Molecular characteristics of water-soluble fullerene derivatives of amino acids and peptides

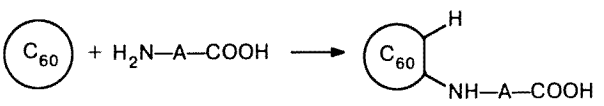
G. I. Timofeeva, V. S. Romanova, and L. A. Lopanova*

A. N. Nesmeyanov Institute of Organoelement Compounds, Russian Academy of Sciences, 28 ul. Vavilova, 117813 Moscow, Russian Federation. Fax: +7 (095) 135 5085

The diffusion of fullerene derivatives of amino acids and peptides in dilute aqueous solutions has been studied. These derivatives can exist in solution both as separate molecules and as associates. The degree of association depends both on the nature of the amino acid or peptide residue and on the concentration of the solution.

Key words: fullerene, amino acid, peptide, solubility in water, diffusion, associates.

The synthesis of *N*-(monohydrofullerenyl)amino acids and -peptides with a free carboxyl group has been reported recently.[1]



Unexpectedly, the fullerene derivatives of glycine (**1**), L-alanine (**2**), glycylglycine (**3**), glycyl-L-alanine (**4**), glycyl-L-valine (**5**), and L-alanyl-L-alanine (**6**) proved to be soluble in water, which made it possible to study the properties of their solutions in detail. The solubility of these compounds in water is quite different: the limiting concentration of compound **1** is 0.07 %, whereas those of compounds **2** and **6** are 2 and 10 times higher, respectively.

In the present work, we report the results of a study of the molecular characteristics and association of the above-mentioned water-soluble *N*-(monohydrofullerenyl)amino acids and -peptides, carried out by sedimentation analysis and by the method of diffusion.

The absolute methods of sedimentation equilibrium[2] and of approaching the sedimentation equilibrium (the Archibald[3] method) are used most frequently for determining molecular characteristics from the sedimentation data. However, in the present case, these methods proved to be inapplicable, because at the high rates of rotation of the rotor (40000 rpm and higher), needed for achieving equilibrium, partial precipitation of the substance occurred, which is characteristic of large nanoparticles or microgels. In addition, the peaks of the sedimentograms broadened during the experiment, which also indicates that solutions of the compounds under consideration are not true solutions and contain large particles. Therefore, to study the molecular characteristics, we used the results[4] of the measurement of the diffusion coefficient *D*.

It is known that measurements of diffusion coefficients in solutions make it possible to calculate the coefficients of the forward friction of molecules and their average hydrodynamic radius, the so-called Stokes radius. If the molecules of the compounds under study exist in solution as spheres and are not solvated, one can easily pass from the Stokes radius to the volume of particles and their molecular weight. In addition, the known diffusion coefficient makes it possible to monitor the stability of the structure of molecules in the solution and its possible changes.

Experimental

The experiments were carried out on a MOM-3180 analytical ultracentrifuge (Hungary) using Philpot—Svennson optics at a rotor temperature of 25±0.1 °C. Water was used as the solvent, concentrations varying in the 0.05—0.15 g dL^{-1} range (higher concentrations afford too dark nontransparent solutions, and lower concentrations cannot be studied due to the small increment of the refraction index). The sedimentation equilibrium was studied using a two-section cell (50000 rpm) at heights of the solution and solvent columns of 3—4 mm (instead of the normally used 12 mm), which substantially accelerated attainment of the equilibrium concentration in the cell.[5] The molecular weight of only one sample, *viz.*, compound **5**, could be measured by this method (Table 1).

The experiments on the measurement of the diffusion coefficient (*D*) were carried out in a boundary-forming cell by layering of the solvent onto a solution of finite concentration within the above-mentioned concentration range. Pictures of the boundary between the pure solvent and the solution were taken at regular intervals, and its broadening was thus monitored. The rotation frequency of the rotor (4000 rpm) was chosen in such a way that sedimentation of the particles did not occur during the experiment and the boundary broadened only due to the diffusion.

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Table 1. Molecular characteristics and sizes of associates and individual molecules of water-soluble fullerenyl derivatives of amino acids

