THEORETICAL CONFORMATIONAL ANALYSIS OF N-ACETYL-L-LEUCINE METHYLAMIDE

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We have previously made a theoretical investigation of the spatial structures of a number of methylamides of N-acetyl-L-(amino acid)s ($CH_3CO-X-NHCH_3$, where X = Gly, Ala, Phe, Val, or Pro), which enabled us to obtain an idea of the potential surface of these molecules and to determine the geometrical and thermodynamic parameters of all the optimum forms [1-4]. In the calculation of molecules containing voluminous substituents on the C^{α} atom (Phe, Val), an interconnection of the conformational states of the main chain and the side chain was revealed. It was found that to each optimum conformation of the main chain correspond energetically favorable orientations of the substituents. In its turn, a side chain affects the position and stability of the optimum forms of the main chain.

In the subsequent consideration of the spatial structure of the main and side chains of the amino-acid residues in proteins of known structure, it was possible to conclude that between their conformational states and the geometry of the optimum forms of the corresponding dipeptides there is a fairly close correlation [5, 6]. In the overwhelming majority of cases precisely those conformations of the residues are realized in proteins that are the most preferred in the isolated simplest fragments. Apart from this, in the hydrophobic residues in proteins the same mutual dependence of the conformational states of the main and side chains is observed as for the methylamides of N-acetyl(α-amino acid)s. The analogy that has been observed in the spatial structure of isolated dipeptide fragments and amino-acid residues in the polypeptide chain shows the necessity in studying the structure of a protein of knowing the conformational possibilities of the simplest model compounds of all the natural α-amino acids.

In the present paper we give the results of a conformational analysis of N-acetyl-L-leucine methylamide. This compound possesses a voluminous substituent at the C^{α} atom, and therefore one of the tasks of the investigation was to elucidate the connection between the conformations of the main and side chains. A comparison of the results obtained with the experimental results on the spatial structure of the leucine residues in proteins enables the role of close interactions in the formation of the conformations of Leu residues in a polypeptide chain to be evaluated. Furthermore, it is important to emphasize how correct it is to regard leucine as a stereochemical analog of alanine [5].

MODEL OF THE MOLECULE AND POTENTIAL FUNCTIONS

The conformational state of N-acetyl-L-leucine methylamide is described by the four angles of rotation around the following bonds: $C^{\alpha}-N$ (φ), $C^{\alpha}-C'$ (ψ), $C^{\alpha}-C^{\beta}$ (χ_1), and $C^{\beta}-C^{\gamma}$ (χ_2) (a model of the molecule is shown in Fig. 1). We have discussed the method of calculating peptide compounds previously [8]. For the bond links of the amide group we used the values proposed by Pauling and Corey [9]. For the valence angles in the main chain we took the mean parameters for a peptide group [1]. In harmony with x-ray structural results for glycyl-L-tyrosine [10], the $C^{\alpha}-C^{\beta}-C^{\gamma}$ angles was fixed at 113°.

In the calculation of conformations, the nonvalent and electrostatic interactions were taken into account, and so was the energy of rotation around the ordinary bonds. The formation of intramolecular hydrogen bonds was not taken into account, since the conformation of N-acetyl-L-leucine methylamide was considered for the case of an aqueous medium. The nonvalent interactions were calculated from the Buckingham potential with the parameters proposed by Dashevskii [1]. The electrostatic energy was calculated by

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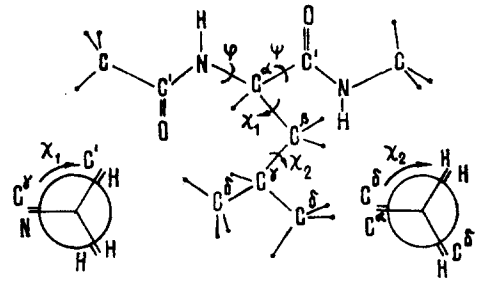

Fig. 1. Model of N-acetyl-L-leucine methylamide in the conformation $\varphi = \psi = -180°$ (positions corresponding to $\chi_1 = \chi_2 = 0$ [7] shown in Newman projections).

