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AN INVESTIGATION OF THE CONFORMATIONAL STATES OF THE METHYLAMIDE OF N-ACETYL-L-HISTIDINE

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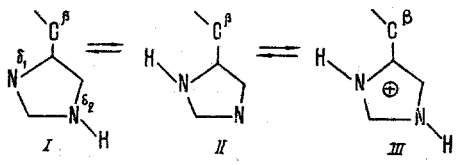
The present communication continues a series of communications devoted to the role of the short-range interactions in the formation of the structure of the peptide chain in proteins [1-7]. As had been reported, methylamides of N-acetylpeptides can serve as suitable objects for the investigation of the specific features of the interaction of the side chains of natural α-amino acids with adjacent peptide groups. In the present case, the conformational states of the methylamide of N-acetyl-L-histidine (AcL-His-NHMe) are considered. The increased interest in an investigation of the spatial structure of His is due to the essential participation of this residue in the acid–base stage of the enzymatic reactions of many proteins (chymotrypsin, elastase, carboxypeptidase, ribonuclease, etc.).

With respect to the nonvalent interactions of the atoms of the side and main chains, the methylamide of N-acetyl-L-histidine is a stereochemical analog of the methylamide of N-acetyl-L-phenylalanine, the calculation of which has been published previously [4]. However, the side chain of His has a number of specific features which may lead to conformational states of this residue substantially differing from the states of Phe and the other analogs of it–Trp and Tyr. Thus, the imidazole ring of histidine, unlike the benzene ring, may take part in considerable electrostatic interactions with the main chain, and also form hydrogen bonds. Under physiological conditions, the imidazole ring may exist in the protonated and in the nonprotonated states in equal measure. The capacity of His for undergoing protonation changes insignificantly if the residue is included in a branched peptide chain [8]. In addition to this, in the neutral form of the imidazole ring, the hydrogen atom can form covalent bonds with the N^{δ_1} and with the N^{ε_2} atoms with almost equal probability. Thus, in the His side chain an equilibrium is set up between the two neutral tautomeric forms (I and II) and the ionic form (III). It is quite realistic to expect stereochemical differences in the interactions of the main chain with the free forms of the side chain. In its turn, the conformational state of the His residue may have a definite influence on the stability of the above-mentioned forms of the imidazole ring. Consequently, we have performed a conformational analysis of the methylamide of N-acetyl-L-histidine with all the possible forms of the side chain.

Model of the Molecule and Potential Functions. A model of the molecule of the methylamide of N-acetyl-L-histidine is shown in Fig. 1. The null values for the listed parameters $\varphi(C^\alpha - N)$, $\psi(C^\alpha - C')$, $\chi_1(C^\alpha - C^\beta)$ and $\chi_2(C^\beta - C^\gamma)$ were selected in accordance with the accepted nomenclature [9]. The strength of the bonds in the main chain were taken as equal to the Pauling–Corey parameters [10], and the valence angles as the average parameters for a peptide group [3]. The geometry of the imidazole ring changed in dependence on its form. For



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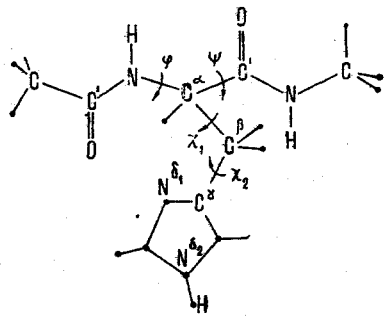


Fig. 1. Model of the molecule of the methylamide of N-acetyl-L-histidine.

form (I) the geometric parameters were taken from a neutron diffraction investigation of L-histidine [11], and for form (III) from the results of an x-ray structural analysis of histidine hydrochloride monohydrate [12]; the lengths of the bonds and the valence angles of form (II) agree with the corresponding values for (I).