C /g dL^{-1}	$D \cdot 10^7$ /cm^2 s^{-1}	$f \cdot 10^8$ /g s^{-1}	M /Da	n	V_{ass} /Å^3	V_0 /Å^3	d_{ass} /Å	d_0 /Å
N-(Monohydrofullerenyl)glycine (**1**, M_0 = 795)								
0.070	5.1	2.72	14200	17.9	17730	990.0	32.4	12.36
0.060	21.7	1.89	4800	6.0	5955	986.0	22.5	12.35
0.050	35.0	1.17	1100	1.4	1406	1004.4	13.9	12.42
N-(Monohydrofullerenyl)-L-alanine (**2**, M_0 = 809)								
0.150	7.8	5.25	101500	125.5	126788	1010.0	62.3	12.45
0.125	15.1	2.74	14400	17.8	18007	1012.0	32.5	12.45
0.075	31.0	1.33	1650	2.0	2061	1010.5	15.8	12.45
N-(Monohydrofullerenyl)glycylglycine (**3**, M_0 = 852)								
0.150	25.6	1.61	2900	3.4	3654	1075	19.1	12.71
0.125	26.6	1.55	2600	3.1	3262	1069	18.4	12.69
0.075	30.4	1.35	1750	2.1	2156	1052	16.0	12.62
N-(Monohydrofullerenyl)glycyl-L-alanine (**4**, M_0 = 866)								
0.125	32.0	1.28	1500	1.7	1838	1062	15.2	12.66
0.100	35.0	1.17	1100	1.3	1403	1105	13.9	12.82
N-(Monohydrofullerenyl)glycyl-L-valine (**5**, M_0 = 894)								
0.150	37.0	1.11	960	1.07	1198	1116	13.2	12.86
0.125	36.6	1.12	990	1.11	1231	1112	13.3	12.85
0.075	38.0	1.08	880/860*	0.98	1104	1122	12.8	12.89
N-(Monohydrofullerenyl)-L-alanyl-L-alanine (**6**, M_0 = 880)								
0.150	14.5	2.83	15980	18.2	19950	1099	33.7	12.80
0.125	14.8	2.78	15029	17.1	18760	1098	33.0	12.80
0.075	14.0	2.94	17755	20.2	22170	1099	34.9	12.80

* The molecular weight was calculated from the data of sedimentation equilibrium.[4]

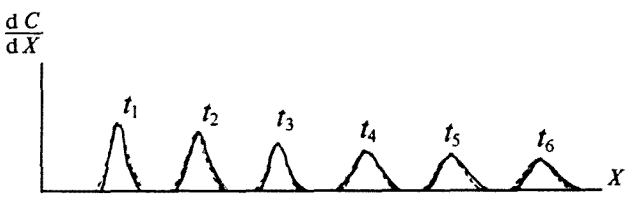

Fig. 1. Diffusion curves of an aqueous solution of *N*-(monohydrofullerenyl)-L-alanine (C = 0.15 g dL^{-1}). The first photograph was taken after 240 s, and the others were taken at 300 s intervals. The dashed lines show the Gaussian curves.

The partial specific volume of particles in the solution $\bar{v}$ was determined by pycnometry. It proved to be the same for the samples studied and equal to 0.752 cm^3 g^{-1}. The density of water ρ_0 = 0.997 g cm^{-3} and its viscosity at 25 °C η_0 = 0.8937 cP were taken from tables.

Figure 1 presents the diffusion curves for compound **2** (C = 0.15 g dL^{-1}) as examples. It can be seen from Fig. 1 that the gradient curves are rather close in their shape to Gaussian curves; therefore, the apparent diffusion coefficient D_c (the diffusion coefficient at a finite concentration) was calculated from the ratio of the area Q below the curve to its maximum ordinate H at the instant of time t.[6]

$$D_c = (Q/H)^2/4\pi t \quad (1)$$

The coefficient D is determined by the coefficient of forward friction f:

$$f = kT/D, \quad (2)$$

where k is Boltzmann constant and T is absolute temperature (K).

For molecules that exist in the solution as spheres, the coefficient of forward friction f is related to the diameter d, according to the Stokes equation, in the following way:

$$f = 3\pi\eta_0 d, \quad (3)$$

where η_0 is the viscosity of the solvent.

If we express the diameter of the sphere in terms of its volume $d = (6V/\pi)^{1/3}$, Eq. (3) can be written as

$$f = 3\pi\eta_0(6V/\pi)^{1/3}. \quad (3a)$$

If the particles are not solvated, *i.e.*, $V = M\bar{v}/N_A$ (M is molecular weight, N_A is the Avogadro number, $\bar{v}$ is the partial specific volume),[7] then, for a sphere we obtain the following expression instead of relation (3a):

$$f = 3\pi\eta_0(6M\bar{v}/\pi N_A)^{1/3}. \quad (4)$$

By solving this equation for M, we derive a formula for the calculation of the molecular weight

$$M = (f/3\pi\eta_0)^3/(6\bar{v}/\pi N_A). \quad (5)$$

Results and Discussion

We used the experimentally measured diffusion coefficients D_c and partial specific volumes $\bar{v}$ and also the relations (2)—(5) presented above to calculate the coefficients of forward motion f, molecular weights M of particles (we called them associates), the number of individual molecules with molecular weight M_0 in an associate, $n = M/M_0$, the volumes of associates and of individual molecules $V_{ass} = V_{sph}$ and $V_0 = V_{ass}/n$, as well as their diameters $d_{ass} = d_{sph}$ и $d_0 = (6V_0/\pi)^{1/3}$ for aqueous solutions of the compounds under consideration with finite concentrations of 0.05—0.15 g dL^{-1} (see Table 1).