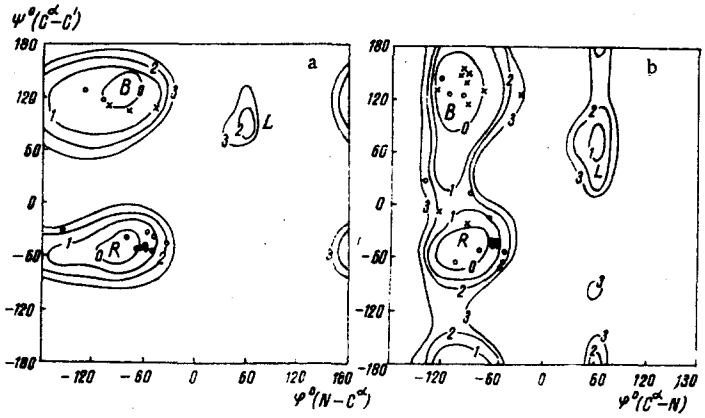

Fig. 2. $\varphi - \psi$ conformational maps of N-acetyl-L-leucine methylamide calculated for two orientations of the side chain: $\chi_1 = \chi_2 = 180°$ (a) and $\chi_1 = \chi_2 = -60°$ (b). On the maps have been marked the conformational points φ, ψ of the Leu residues in myoglobin (·), α-chymotrypsin (×), and carboxypeptidase (o).

Coulomb's law using the partial charges on the atoms found by Scott and Scheraga [11]. The calculation was performed at a value of the dielectric constant $\varepsilon = 10$. The torsional potentials of the bonds in the main and side chains were taken in the following forms [11]:

$$U(\varphi) = 0.3\ (1 - \cos 3\varphi),$$
$$U(\psi) = 0.1\ (1 + \cos 3\psi),$$
$$U(\chi) = 1.5\ (1 + \cos 3\chi).$$

POTENTIAL SURFACE AND OPTIMUM FORMS OF THE MOLECULE

In N-acetyl-L-leucine methylamide with its voluminous hydrophobic side chain the nonvalent interactions of the atoms play the main role, in comparison with the other types of interactions, in determining the spatial structure of the molecule. Consequently, the conformational maps constructed with only the nonvalent interactions taken into account give an extremely full representation of the potential surface. Among the parameters determining the conformation, the most rigid are the angles χ_1 and χ_2, a change in which is connected with the overcoming of barriers of about 3.0 kcal/mole. In view of this, we first constructed the $\varphi - \psi$ conformational maps corresponding to all the minima of the torsional potentials of rotation about the $C^\alpha - C^\beta$ and $C^\beta - C^\gamma$ angles, i.e., corresponding to the nine pairs of values of χ_1 and χ_2 at which $U(\chi_1) = U(\chi_2) = 0$. According to the nomenclature adopted (see Fig. 1), this condition is satisfied by angles χ_1 and χ_2 of 60°, 180°, and −60°. It was found that of the nine variants considered only in two, namely those cases in which χ_1 and $\chi_2 = 180$ and −60°, are there low-energy regions on the $\varphi - \psi$ maps. In the other seven maps, the energy at the minima exceeds the energy of the most favorable conformation by 8-9 kcal/mole. On the $\varphi - \psi$ conformational map with the angles $\chi_1 = \chi_2 = 180°$ (Fig. 2a), the permitted regions R and B are stretched out along the φ direction. This is due to the fact that with these values of χ_1 and χ_2 the lateral substituent is located above the $C^\alpha - C'$ bond and thus limits the freedom of rotation