In the calculation of the potential energy, we took into account the nonvalent and electrostatic interactions of the atoms, hydrogen bonds, and torsional contributions. The nonvalent interactions were considered by means of the Buckingham potential with Dashevskii's parameters [13]. The electrostatic interactions were evaluated by means of the Coulomb law. The values of the charges on the atoms of the main chain and of the side chain in forms (I) and (II) were taken from the work of Poland and Scheraga [14]. There is no information in the literature on the charges for the protonated form (III). We took the charges on the N^{δ_1} and N^{ε_2} atoms as 0.3 electron unit and the H^{δ_1} and H^{ε_2} atoms as 0.6 electron unit each. The dielectric constant was taken as 10, i.e., the value most suitable for the calculation of peptides in an aqueous medium [15].

The hydrogen bond was calculated by means of a Morse-type potential [13]. The energy of a $C=O\cdots H-N$ bond both in the main chain and between the main and side chains in an aqueous medium was taken in accordance with an experimental evaluation by Shellman [16] as 1.5 kcal/mole (at the optimum distance $r_{O\cdots H} = 1.8$ Å). In view of the close values of the energies of $C=O\cdots H-N$ and $N\cdots H-N$ hydrogen bonds in a nonpolar medium [17], we estimated the $N\cdots H-N$ bond in an aqueous medium also as 1.5 kcal/mole. The parameter D in the Morse potential was determined as the difference between 1.5 kcal/mole and the energy of electrostatic interactions in the fragments $C=O\cdots H-N$ and $N\cdots H-N$ at the optimum distance of the atoms. As a result, the values of D proved to be 1.1 kcal/mole for $C=O\cdots H-N$ in the side chain and 0.8 kcal/mole for $C=O\cdots H-N$ between the main and side chains, and 0.9 kcal/mole for $N\cdots H-N$.

The torsional potentials of the $C^{\alpha}-N$, $C^{\alpha}-C'$, and $C^{\alpha}-C^{\beta}$ bonds and the values of the corresponding barriers were taken from Scott and Scheraga's paper [18]. Rotation around the $C^{\beta}-C^{\gamma}$ bond was described by the potential $U_{(\chi_2)} = 0.3\ (1+\cos 6\ \chi_2)$ [19].

Potential Surface and Optimum Conformations. The conformational states of the methylamide of N-acetyl-L-histidine are modelled to a certain extent by the states of the methylamides of N-acetyl-L-phenylalanine and N-acetyl-L-asparagine, which have been considered previously [6]. The His and Phe derivatives each contain an aromatic ring in the side chain, and the His and Asn derivatives each have at C^{γ} a group of atoms capable of being hydrogen-bond donors and acceptors.

Let us consider the potential surface of Ac-L-His-NH-Me with the imidazole ring in form (I). As in the Ac-L-Phe-NH-Me molecule the most preferred values of χ_2 here prove to be 90 and $-90°$, and those of χ_1 -60, 180, and 60°, corresponding to the minima of the torsional potential. This conclusion is confirmed by a consideration of the $\chi_1-\chi_2$ sections at different values of φ and ψ of the main chain fixed in the low-energy regions. A comparison of the $\varphi-\psi$ maps of Ac-L-His-NH-Me with the corresponding $\varphi-\psi$ maps of the methylamide of N-acethy-L-phenylalanine [4] shows that in the nature of their nonvalent interactions the Phe and His (in form I) residues are extremely close to their analogs. In the first and second molecules the regions of low energy for rotamer with $\chi_1 = -60°$ are extended in the vertical direction and with $\chi_1 = 180°$ in the horizontal direction, and for the rotamer with $\chi_1 = 60°$ they are raised in the direction of ψ. The absence of a H atom at N^{δ_1} in the imidazole ring in form (I) makes the contours of the potential surface flatter. The R and B low-energy regions are practically isoenergetic for all three rotamers with respect to χ_1. The energy in region L exceeds the minimum value by ~3 kcal/mole; the position of the side chain with $\chi_1 = 60°$ in conformation L cannot be realized. Since

