CONFORMATIONAL STATES OF METHYLAMIDES OF N-ACETYL α-AMINO ACIDS AND THEIR N-METHYL DERIVATIVES

IV. ASSOCIATION IN SOLUTIONS

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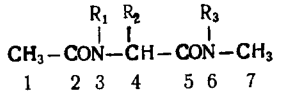
In the preceding papers, we have given the results of an investigation of the spatial structure of a series of methylamides of N-acetyl α-amino acids of the type



$$\underset{1}{CH_3}-\underset{2}{CO}\underset{3}{\overset{R_1}{N}}-\underset{4}{\overset{R_2}{C}H}-\underset{5}{CO}\underset{6}{\overset{R_3}{N}}-\underset{7}{CH_3}$$

with the aid of IR [1] and NMR [2] spectroscopy and the dipole-moment method [3]. In the present paper, we discuss the concentration dependences of the molecular weights and dipole moments of the following compounds:

I.	Ac-L-Ala-NHMe	$R_1=R_3=H,\ R_2=Me$
II.	Ac-L-Ala-NMe$_2$	$R_1=H,\ R_2=R_3=Me$
III.	Ac-L-MeAla-NHMe	$R_1=R_2=Me,\ R_3=H$
IV.	Ac-L-MeAla-NMe$_2$	$R_1=R_2=R_3=Me$
V.	Ac-L-Val-NHMe	$R_1=R_3=H,\ R_2=CHMe_2$

The immediate aim of this investigation was to determine the limits of concentration at which the molecules of the diamides are present in the nonassociated state, which is necessary for the analysis and correct comparison of the results of different physical methods. Furthermore, a quantitative evaluation of the degree of association was required in the interpretation of the NMR spectra [2] obtained at comparatively high concentrations of the samples under investigation.

The results of determinations of the molecular weights of compounds (I-V) in chloroform are given in Figs. 1 and 2 in the form of the dependence of the degree of association $\alpha = (M_e - M)/M$ on the concentration, C (where M_e and M are, respectively, the effective and true molecular weights); N-methylacetamide was investigated for comparison. As can be seen from the figures given, the beginning of intermolecular association and the nature of the $\alpha = f(C)$ curves depend fundamentally on the position and number of the secondary amide groups in the molecule of the monomer, and also on the nature of the side chain R_2.

The curves in Fig. 1 relate to N-methylacetamide and to the diamides (I), (II), and (III); in the case of compounds (IV) with two tertiary amide groups no increase in molecular weight in chloroform solution was observed up to a concentration of 0.3 M.

Among the compounds containing one secondary amide group, the greatest capacity for association is possessed by N-methylacetamide, in which the formation of associates begins earlier and takes place more intensively than in compounds (II) and (III). The deciding role here is obviously played by the size of the molecule and by spatial screening. The fact that the curve for (III) is above that for (II) is apparently due to the greater accessibility for the formation of a H bond of a C-terminal amide group than of a N-terminal

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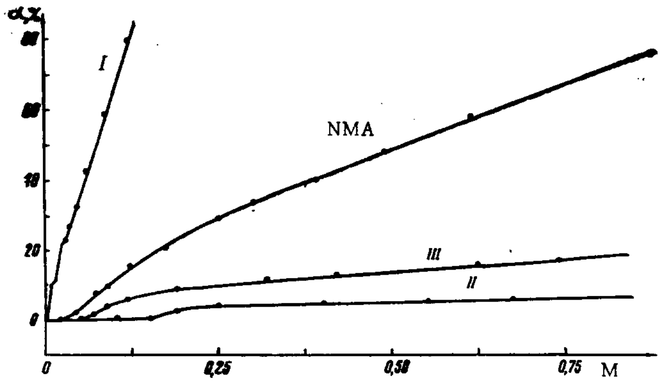


Fig. 1. Concentration dependences of the degrees of association of the methylamide of N-acetyl-L-alanine (I), N-methylacetamide (NMA), the dimethylamide of N-acetyl-L-alanine (II), and the methylamide of N-acetyl-N-methyl-L-alanine (III) in $CHCl_3$ solution.