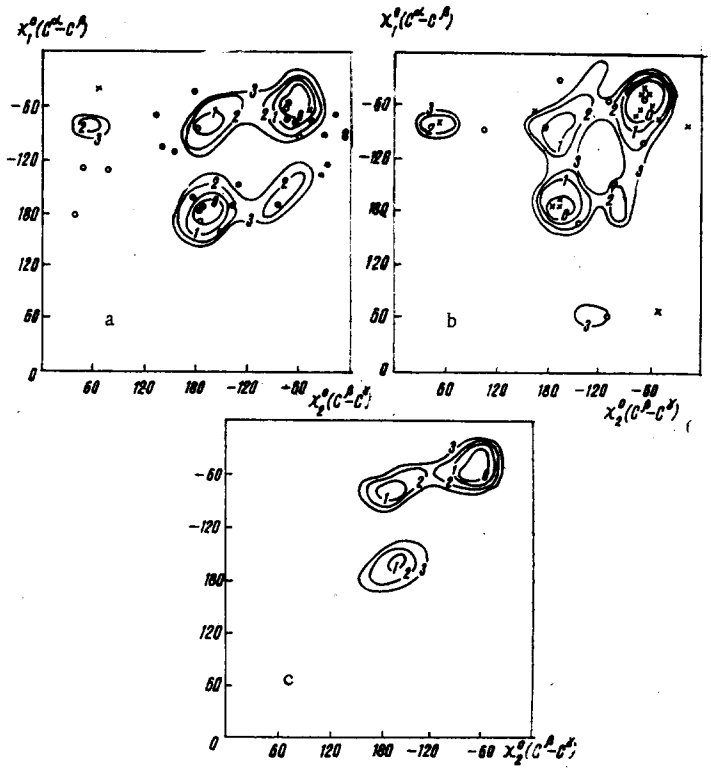

Fig. 3. $\chi_1-\chi_2$ conformational maps of N-acetyl-L-leucine methylamide calculated for the three optimum forms of the main chain R – $\varphi=-100°$, $\psi=-60°$ (a), B – $\varphi=-100°$, $\psi=140°$ (b), and L – $\varphi=60°$, $\psi=60°$ (c). On the maps have been plotted the conformational points χ_1, χ_2 of the Leu residues in myoglobin (·), α-chymotrypsin (×), and carboxypeptidase (o).

around this bond (ψ). Conversely, at the values $\chi_1=\chi_2=60°$ (see Fig. 2b) the low-energy contours R and B on the potential surface are stretched out in the direction of the ψ axis; in this case, the side chain is located above the $C^{\alpha}-N$ bond and thereby limits the region of possible values of the angle φ. With the orientation of the side chain given by $\chi_1=\chi_2=180°$, there is a high barrier on the map separating the R and B regions. At the values of the angles $\chi_1=\chi_2=-60°$, the height of the barrier is considerably smaller and the corresponding values of the angles ψ of the main chain become extremely likely. The L region is less preferred with respect to nonvalent interactions than the R and B regions at all angles of the side chain. The most suitable position in the L conformation is the $\chi_1=\chi_2=-60°$ position.

A generalized conformational map of N-acetyl-L-leucine methylamide constructed by selecting the lowest values of the energy from the two maps mentioned above is very similar to the map of N-acetyl-L-alanine methylamide [1]. This means that the side chain of Leu is adapted to the conformation of the main chain on the whole the freedom of rotation of the main chain in both the first and the second molecule is adequate in practice. Consequently, the leucine dipeptide is a stereochemical analog of the analine dipeptide.

Above, we consider the potential surface of N-acetyl-L-leucine methylamide giving an idea of the conformational possibilities of the main chain of the molecule. To solve the analogous question in relation to the side chain we obtained sections of the $\chi_1-\chi_2$ potential surface at fixed positions of the side chain. As the latter we selected the values of φ and ψ of the optimum forms: R ($\varphi=-100°$, $\psi=-60°$), B ($\varphi=-100°$, $\psi=140°$), and L ($\varphi=60°$, $\psi=60°$). The corresponding $\chi_1-\chi_2$ conformational maps of the side chain are shown in Fig. 3a, b, and c. It can be seen from this figure that the most suitable positions with respect both to enthalpy and entropy are those at $\chi_1=\chi_2=180$ and $-60°$. However, it must be borne in mind that the sections given were obtained with strictly fixed conformations of the main chain. We have plotted $\chi_1-\chi_2$ maps for other values of φ and ψ corresponding to the low energy regions R and B, as well. Although in all cases the orientations of the side chain with $\chi_1=\chi_2=180°$ and $-60°$ were the most preferred, nevertheless, the energy difference of these forms as compared with the other had changed considerably. Furthermore, for a number of conformations of the side chain likewise corresponding to minima of the torsional potentials U (χ_2) the total energy of the molecule is extremely sensitive to deviations of the angles χ_1 and χ_2 for their

TABLE 1. Energies and Geometries of the Optimum Conformations of N-Acetyl-L-leucine Methylamide

