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The mean conformation of *N*-acetylamino acid *N'*-methylamides in dimethyl sulfoxide solution

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The mean conformation of a series of *N*-acetylamino acid *N'*-methylamides in dimethyl sulfoxide (DMSO) was determined by ^{1}H-NMR and ^{13}C-NMR methods. The series investigated consisted of derivatives of DL-Ala, DL-Asn, Gln, His, DL-Ile, DL-Leu, Met, DL-Pro, DL-Phe, Ser, Thr, Trp, Tyr, DL-Val, DL-Nva, and DL-Nle. It was found that the conformational equilibria in DMSO are dominated by C_5 and C_7^{eq} forms. The amounts of these forms in the equilibria were found to be roughly proportional to the Boltzmann probabilities for the occurrence of a definite form, as calculated theoretically by Vasquez et al. (Macromolecules 16 (1983) 1043). Exceptions to this rule were DL-Pro and, to a lesser extent, Ser, Asn and Trp derivatives.

1. Introduction

The mean solution conformation of oligopeptides is relatively easy to establish using NMR. C^{β} chemical shifts (expressed on the hydantoin scale) of the amino acid side chains allow the mean conformational angle ψ to be determined [1]. The mean conformational angle ϕ can be determined from the $^{3}J_{NH^{\alpha}}$ coupling constants [2]. In this paper, we present the results of such an investigation, performed for a series of *N*-acetylamino acid *N'*-methylamides in dimethyl sulfoxide (DMSO) solution. The series consisted of derivatives of DL-Ala, DL-Asn, Gln, His, DL-Ile, DL-Leu, Met, DL-Phe, DL-Pro, Ser, Thr, Trp, Tyr and DL-Val. The corresponding derivatives of DL-norvaline (Nva) and DL-norleucine (Nle) were also investigated. Some selected results of these studies were the subject of a preliminary communication [3].

The dihedral angle ϕ of the *N*-acetyl *N'*-methylamides were determined using the equation of Ramachandran et al. [4]:

$$^{3}J_{NH^{\alpha}} = 6.55 \cos^{2}\theta' - 1.55 \cos\theta' + 1.35$$

where θ' is the dihedral angle in the moiety H-N-C^{α}-H.

The dihedral angle ψ was calculated from the relation:

$$|\theta| = 49.7 + 19.4\Delta_h$$

where θ is the dihedral angle in the moiety C^{β}-C^{α}-C'-O ($\theta = \psi - 60°$) and Δ_h the chemical shift difference between C^{β} resonances for the given amide and corresponding hydantoin, respectively (i.e., the hydantoin of the same amino acid [1].

The conformation of the *N*-acetyl *N'*-methylamides of amino acids has recently been the subject of theoretical work by Vasquez et al. [5]. These authors computed minimal energy conformations and the Boltzmann probabilities for the occurrence of definite conformational states of amides in conformational equilibrium. They found that the conformational states A, C, D, E, F, G and A* have the greatest chance of occurring (for notation of the definite conformational states, see ref. 6). For our purposes, we identified conformational state A with the α- and 3_{10}-helical conformations, C with the γ-turn (C_7^{eq} conformation), E with the extended β-structure (C_5 conformation) and F with the P II (poly-Pro II) structure. The above-mentioned authors found that the confor-

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Table 1

Values of coupling constants $^3J_{NH^\alpha}$ of *N*-acetylamino acid *N'*-methylamides and possible values for the mean conformational angles ϕ (in DMSO; internal standard TMS; 305±2 K, 100 MHz)

Compound	$^3J_{NH^\alpha}$ (Hz)	ϕ (°)
Ac-Thr-NHMe	9.0	−134, −106
Ac-Ser-NHMe	8.5	−141, −99
Ac-DL-Val-NHMe	8.5	−141, −99
Ac-Met-NHMe	8.4	−142, −98
Ac-His-NHMe	8.4	−142, −98
Ac-DL-Phe-NHMe	8.3	−143, −97
Ac-Tyr-NHMe	8.3	−143, −97
Ac-DL-Ile-NHMe	8.2	−144, −96
Ac-Gln-NHMe	8.1	−145, −95
Ac-Trp-NHMe	8.0	−147, −93
Ac-DL-Ala-NHMe	8.0	−147, −93
Ac-DL-Leu-NHMe	7.9	−147, −93
Ac-DL-Asn-NHMe	7.6	−150, −90
Ac-DL-Nva-NHMe	8.0	−147, −93
Ac-DL-Nle-NHMe	8.5	−141, −99

mational equilibria of amides are dominated by the C_5 and C_7^{eq} forms. The greatest probability of the C_5 conformation was predicted for the Tyr derivative. The Boltzmann probability of this conformation of the Tyr derivative was found to be 0.737. The probability for the C_7^{eq} structure is only 0.060. Our idea, therefore, was to use the Tyr derivative as a standard for comparison of the data obtained for the whole series of compounds investigated.

