards, F. M. *J. Biol. Chem.*, 1970, **245**, 305.