Dependence of the degree of association of water-soluble amino acid and peptide derivatives of fullerene[60] on pH and the ionic strength of a solution

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The dependences of the degree of association of water-soluble amino acid and peptide derivatives of fullerene[60] on pH and the ionic strength of a solution were studied by the diffusion method. The degree of association of alanine, serine, arginine, and alanylalanine derivatives of fullerene increases both in acidic and in alkaline media compared to the initial aqueous solution. Using arginine and alanylalanine derivatives of fullerene as an example, it was demonstrated that the degree of association also increases as the ionic strength of a solution increases.

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

Previously, we have demonstrated that water-soluble amino acid and peptide derivatives of fullerene[60] of the general formula $HC_{60}NHXCOOH$, which were synthesized by direct addition of amino acid or peptide to fullerene,[1] can occur in dilute aqueous solutions (C = 0.05÷0.15 g dL^{-1}) both as associates and as isolated molecules.[1–2] The degree of association of the molecules depends on the nature of an amino acid or peptide fragments, *i.e.*, on the hydrophilic-hydrophobic balance of the molecule. The concentration of the compound in a solution also affects the degree of association. Generally, the degree of association decreases as the concentration decreases, but in some cases, it remains unchanged.

As part of continuing studies, in this work we studied the dependence of the degree of association on the concentration of the compound in an aqueous solution for fullerene derivatives of DL-serine (**1**) and L-arginine (**2**). The amino acid fragments of these fullerene derivatives, unlike those studied previously,[2] contain, in addition to a carboxy group, one more functional group. The molecular characteristics of solutions of these compounds were studied using the procedure for measurements of diffusion coefficients (D) reported in Ref. 3.

Experimental

N-(Monohydrofullerenyl)-DL-serine (**1**), *N*-(monohydrofullerenyl)-L-arginine (**2**), *N*-(monohydrofullerenyl)-DL-alanyl-DL-alanine (**3**), and *N*-(monohydrofullerenyl)-L-alanine (**4**) were synthesized according to a procedure reported previously.*

Experiments were carried out on an MOM-3180 analytical ultracentrifuge (Hungary) with Philpot-Swensson optics. The rotor temperature was 25±0.1 °C. Water was used as the solvent. The concentrations were varied within the range of 0.075—0.15 g dL^{-1} (higher concentrations afford dark nontransparent solutions, whereas lower concentrations cannot be studied because of the small increment of the refraction index). The diffusion coefficients were measured in a boundary-forming cell by layering the solvent onto a solution of a finite concentration. Pictures of the boundary between the pure solvent and the solution were taken at certain intervals, and its broadening was thus monitored. The rate of rotation of the rotor (4000 rpm) was chosen so that sedimentation of particles did not occur during the experiment, and the boundary broadened only due to diffusion. It is known that measurements of diffusion coefficients in solutions make it possible to calculate the coefficients of forward friction of molecules and their average hydrodynamic radius, the so-called Stokes radius. Studies by electron and tunneling microscopy demonstrated[4,5] that associates of amino acid and peptide derivatives of fullerene occur in aqueous solutions as ellipsoid particles containing an insignificant amount of the solvent. If the particles of the compounds under study occur in solutions as spheres or approximately spherical particles, one can easily get from the Stokes radius to the volume of particles and their molecular weights.[6] In addition, knowledge of the diffusion coefficients makes it possible to make conclusions about the stability of the molecule in solution and its possible changes.

The partial specific volume of particles in the solution ($\bar{v}$) was determined picnometrically. This characteristic proved to be the same for the samples under study ($\bar{v}$ = 0.752 cm g^{-1}). The density of water (ρ_0 = 0.997 g cm^{-3}) and its viscosity at 25 °C (η_0 = 0.8937 cP) are the tabular values. The coefficients of forward friction f, molecular weights M of particles (we called them associates), the number of individual molecules with the molecular weight M_0 in an associate $n = M/M_0$, the volumes of associates $V_{ass} = V_{sph}$ and of individual molecules $V_0 = V_{ass}/n$, and their diameters $d_{ass} = d_{sph}$ and $d_0 = (6V_0/\pi)^{1/3}$

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Table 1. Molecular characteristics and sizes of associates of water-soluble fullerene derivatives of amino acids in aqueous solutions in relation to the concentration