Conformation	Geometry, deg				Energy, kcal/mole	
	φ	ψ	χ_1	χ_2	U_{nv}	U_{tot}
R	−100	−60	−60	−60	0	0
	−100	−60	−80	−160	0	1,2
	−100	−60	−80	60	3,0	3,0
	−100	−60	180	180	0,3	0,3
	−100	−60	−160	−80	0,8	2,2
B	−120	120	−60	−60	0,2	−0,3
	−120	120	180	180	0,5	0.1
	−120	120	−160	−80	1,0	1,9
	−100	140	−80	−160	0,2	1,3
	−100	140	−80	60	3,3	3,0
	−100	140	60	−140	4,0	3,5
L	60	60	−60	−60	1,2	1,7
	60	60	−80	−160	1,4	3,0
	80	60	180	180	1,9	2,5
	100	60	−160	−80	1,6	3,2

optimum values. For example, on the $\chi_1-\chi_2$ section in the R region (see Fig. 3a), the total energy at $\chi_1=-60°$, $\chi_2=180°$ and at $\chi_1=180$, $\chi_2=-60°$ is 8-10 kcal/mole higher than the energy at $\chi_1=\chi_2=180°$ and $-60°$. Nevertheless, it is sufficient to change the angles χ_1 and χ_2 by only 20° for the difference to fall to 1-2 kcal/mole. Thus, the orientation of the side chain at $\chi_1=-60°$, $\chi_2=180°$ and at $\chi_1=180°$, $\chi_2=-60°$ must be considered as perfectly possible; the values of χ_1 and χ_2 at 60° are less likely (see also [16]).

Table 1 gives the energies and geometric parameters of the optimum conformations of N-acetyl-L-leucine methylamide taking all forms of interactions into account and, separately, with only the nonvalent interactions taken into account. The overall minimum is found in region B.

To estimate the contribution to the stability of the optimum conformations of the entropy of the side chain, we obtained conformational maps of the statistical sums (Z) on rotation at each point φ, ψ around the $C^{\alpha}-C^{\beta}$ and $C^{\beta}-C^{\gamma}$ bonds from −180 to 160°. The statistical sums were calculated with and without allowance for the energy of the main chain. The statistical sums were determined by approximate integration over all the conformations of the side chain:

$$Z=\left(\frac{2\pi}{N}\right)^2\sum_{\chi_1}\sum_{\chi_2}e^{-(E-E_0)/kT},$$

where E_0 is the energy of the overall minimum ($\varphi=-120°$, $\psi=120°$, $\chi_1=-60°$, and $\chi_2=-60°$); and N is the number of subdivisions of 2π − in this case, the values of χ_1 and χ_2 were changed in steps of 20°, and consequently N=18. Knowing the statistical sum it is possible to find the free energy of the conformation from the formula $F=RT\ln Z$ and to estimate its relative stability.

From the values of the statistical sums for Leu obtained without allowing for the energy of the main chain it follows that the entropy of the side chain depends on the angles φ and ψ. However, in the low-energy regions R and B symbaticity in the change of Z is observed.

Below we give for N-acetyl-L-leucine methylamide the values for the side chain without taking the energy of the main chain into account.

φ		−180°	−160°	−140°	−120°	−100°	−80°	−60°
ψ	−60°	0,20	0,20	0,30	0,50	0,50	0,40	0,30
	120°	0,20	0,25	0,30	0,45	0,50	0,40	0,25

Here the dependences of Z on φ at two optimum values of ψ (−60 and 120°) are compared. The statistical sums, and, consequently, the free energies of the side chain in the R and the B conformations are identical. This also relates to the values of Z (~0.3) in dipeptide fragments in the conformations of a standard α-helix and of parallel and antiparallel β-structures. Consequently, the local mobility of the side chain of the Leu residues in poly-L-leucine does not give advantages to any of the canonical forms mentioned above. Thus, the presence of an overall minimum in the B region on the map of the statistic sums of N-acetyl-L-leucine methylamide in the plotting of which the interaction of all the atoms of the molecule was taken into account (Fig. 4) is explained by the preferential nature of the B conformation only with respect to the energy of interaction in the main chain. This form is also more suitable than R from the point of view of the entropy of the main chain; the contours of the high values of the statistical sums outline the largest area in this region of the $\varphi-\psi$ map.