The results of our investigations are summarized in tables 1 and 2. In table 1, the values of the measured $^3J_{NH^\alpha}$ coupling constants and the possible values calculated for angles ϕ are given. Only the negative values of ϕ have been included in table 1, as the other (positive) ϕ values appeared to be very unlikely to be representative as mean conformational angles. It is very difficult to decide which of the two values of ϕ given for every compound in table 1 is the real one. Both values may result from the contributions of the C_5 and C_7^{eq} forms. However, it seems that in the case of derivatives which possess a high Boltzmann probability of occurrence of the C_5 conformation (those of Tyr and Phe) a value of about −140° is more likely. In other cases, where the amounts of the C_7^{eq} form become greater, values ranging from −90 to −106° seem to be more likely. In a preliminary communication [4], we also attributed ϕ values of about −140° to Trp and Ile derivatives. Thus, we assumed that the conformational equilibria should be dominated by the conformation of minimal potential energy; indeed, the C_5 form was found [5] to be such a conformation in the case of Trp and Ile amides. Such an assumption, however, needs further verification.

In table 2 we have summarized the chemical shifts of the C^β atoms, values of the coefficient Δ_h, the absolute values of angles θ and the possible values of angles ψ, calculated from the equation given above. From the two sets of ψ values given in table 2, those located in the range 10 to −20° appear rather unlikely to reflect the conformation occurring in reality. These values characterize a $(i+2)$ residue when it occupies the corner position in a β-turn. Such a structure, however, is not possible in the case of the *N*-acetyl *N'*-methylamides of amino acids. Therefore, of the values listed in table 2 only those between 109 and 139° seem worthy of consideration.

Table 2

C^β chemical shifts, Δ_h values, absolute values of conformational angle θ and possible values of mean conformational angle ψ (in DMSO; 25.142 MHz, 305±2 K; internal standard TMS)

Compound	C^β (ppm)	Δ_h (ppm)	$\lvert\theta\rvert$ (°)	ψ (°)
Ac-His-NHMe	29.55	−0.05	49	109, 11
Ac-DL-Ile-NHMe	36.30	0.03	50	110, 10
Ac-DL-Val-NHMe	30.03	0.52	60	120, 0
Ac-DL-Leu-NHMe	41.17	0.54	60	120, 0
Ac-Thr-NHMe	65.88	0.75	64	124, −4
Ac-Trp-NHMe	27.21	0.82	66	126, −6
Ac-Gln-NHMe	28.01	0.83	66	126, −6
Ac-Met-NHMe	31.67	0.86	66	126, −6
Ac-DL-Ala-NHMe	18.15	1.12	71	131, −11
Ac-DL-Asn-NHMe	37.65	1.13	71	131, −11
Ac-Ser-NHMe	61.49	1.30	75	135, −15
Ac-DL-Phe-NHMe	37.72	1.38	76	136, −16
Ac-Tyr-NHMe	36.98	1.49	79	139, −19
Ac-DL-Pro-NHMe	29.38	2.73	103	163, −43
Ac-DL-Nva-NHMe	34.14	0.95	68	128, −8
Ac-DL-Nle-NHMe	31.50	1.18	73	133, −13

As noted above, the conformational equilibrium of the Tyr derivative should, according to theoretical prediction, be dominated by the C_5 structure. Indeed, the conformational angles ϕ and ψ equal to -143 and $139°$, respectively, which result from our measurements, correspond very well with those typical of the C_5 structure. Assuming that the spectroscopic characteristics observed for the Tyr derivative reflect the situation in which the C_5 structure is the dominating form in the conformational equilibrium, we can anticipate the changes in spectroscopic data which should take place on a shift in the conformational equilibrium occurring. Thus:

(i) An increase in population of the C_7^{eq} form in the equilibrium should be manifested by a marked decrease – as compared to the situation observed for the Tyr derivative – in the mean value of angle θ (decrease in coefficient Δ_h). The coupling constant $^3J_{NH^\alpha}$ should not change significantly. Both a small decrease and small increase in coupling constant are equally possible, depending on the value of the conformational angle ϕ specific for the C_5 and C_7^{eq} conformations of a particular amino acid. For example, in the case where the angles for a given residue are -140 and $-80°$, respectively, the increase in proportion of the C_7^{eq} conformation in the equilibrium should be accompanied by a parallel decrease in $^3J_{NH^\alpha}$. However, for an angle of about $-100°$ for the C_7^{eq} conformation and approx. $-120°$ for the C_5 conformation, the change in equilibrium discussed may be manifested by an increase in $^3J_{NH^\alpha}$.