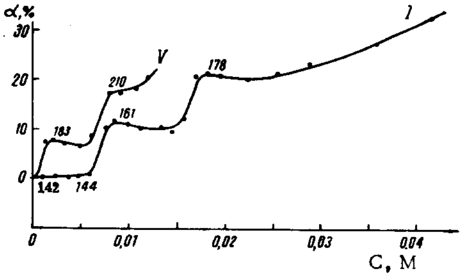


Fig. 2

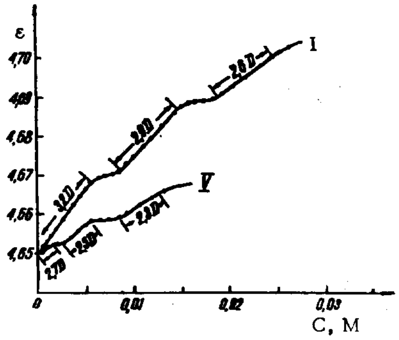


Fig. 3

Fig. 2. Concentration dependences of the degree of association of the methylamide of N-acetyl-L-alanine (I) and the methylamide of N-acetyl-L-valine (V) in $CHCl_3$ solution. The effective molecular weights are given at the inflections of the curves.

Fig. 3. Concentration dependences of the dielectric permeability of the methylamide of N-acetyl-L-alanine (I) and the methylamide of N-acetyl-L-valine (V) in $CHCl_3$ solution. The effective values of the dipole moments (D) are shown on the curves.

group. On the curve of the association of N-methylacetamide it is possible to see several regions in which the dependence of α on C has different natures. In the initial stage (up to ~ 0.03 M), the molecular weight scarcely increases; then in the range of concentrations from 0.03 to 0.12 M the degree of association rises rapidly, and this is followed by a flatter section. A similar nature of the dependence of α on C is possessed by compounds (II) and (III). This feature of the curves is connected with the cooperative nature of association, i.e., with the dependence of the energy of a H bond of the number of molecules entering an associate. The features of the NMR spectra [4] and quantum-chemical calculations [5] show that with an increase in the molecular weight of an associate a H bond becomes more stable. This is also shown by the IR spectra: at a concentration corresponding to the beginning of the association of N-methylacetamide and the formation mainly of dimers, the absorption band of bound NH groups has a maximum at 3370 cm^{-1}, and with a rise in the concentration the maximum shifts to 3290 cm^{-1} [1].

The flat sections of the relation $\alpha = f(C)$ show a disappearance of the cooperativeness of the associative process with an increase in concentration. As can be seen from Fig. 1, the capacity for association of N-methylacetamide falls at a concentration $C > 0.12$ M, that of the diamide (II) at $C > 0.19$ M, and that of (III) at $C > 0.10$ M. The passage to a flatter section of the curve in the compounds mentioned takes place at a comparatively small increase in the concentration of the solution and at low values of the degree of association α, which shows a rapid fall in cooperativeness with an increase in the molecular weight of the associate. Apparently, the additional gain in energy of the H bond through the nonadditivity of the process has an appreciable influence only on the formation of low-molecular-weight associates – tri-, tetra-, and, possibly, penta- and hexamers – while the greatest effect is observed on passing from the dimer to the trimer.

The curves of the association of the methylamides of N-acetyl-L-alanine (I) and N-acetyl-L-valine (V) (see Figs. 1 and 2) have an extremely peculiar nature. In curve 1, after the monomeric section, there is a fairly sharp increase in the degree of association in the range of concentrations from 0.006 to 0.008 M; with a change in C from 0.008 to 0.016 M, the value of α scarcely rises, and with a further increase in the concentration in the range from 0.016 to 0.018 M there is another sharp rise in α; then, after a small section in which α is constant, there is a monotonic rise in the degree of association with an increase in the concentration which, however, is more pronounced than for N-methylacetamide, (II), and (III). The dependence of α on C for compound (V) is similar in nature, with the only difference that in the latter the jumps in association are found at lower concentrations (0.0008-0.0012 and 0.006-0.008 M).

For compounds (I) and (V), the dependence of the dielectric constant ε on the concentration is shown in Fig. 3. The curves $\varepsilon = f(C)$ have two inflections, and these are at the same concentrations at which the jumps on the $\alpha = f(C)$ curves are observed. In the monomeric sections, the dipole moments of (I) and (V) do not change; after the first inflection, they decrease considerably and then remain constant to the second inflection where they again decrease abruptly. After the second inflection, the values of the dipole moments first remain constant and then begin to decrease monotonically.

The existence of several stepwise changes in the effective values of the molecular weights and of the dipole moments of (I) and (V) shows that with a rise in the concentration of these compounds the formation of associates of different types takes place.