Compound	M_0	C /g dL^{-1}	$D\cdot10^7$ /cm^2 s^{-1}	$f\cdot10^8$ /g s^{-1}	M /Da	n	V_{ass}/Å^3	V_0/Å^3	d_{ass}/Å	d_0/Å
1	825	0.150	10.0	4.11	48700	59.1	60850	1030	48.8	12.52
		0.125	10.3	3.99	44600	54.0	55700	1030	47.4	12.53
		0.075	10.2	4.03	45920	55.7	57330	1029	47.8	12.52
2	894	0.150	12.5	3.29	24950	27.9	31140	1116	39.0	12.87
		0.125	13.7	3.00	18950	21.2	23660	1116	35.6	12.87
		0.075	14.2	2.89	17000	19.0	21250	1118	34.4	12.87

were calculated according to equations reported in Ref. 2 using the experimentally measured diffusion coefficient D_c and partial specific volumes $\bar{v}$ (Table 1).

Results and Discussion

As can be seen from Table 1, the behavior of compounds **1** and **2** in the concentration range under study is different. The degree of association of compound **1** (in the concentration range studied) is virtually independent of the concentration, and associates consist of about 60 molecules, whereas the degree of association of compound **2** is approximately halved upon dilution of the solvent by a factor of one and a half. It is noteworthy that the degree of association of compound **2** is from two to three times lower than that of compound **1**. These results agree well with the data on the solubility of compounds **1** and **2** in water (1.6 mg mL^{-1} and 20 mg mL^{-1}, respectively). For poorly soluble compound **1**, the concentration range under study is close to the limiting solubility, which is responsible for the high degree of association and its independence from the concentration. For compound **2** whose solubility is an order of magnitude higher, these concentrations are not high, and therefore, the initial degree of association is lower and depends on the concentration.

Note that the diameters of the individual molecules calculated from our experimental data agree well both with the diameters of the molecules of amino acid and peptide derivatives of fullerene determined previously[2] and with the calculated values.

A knowledge of the dependence of the degree of association of amino acid and peptide derivatives on pH of the solution is of great importance in further physicochemical studies and, particularly, in studies of the biological activity of amino acid and peptide derivatives of fullerene. We studied the dependence of the degree of association of compounds **1** and **2** and fullerene derivatives of DL-alanyl-DL-alanine (**3**) and L-alanine (**4**) at the concentration of 0.15 g dL^{-1} on pH of solutions by the diffusion method. As can be seen from Table 2 and Fig. 1, when a solution is acidified to pH 3—4, the degree of association increases by approximately an order of magnitude. An increase in pH of the solution to approximately 5 decreases the degree of association of all compounds under study. For compounds **2**—**4**, the lowest degree of association is observed at pH 5, whereas for compound **1**, the lowest degree of association is observed at pH ~8. Further increase in pH causes an increase in the degree of association, particularly in the case of derivative **1**. In the latter case, this change is comparable with the increase in the degree of association upon acidification.

The observed dependence of the degree of association on pH of the solution can be explained as follows. Previously, studies by electron and tunneling microscopy[4,5] demonstrated that in the concentration range under study, amino acid and peptide derivatives of fullerene are self-organized to form ellipsoid associates containing insignificant amounts of water. The presence of a hydrophobic (fullerene) fragment, which is virtually insoluble in water, in the molecules makes it possible to suggest the mechanism of dissolution of surfactants for these compounds. The hydrophobic residues of the molecules are self-organized so that they are located inside associates. The hydrophilic portion of the molecule is located in water. The higher the solubility of the amino acid or peptide residue, the higher is the solubility of the fullerene derivative. However, the basicity of the amino

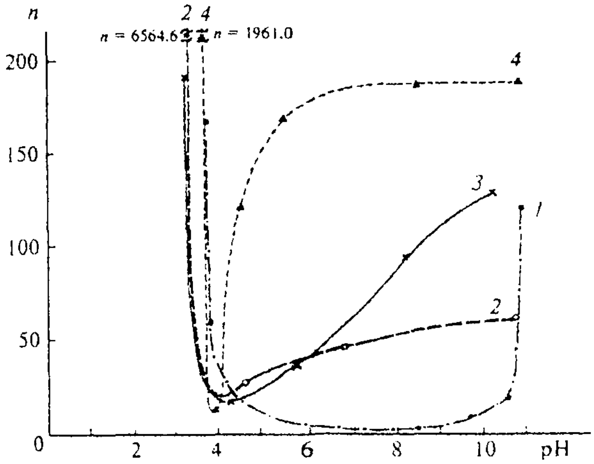

Fig. 1. Curves of the dependences of the degree of association n on pH of solutions of the fullerene derivatives of DL-serine (*1*); L-arginine (*2*); DL-alanyl-DL-alanine (*3*); and L-alanine (*4*).