CONFORMATIONAL STATES OF LEUCINE RESIDUES IN PROTEINS

In this section we consider the question of the degree of correspondence of the spatial structure of leucine residues in proteins to the conformational possibilities of N-acetyl-L-leucine methylamide. On the maps of the isolated dipeptide given in Fig. 2a, and b the conformational points φ, ψ of Leu residues in myoglobin, α-chymotrypsin, and carboxypeptidase have been plotted at the corresponding values of χ_1

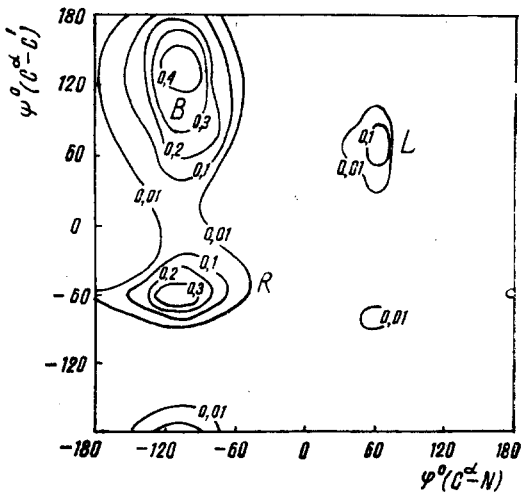

Fig. 4. $\varphi-\psi$ conformational map of the statistical sums of the side chain of N-acetyl-L-leucine methylamide.

and χ_2.* As can be seen from Fig. 2, practically all the residues fall in low-energy regions on the map. Not one residue has the L conformation.

The distribution of the Leu residues in the low-energy regions, especially in R, deviates from the mean statistical distribution of the conformations of the dipeptide. In the R region, as a rule, the values of the angle φ of the Leu residues (−70 to −40°) are considerably greater than in the optimum form of N-acetyl-L-leucine methylamide ($\varphi \sim -100°$). This is due to the influence of the neighboring residues and of the incorporation of the Leu into the α-helix [5].

The difference in the energy of the optimum form R of the dipeptide ($\varphi = -100°$, $\psi = -60°$) and the form having the parameters of the standard α-helix ($\varphi = -57°$, $\psi = -48°$) does not exceed 1 kcal/mole. The comparatively large number of Leu points in the region of the low R−B barrier in the majority of cases is connected with the entry of the residue into a 10-membered ring with a hydrogen bond of the 1−4 type [5].

We mentioned above that the low-energy contours on the $\varphi-\psi$ map depend on the orientation of the side chain of the N-acetyl-L-leucine methylamide. With the fixing of the side chain in the position $\chi_1 = \chi_2 = 180°$ (and also $\chi_1 = 180°$, $\chi_2 = 60°$), the R and B regions are extended along the φ axis, and at $\chi_1 = \chi_2 = -60°$ they are extended along the ψ axis. In the case of Leu residues in proteins, the same interdependence of the conformational states of the side and main chains is observed as in the dipeptide. Where, in the side chain, the values of the angles χ_1 and χ_2 are ~180°, on the $\varphi-\psi$ conformational map in correspondence with the profile of the potential surface a large scatter of the points with respect to the angle φ and a small scatter with respect to the angle ψ is observed and conformations in the space between the R and B regions (ψ = −20 to 60°) are absent; at the values $\chi_1 = \chi_2 = -60°$, conversely, the greatest scatter of the points is seen with respect ψ (Fig. 2a, b).

An important role of close interactions is also observed in an analysis of the conformations of the side chains of the leucine residues in protein. The two most favorable positions of the side chain in N-acetyl-L-leucine methylamide ($\chi_1 = \chi_2 = 180$ and −60°) are found most frequently for Leu in proteins.

The conformational points of the side chains of Leu residues in proteins have been plotted on the sections of the $\chi_1-\chi_2$ potential surface at $\varphi = -100°$, $\psi = -60°$ (see Fig. 3a) and $\varphi = -100°$, $\psi = 140°$ (see Fig. 3b). In a considerable majority of the cases, the points fall in the region of low energy of the side chain of the leucine dipeptide. This shows a satisfactory correspondence of the positions of the side chains of the Leu residues in proteins with the optimum forms of the isolated model compound. The presence of a number of points outside the regions of minimum energy is, in our view, due above all to the inaccuracy of the experimental determination of the angles χ_1 and χ_2 (the mean error amounts to 20-30°). The fact that many of those Leu residues, the parameters χ_1 and χ_2 of which have been plotted on the map (see Fig. 3a, c), have angles φ and ψ of the main chain differing very substantially from the values at which the $\chi_1-\chi_2$ sections were calculated is also of definite significance. In the plotting of the points χ_1, χ_2, all the conformations of the side chain were separated into only two groups, R and B. Finally, the scatter may also be connected with a real deviation of the angles χ_1 and χ_2 from the optimum values because of the interaction of the Leu residues with remote sections of the peptide chain. However, we assume that the deviations for this reason cannot be so pronounced.