Making use of Figs. 2 and 3, let us evaluate the percentage of monomeric (m) and associated (1−m) molecules for different values of the degree of association α at given sizes of the associate (n). Since the observed molecular weight is inversely proportional to the total molar concentration of monomeric and associated forms, then we have

$$\frac{M_e}{M} = \frac{1}{m + \frac{1-m}{n}} \cdot$$

In view of the fact that $\alpha = (M_e/M) - 1$, we obtain

$$m = \frac{1 - \frac{\alpha}{n-1}}{1+\alpha} \cdot$$

Table 1 gives the proportion of monomer on the formation of di-, tri-, and tetrameric associates and the values of α corresponding to different sections of the curve $\alpha = f(C)$ for compound (I). The same table gives the effective dipole moments (μ_{obs}) found under conditions corresponding to the given values of α, and the values of the dipole moments of a different type of associates (μ_{ass}) calculated from the formula $\mu^2_{obs} = \mu^2_{mon} + [(1-m)/n]\mu^2_{ass}$. In all cases, μ_{mon} was taken as 3.2 D, i.e., the dipole moment observed at $\alpha = 0$. The results of calculation have shown that only the values of the moments of the dimeric associates (n = 2) are effective. In fact, the moments of associates of other types, calculated on the assumption that in each case an equilibrium exists only between the monomer and the trimer or between the monomer and the tetramer, and so on, had imaginary values. Although the figures in the table do not, in principle, exclude the existence of associates with n > 2, nevertheless, they show that in a solution of compound (I) in $CHCl_3$ at concentrations corresponding to the jumps on the curves $\alpha = f(C)$ and $\varepsilon = f(C)$ low-molecular-weight, mainly dimeric, associates predominate in the equilibrium.

Before investigating the possible reasons for the jump-like changes in the apparent molecular weights and dipole moments, and also the types of associates of (I) and (V) formed under these conditions, let us dwell briefly on the conformations of these compounds in the nonassociated state. Experimental results and those of a theoretical analysis show that in highly dilute $CHCl_3$ solutions compounds (I) and (V) exist in folded and extended forms [1, 6, 7]. The folded forms can exist in two conformations with intramolecular H bonds – M ($\Phi \sim 120°$, $\Psi \sim 240°$) and H ($\Phi \sim 240°$, $\Psi \sim 120°$), respectively with the pseudoequatorial and the pseudoaxial orientation of the side chain (R_2) with respect to the seven-membered ring. Among the extended

TABLE 1. Relative Proportions of the Monomers m and the Dipole Moments of the Associates at Different Values of α and n for the Methylamide of N-Acetyl-L-alanine (I)

C, M ($CHCl_3$)	α	m			μ_{obs}, D	μ_{ass}, D		
		$n=2$	$n=3$	$n=4$		$n=2$	$n=3$	$n=4$
0,005	0	1	1	1	3,2	—	—	—
0,009	0,12	0,79	0,84	0,86	2,9	3,05	*i*	*i*
0,018	0.22	0,64	0,73	0,76	2,6	1,17	*i*	*i*
0,042	0,33	0,50	0,63	0,67	2,3	0,68	*i*	*i*

forms in the case of compounds (I) and (V), conformations present in region B of the steric maps [6, 7] predominate.

A theoretical analysis [6] shows that from the point of view of the nonvalent interactions and the entropy factor, the folded forms H and M of compounds (I) and (V) are less advantageous than the extended B forms. Their formation in strongly diluted nonpolar and weakly polar solutions is due to the reduction in the free energy through the intramolecular H bond. In polar media, the H bond is appreciably weakened [7], and the equilibrium is shifted to the sterically more favorable open conformations. This conclusion of the theoretical analysis is fully confirmed by the experimental results [1, 3]. Furthermore, the results of a calculation [6] permit the prediction of a decrease in the stability of the folded forms as compared with the extended with an increase in the volume of the side chain (R_2). Among the folded forms, according to [6], the H conformation has a somewhat lower potential energy than the M conformation, but it is inferior to the latter with respect to entropy; consequently, the values of the free energies of the M and H forms may be extremely close. However, one of the reasons for the specific nature of the curves of the association of (I) and (V), in our opinion, is connected with the presence in solutions of these compounds of an equilibrium between the folded and extended forms. Nevertheless, the stepwise nature of the curves of (I) and (V) cannot be explained by this factor alone. In $CHCl_3$, compound (III) also contains a considerable amount of the folded forms [1], but nevertheless its curve (see Fig. 1) does not show a jump-like change in the degree of association. Evidently, a large role in the anomalous relation $\alpha = f(C)$ for compounds (I) and (V) is played by the free $N^{(3)}H$ group [which is absent from (III)] and the free $C^{(5)}O$ group. In the folded forms, because of the direct interaction between the two amide groupings in the seven-membered ring these groups have a greater tendency to intermolecular interactions of the corresponding groups of the extended forms. In view of this, with an increase in the concentration of solutions of (I) and (V) one may expect, in the first place, the formation of dimeric associates of the folded forms, which takes place at definite threshold values of C for such conditions. In the dimerization of the M and H forms by the routes shown in Fig. 4, the four amide groups become involved through H bonds in the folded system. As the result of the direct formation of two intermolecular H bonds (with the presence of two intramolecular H bonds) and the cooperative nature of association, this type of dimer is energetically more favored than the other possible types of dimers of the folded forms and, particularly, than the dimers of the extended forms. The dimers shown in Fig. 4 are stabilized not only by the H bonds but also by electrostatic interactions, since the atoms in the amide groups opposite to one another have opposite charges.