The analysis of the data presented in Table 1 and in Fig. 2, which shows the concentration dependence of the apparent diffusion coefficients D_c, makes it possible to draw some conclusions concerning the different hydrodynamic behavior of the compounds under consideration. For example, the diffusion coefficients of compounds **1** and **2** depend appreciably on the concentration (see Fig. 2), the D_c value sharply increasing with a decrease in the concentration, although in experiments with an artificial boundary, an opposite situation normally occurs, that is, the diffusion coefficient D_c measured at a finite concentration of a solution increases with increase in the concentration, since the gradient of the osmotic pressure increases simultaneously with the concentration. For the other samples (compounds **3**—**6**), the diffusion coefficients D_c depend only slightly or not at all on the concentration. This fact makes it possible to conclude that the dramatic increase in D_c with a decrease in the concentration of compounds **1** and **2** is associated with a change in the sizes of the particles or, more precisely, with their instability in solution, rather than with concentration or electrolyte effects. This conclusion is illustrated by the values for the molecular characteristics and sizes listed in Table 1.

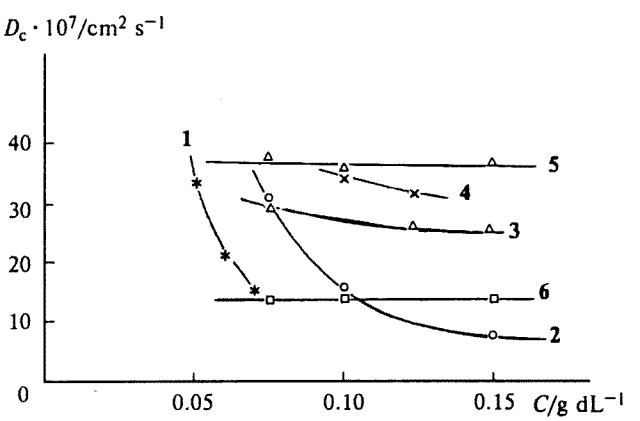

Fig. 2. Concentration dependence of the diffusion coefficients D_c of aqueous solutions of *N*-(monohydrofullerenyl)amino acids. The numbers of the curves correspond to the numbers of compounds in Table 1.

For example, in the case of compound **5**, the average value $D_c = 37.0 \cdot 10^{-7}$ $cm^2\ s^{-1}$ is the largest, compared to those of the other samples. The molecular weights determined by the method of sedimentation equilibrium ($M = 860$) and calculated from the diffusion data ($M_{av} = 880$) are practically identical and correspond to the molecular weight M_0 of an individual molecule, whose diameter (~13 Å) and volume (~1100 $Å^3$), calculated from the diffusion data, are in good agreement with the values obtained on the basis of the geometric dimensions of the monofullerene derivative of this peptide. This unambiguously indicates that in the concentration range chosen, no associates are formed.

In the case of compounds **3** and **4**, association occurs to a small extent. The molecular weights based on the diffusion data indicate that in the given range of concentrations, associates consist of two or three individual molecules and tend to be destroyed as the concentration decreases.

In the case of compound **6**, the $D \approx 14.5 \cdot 10^{-7}$ $cm^2\ s^{-1}$ value, average over three concentrations, corresponds to stable associates with $M = 16200$ (±5 %) and with a diameter of 34 Å, which are not destroyed when the solution is diluted twofold and contain ~18 individual molecules. Possibly, in the case of this sample, the concentration $C = 0.075$ g dL^{-1} is still too high for the destruction of the associates, while $C = 0.15$ g dL^{-1} is not high enough for the formation of larger associates. In fact, as shown by electron microscopy,[8] particles of diameter greater than 160 Å are formed in more concentrated solutions (0.3 g dL^{-1}).

In the case of compounds **1** and **2**, we observed the formation of unstable associates, whose size depended on the concentration. For example, a solution of compound **2** with $C = 0.15$ g dL^{-1} contains associates whose volume is ~126000 $Å^3$, molecular weight is greater than 100000 Da, and which consist of 125 individual molecules. The diameter of these associates is more than 60 Å. As the solution is diluted, the size of the associates decreases, and at $C = 0.075$ g dL^{-1}, they consist of two molecules, their sizes approaching those of individual molecules. It may be suggested that at $C > 0.15$ g dL^{-1} the associates will be even larger, while at $C < 0.075$ g dL^{-1} compound **2** will exist in a solution as individual molecules. In other words, each concentration of this fullerenyl derivative is matched by a particular average degree of association of the molecules, corresponding to an average molecular weight, and the lower the concentration, the smaller the number of individual molecules incorporated in the associate. In the case of compound **1**, the characteristic features are similar but are observed at lower concentrations, because the solubility of compound **1** in water is lower than that of compound **2**.

Thus, the study of the diffusion of various *N*-(monohydrofullerenyl)amino acids and -peptides in dilute aqueous solutions made it possible to show for the first time that these compounds can exist in solution both as separate molecules and as associates, the degree of association being independent of the concentration of the solution in some cases and being determined by the concentration in other cases. The different behavior of the compounds studied in solution correlates with their solubility in water, which is governed, like the association, by various relationships between the hydrophobic and hydrophilic fragments in the molecule. The calculation of the molecular weight, the volume, and the diameter of the associates made it possible to calculate the degree of association n, and also the diameter of individual molecules, which is in satisfactory agreement with the data obtained on the basis of a theoretical model.

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