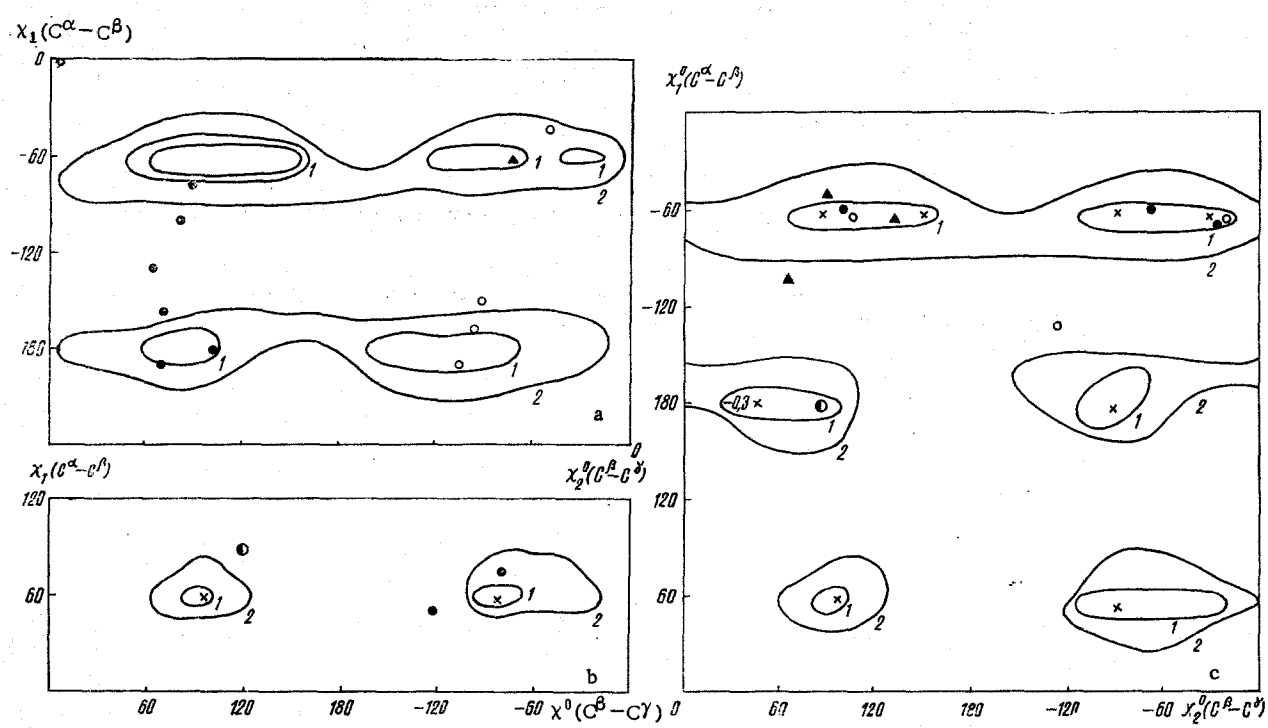
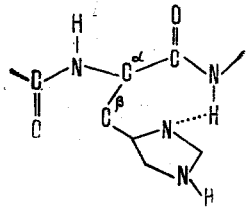


Fig. 2. Sections of the $\chi_1 - \chi_2$ potential surface of the methylamide of N-acetyl-L-histidine at the following values of the angles of rotation in the main chain: a – $\varphi = -120°$, $\psi = -60°$ ($\chi_1 > 120°$); b – $\varphi = -60°$, $\psi = -40°$ ($\chi_1 < 120°$); c – $\varphi = -120°$, $\psi = -160°$. On the maps are shown the positions of the side chains of the His residues in myoglobin (●), α-chymotrypsin (◐), carboxypeptidase (○), and ribonuclease S (▲) The symbol x shows the minima.

the conformational states of the molecule in an aqueous medium are being considered, we shall not devote special attention to an analysis of convoluted forms with hydrogen bonds within the main chain of types of M and H, which are not realized under these conditions [15].