In the association of the M and H forms, dimers of three types are possible: H : H, H : M, and M : M. The formation of the H : H and H : M dimers by the proposed scheme is not associated with any steric complications whatever. Dimerization of the M : M type is less favorable in this respect because of the repulsion of the side chains (R_2) and the relative position of the $N^{(3)}H$ and $C^{(5)}O$ groups (angle between them close to 90°), which is unfavorable for the formation of H bonds. The dimers mentioned above differ from one another in their energy and, consequently, may have different threshold concentration values. Furthermore, they are not equivalent in their conformational possibilities which, as we assume, is shown in the nature of the concentration curves of compounds (I) and (V): the formation of dimers of the folded forms is favored only in the initial stage of association. With an increase in the concentration they become less preferred than high-molecular-weight associates of the extended forms in which practically complete saturation with H bonds may also take place. The advantage of the latter over dimers of the folded forms consists in the fact that their component monomeric forms are more favorable than the M and H forms with respect to nonvalent interactions. Consequently, it may be assumed that an increase in concentration will be accompanied by a shift in the equilibrium towards high-molecular-weight associates. They can be formed by the direct

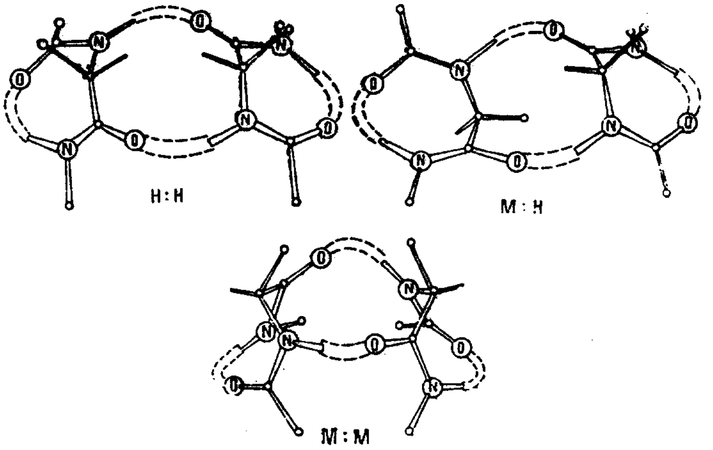


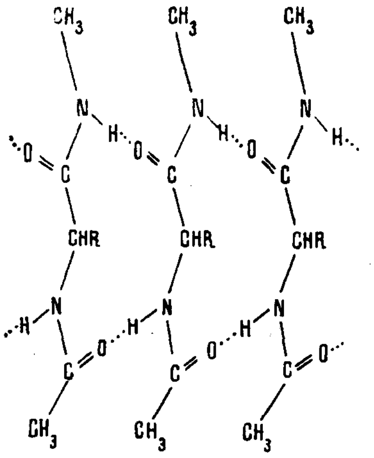
Fig. 4. Structures of the dimeric associates of the folded forms H : H, H : M, and M : M. The dashed lines denote H bonds.

association of the extended conformations that arise in solution and by the condensation of dimers of the extended forms with the rupture of the intramolecular H bonds and the corresponding conformational transition. The second route is most likely only for the metastable dimers of the M : M type. As is well known [6], the M form is located on the conformational map near the low-energy region B, and therefore the M : M → B : B transition is not associated with the overcoming of any appreciable barrier whatever. Conversely, the H form remains far from low-energy regions and is separated from them by high barriers. For this reason and also because of the low stability of the M : M dimer mentioned above, the transitions H : H → B : B and H : M → B : B are less likely than the transition M : M → B : B.