Table 2. Molecular characteristics and sizes of associates of water-soluble fullerene derivatives of amino acids in relation to pH of the solution (C = 0.15 g dL^{-1})

Compound	M_0	pH	$D\cdot10^7$ /cm^2 s^{-1}	$f\cdot10^8$ /g s^{-1}	M /Da	n	V_{ass} /Å^3	V_0 /Å^3	d_{ass} /Å	d_0 /Å	$\eta_0\cdot10^{-3}$ пз	$\bar{\upsilon}$ /cm^3 s^{-1}
1	825	3.75	7.1	5.79	137200	166.5	171000	1021	68.7	12.50	8.938	0.746
		3.90	10.0	4.11	48700	59.1	60850	1030	48.8	12.53	8.937	0.752
		8.54	28.8	1.43	2200	2.7	2724	1016	17.3	12.48	8.939	0.744
		9.68	18.5	2.22	8040	9.8	9634	988	26.4	12.36	8.941	0.722
		10.64	15.1	2.72	15100	18.4	17580	958	32.3	12.22	8.945	0.700
		11.04	8.1	5.07	98800	120.0	114000	950	60.2	12.20	8.950	0.695
2	894	3.36	2.0	20.25	5870000	6564.6	7269000	1107	240.3	12.80	8.938	0.746
		4.70	12.5	3.29	24950	27.9	31140	1160	39.0	12.87	8.937	0.752
		6.80	10.6	3.90	42000	46.9	51940	1107	46.3	12.84	8.939	0.746
		10.80	9.8	4.18	55200	61.7	63670	1032	49.5	12.54	8.952	0.695
3	880	3.27	6.6	6.20	169000	192.0	207700	1082	73.5	12.74	8.938	0.740
		4.32	14.5	2.83	15980	18.2	19950	1098	33.6	12.80	8.937	0.752
		5.85	11.5	3.57	32350	36.8	39970	1087	42.4	12.76	8.939	0.744
		8.25	8.5	4.84	82500	93.8	98920	1055	57.4	12.63	8.941	0.722
		10.31	7.7	5.31	112600	128.0	130800	1022	63.0	12.50	8.945	0.700
4	805	3.74	3.1	13.10	1586500	1961.0	1965000	1002	155.4	12.42	8.938	0.746
		3.95	7.8	5.27	102700	127.0	128200	1009	62.5	12.44	8.937	0.752
		4.58	7.9	5.19	98800	122.4	122400	1003	61.6	12.20	8.941	0.746
		5.70	7.3	5.66	137000	169.3	159200	940	67.3	12.15	8.945	0.700
		8.54	7.0	5.85	150700	186.2	175100	940	69.4	12.15	8.950	0.695
		10.9	7.0	5.87	152930	189.0	176470	933	69.6	12.13	8.950	0.695

group of fullerene derivatives of amino acids and peptides is substantially lowered due to the strong electron-withdrawing effect of fullerene, which is called an "electron sponge." Therefore, unlike initial acids, which are inner salts, their fullerene derivatives are true acids and form zwitterions, if at all, only in the case where the molecule contains nitrogen atoms, which are not bonded to fullerene. The degree of dissociation of the carboxy group depends on the nature of the amino acid or peptide residue. In some cases, zwitterions can be formed in the side chain.[7] These zwitterions are more soluble in water than the neutral molecule. Of all the compounds under study, derivative **2**, which can form zwitterions because of the presence of the guanidine group in the molecule, exhibits the highest solubility.[7] Addition of an acid to the solution substantially decreases the degree of dissociation of the carboxy group due to the shift of the equilibrium to neutral molecules. A decrease in the hydrophilicity of the molecule at the same concentration leads to an increase in the number of molecules in an associate, which makes it possible to increase the hydrophilic portion of the associate. When the solution is strongly acidified, the degree of association increases almost in all cases, yielding a precipitate, which can be solubilized by alkalization.