Let us turn to the $\chi_1 - \chi_2$ section plotted at the optimum values of φ and ψ in the R and B regions (Fig. 2). For the R region, in the range $\chi_1 = 120$–$360°$ the map was calculated at $\varphi = -120°$, $\psi = -60°$, and in the range $\chi_1 = 0$–$120°$ it was calculated at $\varphi = -60°$, $\psi = -40°$, which corresponds for three rotamers with respect to χ_1 to the positions of the minima in this region on the $\varphi - \psi$ maps [4]. Above all, one may note the limited nature of rotation around the $C^\alpha - C^\beta$ bond; motion along the χ_2 coordinate has a considerably greater freedom. The permissible changes in χ_2 are a maximum at $\chi_1 = -60°$. Both in the R and in the B region for the rotamer with $\chi_1 = -60°$ minima are found at $\chi_2 = 90°$ at $-90°$ and also at 150° and $-30°$. The latter two minima are due to U_{tors}, since the barriers separating the trough at $\chi_2 = 90°$ and 150°, and also at $\chi_2 = -90°$ and $-30°$ have a height of $U_0(\chi_2)$. The freedom of movement with respect to χ_2 at $\chi_1 = 180°$ and 60° is more limited. With two exceptions, the minima for these rotamers are found at $\chi_2 = 90°$ and $-90°$. The dependence of the conformational freedom of each of the χ_1 rotamers of the methylamide of acetyl-N-L-histidine considered here in its qualitative aspect is confirmed by a quantitative calculation of the free energies of the χ_1 rotamers of the methylamide of N-acetyl-L-phenylalanine [4]. On the $\chi_1 - \chi_2$ sections (see Fig. 2) there are two specific minima with $\chi_1 = -60°$ and $\chi_2 = -30°$ (R) and with $\chi_1 = 180°$ and $\chi_2 = 30°$ (B). In the Phe derivative, the position $\chi_1 = 60°$, $\chi_2 = -30°$ is forbidden ($U_{nonv} > 10$ kcal/mole) because of the repulsions of the hydrogen atoms of the main chain and of the benzene ring. Conversely, the His side chain in this case forms a hydrogen bond between the N–H of the main chain and the unshared electron pair of the $N^{\delta}{}_1$ atom of the imidazole ring (Scheme 1). The formation of an analogous hydrogen bond is also possible in region B at $\chi_1 = 180°$ and $\chi_2 = 30°$. However, the energy of this conformation is less than the energy of the number of other conformations without hydrogen bonds by only 0.3 kcal/mole). The nonvalent interactions of the nitrogen atoms, which prove to be close to one another because of the arrangement of the N–H and H ··· $N^{\delta}{}_1$ bonds at an angle of ~ 90°, have a destabilizing influence.



Scheme 1

The geometric and energy parameters of the optimum conformations of the methylamide of N-acetyl-L-histidine with the imidazole ring in form I are given in Table 1. In the R conformations the total energies are somewhat higher than the energy of the nonvalent interactions, and in conformations B the opposite pattern is observed. There is no doubt that this is the consequence of electrostatic interaction. The optimum conformations with the angles χ_2 and $\chi_2 + 180°$ are close to isoenergetic, i.e., the unsymmetrical charge distribution in the imidazole ring is levelled out in the interaction of the side and main chains. The most suitable conformations of the molecules in Form I are R with $\chi_1 = -60°$, $\chi_2 = 90°$ and B with $\chi_1 = -60°$, $\chi_2 = 90°$; $\chi_1 = 180°$, $\chi_2 = 30°$ and $-90°$; and $\chi_1 = 60°$, $\chi_2 = -90°$. Lewis et al. [20] have come to a similar conclusion.

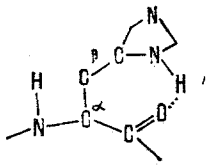
Let us consider the conformational states of the methylamide of N-acetyl-L-histidine with the side chain in form II in which the H atom forms a covalent bond with $N^{\delta}{}_1$. In spite of the great external similarity with the methylamide of N-acetyl-L-phenylalanine, in this form the molecule possesses greater conformational specificity than in form I. The presence of the $H^{\delta}{}_1$ atom leads to a contraction of the $\chi_1 - \chi_2$ contours and to the equalization of the energies in the regions that are relatively strained in the Phe derivatives, for example, at $\chi_2 = -30°$. Rotamers with respect to the angle χ_1 in the R and B regions become nonequivalent in relation to the energy of nonvalent interactions; the conformations with $\chi_1 = 180°$ and 60° are approximately 0.5 kcal/mole less suitable than with $\chi_1 = -60°$. In the Phe case, these three states are favorable [4]. A fundamental contribution to differentiation of the forms is made by electrostatic interactions. Thus, while for U_{nonv} the energy of conformation R, $\chi_1 = -60°$, $\chi_2 = -90°$ is lower than the energy of B, $\chi_1 = 180°$, $\chi_2 = -90°$, when the electrostatic interactions are taken into account the position changes and the energy of the R conformation rises by ~1 kcal/mole (see Table 1). This is due to the fact that in the B conformation with $\chi_1 = 180°$ the electrostatic interaction of $N^{\delta}{}_1 - H$ with C=O have a stabilizing effect; in R with $\chi_1 = -60°$, conversely, an electrostatic repulsion of the $N^{\delta}{}_1 - H$ and N–H of the side chain takes place. It is just for this reason that the global minimum of the potential surface of Ac-L-His-NH-Me in form II is shifted into the B regions; in the case of form I the R and B conformations with the lowest energies are identical. Among the B conformations the rotamers with $\chi_1 = -60°$ are less favorable than those of $\chi_1 = 180°$. In the Ac-L-Phe-NH-Me molecule the conformations mentioned have practically similar energies.