(ii) The increase in proportion of the α- and 3_{10}-helical conformations in the equilibrium should give rise to an increase (as compared with the situation observed for the Tyr derivative, where the C_5 structure dominates the equilibrium) in mean value of angle θ and a marked fall in the value of $^3J_{NH^\alpha}$.

(iii) The P II conformation cannot be differentiated from the C_5 type on the grounds of NMR measurements due to the very similar values of both $^3J_{NH^\alpha}$ and θ.

It can be seen from tables 1 and 2 that in our case the changes in NMR parameters correspond to: (i) variant, indicating that in comparison to Tyr and Phe derivatives, in the conformational

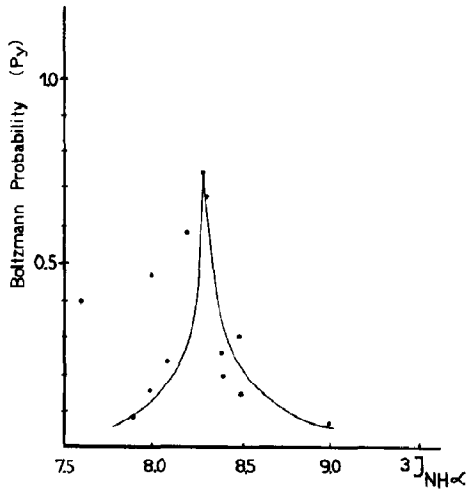

Fig. 1. Plot of coupling constants $^3J_{NH^\alpha}$ of *N*-acetylamino acid *N'*-methylamides vs. Boltzmann probabilities [4] of occurrence in the C_5 conformation.

equilibria of other amides greater amounts of C_7^{eq} forms appear. Such a situation should be anticipated on the grounds of the theoretical work of Vasquez et al. According to these authors, the sums of the Boltzmann probabilities for the C_5 and C_7^{eq} conformations are in excess of 0.62 for the majority of amides. Only for the His and Gln derivatives do these amount to a lesser value, viz., 0.562 and 0.619, respectively. The probabilities of occurrence of the C_5 and C_7^{eq} forms vary, however, from one case to another. As mentioned above, the greatest Boltzmann probability of the C_5 structure occurring was expected for the Tyr derivative. The lowest value was anticipated for Thr (0.074); in this case, however, the probability for the C_7^{eq} form increases to 0.785.

A peculiar regularity may be observed on constructing plots of $^3J_{NH^\alpha}$ of the amides vs the probability for the C_5 and C_7^{eq} conformations, adopted from the work of Vasquez et al. (see figs. 1 and 2). This feature may, of course, be purely fortuitous. Yet it might also reflect the situation actually occurring in DMSO solution; these views suggest that the changes in proportion of the C_5 and C_7^{eq} conformations are the most significant factor determining the mean conformations of *N*-acetylamino acid *N'*-methylamides in DMSO.

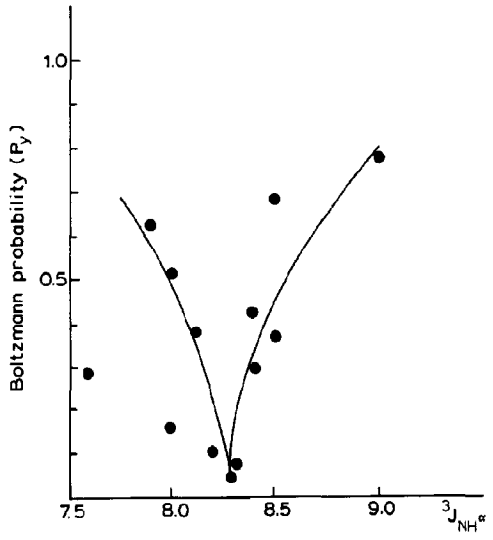

Fig. 2. Plot of coupling constants $^3J_{NH^\alpha}$ of *N*-acetylamino acid *N'*-methylamides vs. Boltzmann probabilities [4] of occurrence in the C_7^{eq} conformation.