Thus, from an analysis of the spatial structure of main and side chains of the Leu residues in proteins an analogy can be clearly seen between their conformational states and the geometry of the optimum forms of N-acetyl-L-leucine methylamide.

*The values of the angles of rotation in myoglobin have been given by Watson [12] and those of α-chymotrypsin by Birktoft et al. [13]. The angles in carboxypeptidase calculated from the coordinates of the atoms [14] were kindly supplied to us by S. G. Galaktionov (Institute of Heat and Mass Exchange of the Academy of Sciences of the Belorussian SSR).

Recently, a paper by C. G. Galaktionov et al. [15] was published on the calculation of N-acetyl-L-leucine methylamide by means of atom–atom potentials. According to these authors [15], the permissible values of χ_1 are $-60°$, 180° and, to a smaller extent 60°, and of χ_2 only $-120°$, i.e., the angle corresponding to the maximum in the torsional potential. This result contradicts our results and the nature of the distribution of the values χ_1 and χ_2 in Leu residues in proteins. The erroneous conclusion of C. G. Galaktionov et al. is apparently due to the use of extremely rigid potentials of the nonvalent interactions with the CH_3 group, which is considered in [15] as a generalized atom.

SUMMARY

1. A conformational analysis of N-acetyl-L-leucine methylamide has been performed. The interdependence of the conformational states of the main and side chains of the molecule has been investigated. The most suitable orientation of the side chain corresponds to the values of the angles $\chi_1 = \chi_2 = 180°$ and $-60°$. The overall minimum is located in the B region.

2. The validity of the assignment of leucine to the alanine stereochemical series has been shown.

3. To evaluate the contribution to the stability of the optimum conformations for the entropy of the side chain, conformational maps have been obtained of the statistical sums with a variation at each point of the angles φ and ψ of rotation around the $C^{\alpha}-C^{\beta}$ and $C^{\beta}-C^{\gamma}$ bonds from 0 to $-360°$.

4. The optimum forms of N-acetyl-L-leucine methylamide have been compared with the conformations of leucine residues in proteins. A satisfactory agreement has been obtained between the theoretical and experimental values.

LITERATURE CITED

1. E. M. Popov, G. M. Lipkind, S. F. Arkhipova, and V. G. Dashevskii, Molekul. Biol., 4, 622 (1968).
2. G. M. Lipkind, S. F. Arkhipova, and E. M. Popov, Izv. Akad. Nauk SSSR, Ser. Khim., No. 2, 315 (1970).
3. G. M. Lipkind, S. F. Arkhipova, and E. M. Popov, Zh. Strukt. Khim., 11, 121 (1970).
4. G. M. Lipkind, E. M. Popov, and S. F. Arkhipova, Molekul. Biol., 4, 331 (1970).
5. E. M. Popov and G. M. Lipkind, Molekul. Biol., 5, 624 (1971).
6. G. M. Lipkind and E. M. Popov, Molekul. Biol., 5, 667 (1971).
7. Biochim. Biophys. Acta, 229, 1 (1971).
8. E. M. Popov, V. G. Dashevskii, G. M. Lipkind, and S. F. Arkhipova, Molekul. Biol., 4, 612 (1968).
9. L. Pauling, The Nature of the Chemical Bond, 3rd ed., Cornell University Press, New York (1960).
10. D. W. Smith and E. H. Wibenga, Acta Crystallogr., 6, 531 (1953).
11. R. A. Scott and H. A. Scherage, J. Chem. Phys., 45, 1091 (1966).
12. H. C. Watson, Progr. Stereochem., 4, 299 (1969).
13. J. J. Birktoft, B. W. Matthews, and D. M. Blom, Biochem. Biophys. Res. Commun., 36, 131 (1968).
14. N. N. Lipscomb, G. N. Reeke, and J. A. Hartsuck, Phil. Trans. Roy. Soc., 257, 177 (1970).
15. S. G. Galaktionov, S. A. Sherman, G. V. Nikiforovich, L. I. Kirnarskii, and I. P. Lagoda, in: Conformational Calculations of Complex Molecules (ed. by A. I. Kitaigorodskii) [in Russian], Minsk (1970), p. 61.
16. V. Sasisekharan and P. K. Ponnuswany, Biopolymers, 10, 583 (1971).