An intermolecular hydrogen bond in the form II is possible at $\varphi = -140°$, $\psi = +40°$, $\chi_1 = 60°$, and $\chi_2 = 90°$, i.e., in the region of the isthmus connecting the R and B regions. The minimum arises where in Ac-L-Phe-NHMe the energy of the nonvalent repulsions is considerable (>5 kcal/mole). However, in an aqueous medium the role of the hydrogen bond (Scheme 2) consists only in eliminating the strong nonvalent repulsions. The energy of such a conformation is ~1.0 kcal/mole higher than that of the global minimum (see Table 1). The formation of a hydrogen bond of such a type is also possible in the methylamide of N-acetyl-L-asparagine.

The protonation of the imidazole ring of His does not introduce appreciable changes into the conformational state of the molecule as compared with form II. It may be noted that there is a symbatic change of energy in the two forms; the values of U_{nonv} and $U_{tot} - U_{nonv}$ in the corresponding conformations are practically equal. In form III, as in II, electrostatic repulsions destabilize the R conformation of the main chain. The global minimum of form III of Ac-L-His-NHMe corresponds to conformation B with $\chi_1 = -60°$ and $\chi_2 = 90°$. The symbatic nature of the difference in the energies of the nonvalent and total interactions in the two forms with $H^{\delta}{}_1$ atoms is due to the fact that the total charge on the $N^{\delta}{}_1 - H$ group is approximately the same in the two cases (0.2 in II and 0.3 in III). However, when the H atoms of the main and side chains are so close that it becomes impossible to consider the N–H group as a whole as the carrier of a point charge, the presence in the protonated form of a large positive charge on the $H^{\delta}{}_1$ atom leads to substantial effect. This takes place, for example, in the R conformation at $\chi_1 = 60°$, where $U_{tot} - U_{nonv} \approx 1.5$ kcal/mole (see Table 1). Thus, if the His residue in a peptide chain is present in the protonated form, then when the conformation of the main chain is R the realization of the rotamer with $\chi_1 = 60°$ is unlikely.

TABLE 1. Geometric Parameters and Energies of the Optimum Conformations of the Methylamide of N-acetyl-L-histidine