One can observe from figs. 1 and 2 that deviations from regularity appear for the Asn and Trp derivatives. In the case of the Asn derivative – compared to the Tyr derivative as our standard – a distinct decrease in $^3J_{NH^\alpha}$ is evident; at the same time changes in Δ_h are only very slight. This suggests that, in this case, greater amounts of helical conformations may be present in the equilibrium. It is possible that the same holds true for the case of the Trp derivative.

The coefficients Δ_h for the series of amides investigated show no such regular dependence on the Boltzmann probabilities of the C_5 and C_7^{eq} conformations, in contrast to the coupling constants. Nevertheless, it is worth noting that the Tyr and Phe derivatives, for which the lowest probability of occurrence of the C_7^{eq} conformation was found (0.060 and 0.077, respectively) show the largest values for Δ_h (1.49 and 1.38, respectively). The probability for the C_7^{eq} conformation increases for all other amides; table 2 shows that – in agreement with our anticipation – the coefficients Δ_h are distinctly smaller than in the case of the Tyr and Phe derivatives.

Nevertheless, interesting discrepancies remain between the expected situation and that found in reality for the Tyr and Ser amides. Based on the results of Vasquez et al., we can expect the mean conformation of these two compounds to be similar. In both cases, the equilibrium should be dominated by the C_7^{eq} conformation (Boltzmann probability for this conformation: 0.789 for the Thr derivative and 0.689 for the Ser derivative). Yet, the Δ_h values of the Ser and Thr derivatives, differ significantly and attain values of 0.75 and 1.30 for the Thr and Ser derivatives, respectively. Thus, the position of the C^β resonance of the Ser derivative indicates that, in this case, much less of the C_7^{eq} conformation exists in the equilibrium than for the Thr derivative.

The largest discrepancy between the expected situation (on the grounds of theoretical calculations) and that observed for DMSO solution was found for Ac-Pro-NHMe. According to the results of Vasquez et al., the mean conformation of this compound should be dominated by the C_7^{eq} form (Boltzmann probability for this conformation: 0.739). However, the highest Δ_h value that we observed for the entire series was that of the Pro derivative (2.73 ppm). The corresponding values possible for the mean conformational angle ψ calculated using our approach equal 163 and $-43°$. The latter value appears more probable, corresponding to a *cis'* orientation of the Pro-carbonyl group. We have also observed a similar mean conformation for Pro in the case of tetrapeptides of the general formula $(Ala)_3Pro$, where Pro occupies two or three positions of the peptide chain [7]. In the analogously constructed Thr tetrapeptides, a close similarity was observed between the mean conformation of the Thr residue and of Pro in proline-containing tetrapeptides of general formula $(Ala)_3Pro$ [8]. Also, the mean conformation of Pro in tuftsin (a tetrapeptide, Thr-Lys-Pro-Arg) and that of Thr in Thr^3-tuftsin are similar [9]. We explained this resemblance by proposing that in the case of the threonine residue a proline-like quasi-cyclic structure is formed by the hydrogen bond between the Thr amide hydrogen and the oxygen atom of the Thr hydroxyl group.

It is of interest that this similarity does not occur with the *N'*-methylamides of acetylated Pro and Thr.

For Ac-Pro-NHMe in DMSO, *cis-trans* isomerism involving the Pro–amide bond occurs. From the relative intensities of the *cis* and *trans* resonances the proportion of the *cis* form present in the equilibrium can be determined to be approx. 28% (the discussion above concerns only the *trans* form of the compound).

In addition to the 14 derivatives of proteinaceous amino acids, we have also investigated the *N'*-methylamides of *N*-acetyl-DL-norvaline (Nva) and *N*-acetyl-DL-norleucine (Nle). The parameters obtained, Δ_h and $^3J_{NH^\alpha}$, demonstrate that the conformational situation here is similar to that observed for other compounds, i.e., that the C_5 and C_7^{eq} conformations probably prevail in the equilibria. It appears interesting, however, to compare the NMR parameters obtained for the Nle derivative with those of Met and Leu:

	$^3J_{NH^\alpha}$	Δ_h
Met	8.4	0.86
Leu	7.9	0.54
Nle	8.5	1.18

As one can see, the mean conformation of the Nle derivative is closer to that of the Met derivative than that of Leu. Whereas in the case of the Leu derivative, an appreciable proportion of folded C_7^{eq} structure probably appears in the equilibrium, for the Met and (even more so) Nle derivatives the extended forms are favoured.