Addition of a small amount of an alkali (in an approximately equimolar ratio) to a solution of fullerene derivative of amino acid or peptide affords a salt, which dissociates more readily than the acid, which in turn causes an increase in the solubility of compounds and a decrease in the degree of association. (The value of pH at which the degree of association is the lowest depends on the nature of the amino acid or peptide fragment of the molecule). Further increase in pH causes an increase in the degree of association of fullerene derivatives, which apparently is associated with a decrease in the degree of association of the salt.

At lower concentrations (0.075 g dL^{-1}), the same tendency for a change in the dependence of the degree of association of the molecules on pH is observed. However, at high pH, this dependence is less pronounced (Table 3). At low concentrations, dissociation of the molecules is the prevailing process. Therefore in alkaline dilute solutions, a change in the degree of association is insignificant and depends only slightly on pH.

The ionic strength of the solution also substantially affects the degree of association of compounds **2** and **3**. It is seen from Table 4 that for compound **3**, even a slight increase in the ionic strength leads to an increase in the degree of association. For compound **2**, this dependence is more complicated. If an amount of KCl added in a molar ratio is comparable with the concentration of the fullerene derivative, the degree of association of compound **2** first decreases and then increases as the ionic strength of the solution increases. This difference in the behavior of compounds **2** and **3** is, apparently, determined by the fact that compound **2** virtually occurs as zwitterions. Therefore, a slight increase in the ionic strength of the solution favors an increase in its solubility, which in turn leads to a decrease in the

Table 3. Molecular characteristics and sizes of associates of water-soluble fullerene derivatives of amino acids in relation to pH of the solution (C = 0.075 g dL^{-1})

Compound	pH	$D \cdot 10^7$ /$cm^2 s^{-1}$	$f \cdot 10^8$ /$g s^{-1}$	M /Da	n	V_{ass} /Å^3	V_0 /Å^3	d_{ass} /Å	d_0 /Å
1	3.57	9.5	4.33	61500	74.6	70951	951	51.4	12.2
	3.86	10.2	4.03	45920	55.7	57330	1029	47.8	12.5
	9.05	27.7	1.48	2480	3.0	2863	954	17.6	12.2
	10.37	17.2	2.39	10360	12.6	11950	948	28.4	12.2
3	3.27	6.8	6.04	161100	183.0	193500	1057	71.8	12.6
	4.50	14.0	2.94	17700	19.4	22266	1147	34.9	13.0
	7.49	12.6	3.26	25400	28.8	30411	1056	38.7	12.6
	9.80	12.6	3.26	25400	28.8	30411	1056	38.7	12.6
4	4.43	3.9	10.54	855600	1057.6	1025700	970	125.1	12.3
	5.82	7.66	5.36	116500	144.0	135400	940	63.7	12.1
	7.77	11.44	3.59	33900	41.9	40640	970	42.6	12.6

Table 4. Molecular characteristics and sizes of associates of water-soluble fullerene derivatives of amino acids in relation to the ionic strength of the solution (C = 0.15 g dL^{-1})

Compound	$I \cdot 10^2$	$D \cdot 10^7$ /$cm^2 s^{-1}$	$f \cdot 10^8$ /$g s^{-1}$	M /Da	n	V_{ass} /Å^3	V_0 /Å^3	d_{ass} /Å	d_0 /Å
3	0.17	14.7	2.80	12900	14.6	19130	1310	33.2	13.6
	0.34	10.6	3.88	34400	39.1	51040	1305	46.0	13.6
	0.51	9.7	4.24	44900	51.0	66600	1306	50.3	13.6
2	0.17	9.9	4.37	49300	55.2	73180	1326	51.9	13.6
	0.34	12.9	3.60	27600	30.9	41030	1328	42.8	13.6
	0.51	14.8	2.78	12600	14.1	18750	1330	33.0	13.6
	5.00	6.90	6.00	163200	185.2	188300	1032	71.1	12.4
	10.00	6.0	6.85	243800	272.7	281400	1032	81.3	12.5

degree of association. However, a sharp increase in the ionic strength of the solution and, correspondingly, an increase in the amount of counter-ions causes a decrease in the solubility of compound **2** and leads to an increase in the degree of association of the compound dissolved. Compound **3** exhibits a lower solubility than compound **2** and occurs as zwitterions to a lesser extent. Therefore, even a slight increase in the ionic strength of the solution causes an increase in the degree of association.

Therefore, the degree of association of fullerene derivatives of amino acids and peptides essentially depends both on the ionic strength of the solution and on its pH.

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