φ, ψ Region	Geometric parameters (degrees)				Form of the imidazole ring					
					I		II		III	
	φ	ψ	χ_1	χ_2	U_{nonv}	U_{tot}	U_{nonv}	U_{tot}	U_{nonv}	U_{tot}
R	−140	−60	−60	90	0	0	−0,1	0,7	−0,2	0,7
	−140	−60	−60	150	0,4	0,1	1,0	1,0	0,3	1,0
	−120	−60	−60	−90	0	0,4	0	0,3	0	0,2
	−120	−60	−60	−30	0,5	0,9	0,5	0,4	0,3	0,3
	−120	−60	180	30	—	—	—	—	0,7	1,0
	−120	−60	180	90	0,3	0,6	0,5	0,7	0,4	0,8
	−120	−60	180	−150	—	—	—	—	1,4	1,4
	−120	−60	180	−90	0,4	0,4	0,2	0,7	1,0	1,0
	−100	−40	60	90	0,7	1,0	0,8	1,2	0,4	1,8
	−100	−40	60	−90	0,7	0,4	0,9	1,7	0,6	2,3
	−100	−40	60	−30	>10	1,3	—	—	—	—
B	−140	140	−60	90	0,1	0	0	0,2	−0,2	0
	−120	140	−60	150	0,4	0	0,9	0,6	—	—
	−140	140	−60	−90	0,4	0,1	0,1	0,3	0	0,2
	−120	140	−60	−30	0,6	0,5	0,6	0,2	—	—
	−120	160	180	30	>5	−0,3	—	—	—	—
	−120	140	180	90	—	—	0,4	0,4	0,5	0,9
	−120	140	180	−90	0,5	0	0,1	0	0,3	0,2
	−120	160	60	90	0,6	04	0,4	0,7	0,4	0,4
	−120	160	60	−90	0,5	0	0,4	0,7	0,2	0,5
	−140	40	60	90	—	—	3,0	1,1	5,4	1,1
L	60	60	−60	90	1,5	2,5	1,3	2,1	1,3	2,3
	60	60	−60	−90	1,4	2,1	1,4	2,3	1,4	2,7
	60	100	180	90	2,0	3,0	2,1	3,9	2,3	3,4
	60	100	180	−90	2,1	3,9	2,0	2,5	3,0	2,0



Scheme 2

On comparing the energies of the optimum conformations of the methylamide of N-acethyl-L-histidine in forms I and II, it is easy to see that the number of conformations comparable in energy with the global conformation is extremely large in the first case but does not exceed two or three in the second case. Thus, in the methylamide of N-acetyl-L-histidine because of differences in the interactions between the main and side chains in forms I and II the equivalence of the two tautomers that exists in ordinary imidazole may be disturbed. In order to approach the estimation of this type of effect, we have calculated the statistical sums $z = \Sigma e^{-(E-E_0)/kT}$ and the difference in the free energies of I and II. Here the energy of interactions in the side chain itself due to its different geometries in the two forms were not taken into account. This enabled conformations I and II to be brought to a single scale. E_0 represents the energy of the global conformation of the molecule in form II. The difference in the energies of this conformation and of the most suitable conformation in form I is +0.3 kcal/mole. The statistical sum when the side chain is present in form I is higher than when it is in form II (7.2 and 6.3, respectively). Whence

$$\Delta F = F_{I} - F_{II} = -kT \ln \frac{z_I}{z_{II}} = -0.1,$$

i.e., in a peptide chain the tautomeric equilibrium of the imidazole ring of His is, for stereochemical reasons, somewhat displaced in the directions of form I (according to a rough evaluation, the proportion of I is 55% and the II 45%).

A comparison of the conformational states of the His residues in proteins* with the calculated characteristics of the methylamide of N-acetyl-L-histidine does not show any contradictions whatever, which indicates

*We had available the values of φ, ψ, χ_1, and χ_2 for the His residues in myoglobin [21], chymotrypsin [22] carboxypeptidease [23], and ribonucleases S calculated from the coordinates of the atoms [24].

the large role of short-range interaction in the formation of the conformations of His in a peptide chain. The conformations of the main chains (φ, ψ) of the His residues in proteins are concentrated within the 2-kcal/mole contour on a dipeptide map. In four proteins, not one His residue is present in the L conformation. The low probability of its realization is predicted by calculation. So far as concerns distribution with respect to the angle χ_1, the rotamer with $\chi_1 = -60°$ is represented in the greatest proportion. Its energy advantageousness also follows from the calculation (see Fig. 2). On the $\chi_1 - \chi_2$ sections practically all the conformational points of the side chain fall in the low-energy region and are grouped close to $\chi_1 = -60°$, 180° and $\chi_2 = 90° - 90°$ (see Fig. 2).

SUMMARY

1. All the conformational states of the methylamide of N-acetyl-L-histidine have been calculated.

2. It has been shown that in the case of the tautomer of the imidazole ring with a hydrogen atom on the N^{ε_2} atom, form R of the main chain is preferred, and in the tautomer with the proton on N^{δ_1} and also in the protonated state of the side chain B forms are preferred.

3. The conformational equilibrium is displaced in the direction of form I.

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