The mean conformation in DMSO of Ac-DL-Nva-NHMe also appears to be more similar to that of the Ala derivative than that of Val. This becomes apparent on comparison of the NMR parameters for these compounds:

	$^3J_{NH^\alpha}$	Δ_h
Val	8.5	0.52
Nva	8.0	0.95
Ala	8.0	1.12

Thus, derivatives of branched-chain amino acids (Val, Leu) exhibit a markedly greater tendency to adopt folded conformations than their unbranched counterparts (Nva, Nle), for which the C_5 conformation is preferred.

Analysis of the data suggests that the picture observed for *N*-acetylamino acid *N'*-methylamides in DMSO is generally consistent with that predicted from theoretical calculations, i.e., the conformational equilibria are dominated by extended C_5 and C_7^{eq} conformations. A somewhat different situation appears to be the case for the Asn and Trp derivatives, in which greater proportions of helical forms are present in the equilibria. In contrast to the theoretical prediction, different mean conformations were observed for Thr and Ser *N*-methylamides. It was also found that, in DMSO, Ac-DL-Pro-NHMe does not adopt the C_7^{eq} structure – theoretically, the preferred minimum energy conformation – and differs with respect to conformational properties from the corresponding threonine derivative.

The *N'*-methylamides of *N*-acetylamino acids have been the subject of several studies. On the basis of infrared [10], dipole moment [11] and ultraviolet and CD [12] measurements, it has been concluded that for derivatives of Ala and Val in nonpolar solvents, equilibria between folded (presumably of the C_7^{eq} type) and unfolded forms exist in polar media, the equilibria are shifted towards the unfolded forms. Madison and Kopple [13] observed that the C_7^{eq} conformation of Ac-Ala-NHMe, which is present in chloroform solution, is altered to that of α_R and P II in water; the proportion of the C_5 form remains approximately constant during this transition. In CCl_4 solutions of this compound, the C_5 and C_7^{eq} conformations are present. Recently, Maloň et al. [14] carried out a CD investigation of the *N'*-methylamides of Ac-Ala, Ac-Val and Ac-Leu. They concluded that in solvents of low polarity, the C_7^{eq} conformation prevails for Ala and Leu derivatives, whereas C_5 dominates in the case of the Val derivative. According to these authors, the population of the C_7^{eq} form diminishes rapidly with increasing solvent polarity and conformers of the α_R- and 3_{10}-helical type become important.

Our results generally agree well with these observations. In DMSO solution, the conformational equilibria of amides are mostly determined by the presence of the C_5 and C_7^{eq} forms. The relative amounts of these forms vary, however, from one case to another; with some exceptions as discussed above, the amounts of these conformers in the equilibria appear to be proportional to the

Boltzmann probabilities calculated for definite conformations by Vasquez et al.

2. Experimental

All *N*-acetylamino acid *N'*-methylamides were synthesized from the corresponding free amino acids by successive esterification, acetylation and aminolysis by means of methylamine. Analytical data for derivatives of DL-Ala, His, Ile, DL-Leu, Met, DL-Phe, Trp and DL-Val have been reported elsewhere [3]. Data for the other derivatives are summarized below:

Ac-DL-Asn-NHMe: m.p. 260 °C; %N: calcd., 16.0; found, 16.1.

Ac-Glu-NHMe: m.p. 233.5 °C; $[\alpha]_{578}^{20} - 9.9°$ (methanol); %N: calcd., 20.9; found, 21.1.

Ac-Ser-NHMe: m.p. 117 °C; %N: calc., 17.5; found, 17.7.

Ac-DL-Pro-NHMe: oil; %N: calc., 16.5; found, 16.8.

Ac-Thr-NHMe: m.p. 164.5 °C; $[\alpha]_{578}^{20} - 161.5°$ ($CHCl_3$); %N: calc., 16.0; found, 16.1

Ac-DL-Nva-NHMe: m.p. 160 °C; %N: calc., 16.3; found, 16.0.

Ac-DL-Nle-NHMe: m.p. 171 °C; %N; calc., 14.9; found, 15.1.

2.1. Spectroscopic measurements

NMR measurements were recorded on Tesla BS-567-A and Jeol JNM/PS/PFT instruments. For ^{1}H-NMR spectra a frequency of 100 MHz was used, 25.142 MHz being used for ^{13}C-NMR. For ^{1}H-NMR and ^{13}C-NMR spectra measurements were made at the same concentrations, ranging from 10 to 30 mg/dm^3. Spectra were recorded at a temperature of 305 ± 2 K using TMS as internal standard. The coupling constants $^3J_{NH^\alpha}$ were measured to an accuracy of ± 0.2 Hz.

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