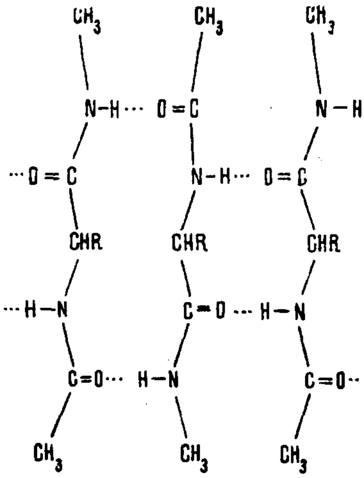
Thus, in agreement with the considerations put forward above, the specific nature of the concentration dependences of compounds (I) and (V) are due to the predominant formations of dimers of the folded forms at low concentrations. The two jumps on the $\alpha = f$ (C) and $\varepsilon = f$ (C) curves correspond to the successive formation of the dimers H : H and H : M. The sections of the curves with constant values of α and μ following after each jump show the absence of additional association in these concentration ranges. If the formation of dimers of the M : M type takes place, it must proceed at higher concentrations and does not lead to the appearance of a new jump because of the low stability of the dimer, the ease of the conformational transition M : M → B : B, and the incipient association of the extended forms.

A complicating factor in this explanation is that the dimerization of (I) and (V) does not take place to the extent of 100% in the region of the first jump, as was to be expected for the formation of an associate of a definite type, and it has a plateau at a considerably lower degree of association. However, cases are known in the literature [8-11] in which the association of compounds for which the direct formation of several types of associates is possible also takes place stagewise without the complete transition of the monomeric molecules into the associated state. Thus, Suzuki et al. [11] have shown that in a highly dilute CCl_4 solution formanilide exists in the trans and cis forms in approximately equal proportions. An increase in the concentration of the solution leads primarily to the dimerization of the molecules with cis-amide groups; practically no association of the trans-formanilide is observed at first. With a further increase in the concentration, dimerization of the cis forms first slows down and then ceases, while the association of the trans forms increases and becomes dominating at high concentrations. The stepwise decrease in the corresponding concentration ranges of the dipole moments agrees with the explanation of the $\alpha = f$ (C) curves given above. The dimers H : H and M : H formed in these regions have lower values of the resulting dipole moments than the nonassociated state.

The fall in the dipole moments in the region of high concentrations is connected with dimerization of the M : M type and, mainly, with the formation of associates of the open forms. The associates of the extended conformations may be of two types (A and B), which consist of fragments of the well-studied parallel and antiparallel β-pleated sheets.



A



B

In associations of type A, the dipole moments of the monomeric subunits have the same direction, and therefore the resulting dipole monent must be close to the moment of the monomer or have a somewhat greater value [12, 5]. In associations of type B, the monomeric subunits are oriented in the opposite directions and, consequently, the resultant moment should decrease. In view of this, it is natural to connect the fall observed in the effective dipole moments of compound (I) and (V) at high concentrations with the association of the extended conformations in the form of the antiparallel β structure. According to the results of x-ray structural analysis [13] and of IR dichroism [14] it is just this type of association that is possessed by the crystalline methylamide of N-acetyl-DL-norleucine. Similar structures of associates have recently been detected in solutions of the related oligopeptides $Me_3COCO-Ile_4-OMe$ and $Me_3COCO-Ile_6-OMe$ [15, 16].

We have also determined the molecular weights of compound (I) and of N-methylacetamide in aqueous solutions. The results of measurements show that in water neither compound shows appreciable association up to a concentration of ~ 3.0 M.

EXPERIMENTAL

To determine molecular weights in solution, we used the method of gas–liquid osmometry [17]. The temperature coefficient of the thermistors was 6% per degree at a resistance R = 33 kΩ; the sensitivity with respect to ΔR was ~ 0.1Ω. The values of the molecular weights in $CHCl_3$ were found with an accuracy of ~ 1% for the range of concentrations from 0.01 to 0.5 M and ~ 2% for concentrations above this range. The molecular weights were calculated from the formula

$$M_{sub}=\frac{m_{sub}\cdot(1-N_{st})\cdot M_{sol}}{m_{sol}N_{st}},$$

where m_{sub} is the weight of the substance under investigation; m_{sol} is the weight of the solvent; M_{sol} is the molecular weight of the solvent; and N_{st} is the molar fraction of the standard substance for a given value of ΔR of the solution.

Naphthalene was used as the standard in chloroform and the dimethyl ether of ethylene glycol as the standard in water.

We have given the results of the determination of dipole moments in the experimental part of a preceding paper [3].

SUMMARY

1. The concentration dependences of the molecular weights and dipole moments of methylamides of N-acetyl α-amino acids and their N-methyl derivatives in chloroform have been studied.

2. The concentration limits at which the molecules are present in the unassociated state have been determined.

3. The jump-like nature of the concentration dependences of the molecular weights and dipole moments observed at the beginning of the association of the methylamides of N-acetyl-L-alanine and N-acetyl-L-valine is due to the dimerization of the folded conformations.

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