Investigation of structures of micelles of a fullerene derivative of alanine in aqueous solutions by tunneling scanning microscopy

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Structures of micelles of *N*-(monohydrofullerenyl)-L-alanine and their associates were studied by tunneling scanning microscopy in the regime of a profilometer with a 10-nm resolution along the vertical line. The data obtained show that the micelles of the compound studied are anisodiametric particles 0.5—10 μm in size containing negligible amounts of water. The thickness of the micelles depends on their lengths.

Key words: fullerene, amino acids, tunneling scanning microscopy, associates.

It has been established previously that fullerene derivatives of amino acids and dipeptides form micelles in aqueous solutions, their sizes varying from 0.5 to 10 μm at various concentrations.[1] An electron microscopic study using a scanning microscope shows that micelles exist as both individual particles and associates.

It is assumed that in aqueous solutions fullerene derivatives are self-organized in such a way that hydrophilic amino acid or peptide fragments are arranged on the micellar surface, while the hydrophobic fullerene regions are localized inside micelles. Perhaps, the shell of a micelle is a bilayer (as in liposomes) in which hydrophilic fragments of the molecule are directed both outward and inward the micelle. The micelle itself can be filled with a solvent or be a conglomerate of amino acid and peptide derivatives of fullerene, which either contains a small amount of a solvent or no solvent at all.

In the present work, structures of micelles of *N*-(monohydrofullerenyl)-L-alanine (**1**) and their associates were studied by tunneling scanning microscopy.

Experimental

The principle of action of a tunneling scanning microscope is based on a strong dependence of the tunneling current on the width of the tunneling gap. The instrument is designed for obtaining microtopographic images of conducting surfaces in air with resolution in a nanometer range. Objects were studied in a tunneling scanning microscope in the regime of a profilometer with a vertical 10-nm resolution. An RTP-1 raster profilometer (St. Petersburg University, Russia[2]) was used, which made it possible to obtain three-dimensional images and to study surface profiles by tracing the surface of the object at any chosen region. Several experiments were carried out on an S-2500 scanning electron microscope (Hitachi, Japan).

Samples of a solution of compound **1** with concentrations of 1.6 g L^{-1} were prepared by two different methods: (1) by drying samples in air and (2) by freeze-drying under conditions used in the electron microscopy of biological objects.[3,4] In both cases, a solution of fullerene derivative **1** was applied onto a freshly split mica surface that is considered atomically smooth.[5] In transmission electron microscopy, this method is used for observation of individual macromolecules of synthetic polymers and biopolymers.[5,6] The freeze-drying method allows one to fix the structure of a micelle in solution, because this excludes the action of surface tension forces resulting in the compression of particles that previously contained a solvent. A 20-nm thick layer of gold was simultaneously deposited *in vacuo* onto all objects to create the conducting surface.

Results and Discussion

The electron microphotograph of the fullerene derivative obtained with an S-2500 scanning electron microscope is presented in Fig. 1. The three-dimensional image (obtained on an RTP-1 tunneling scanning microscope) of the relief of the mica surface with micelles of fullerene derivative **1** applied from the solution is presented in Fig. 2.

The profilograms of the differently prepared micelles corresponding to the tracing direction indicated in the image of particles are presented in Figs. 3 and 4. It is seen from Fig. 3 that the heights of particles depend on their lengths. In particular, a micelle 1.5 μm in diameter has a height of 0.15 μm, while a micelle 0.5 μm in diameter has a height of 0.05 μm. The data obtained show that the heights of air-dried micelles are approximately eight- to tenfold less than their lengths.

When micelles were dried by the freeze-drying method, the same regularity is observed: the height of the micelle decreases as its length decreases. However, the diameter of the micelle is three- to fivefold greater than its height for both large and small particles.

Thus, the particle is flattened upon drying in air. When the freeze-drying method is used, micelles retain the initial structure.[3] However, it is possible that some

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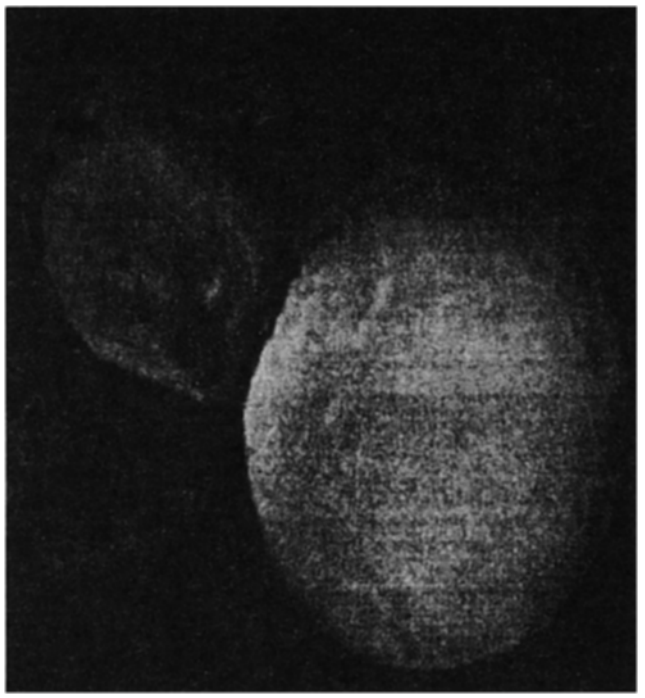

Fig. 1. Electron microphotograph obtained by an S-2500 scanning electronic microscope (Hitachi) (freeze-drying method).

decrease in sizes compared to the real sizes of particles in solution takes place.

It is most likely that the micelle is a flattened anisodiametric particle, whose edges are thinner than the central regions. This is indicated by the profilograms.

It has been shown previously that drying in air of biological objects and polymeric systems containing a solvent results in a change in the real structure of the sample. The pressure exerted by surface tension forces on the surface of a very small biological object was calculated.[7] In particular, it is 160 kg cm^{-1} when a virus is dried in air. Porous materials are deformed under the action of capillary contraction forces that appear due to the surface tension.[8] The shrinkage strain in gelatin gel reaching 300 atm results in a change in the initial structure and formation of dense films. The difference in the results of study of fullerene derivatives prepared by two different methods can be explained by the following factors: (1) the associate of **1** contains the solvent; therefore, sublimation of the frozen solvent (freeze-drying method) makes it possible to retain the form of the particle and (2) the associate contains pores or holes that flatten upon drying in air due to surface tension forces. The cryogenic method of preparation allows one to retain the form of the associate. Taking into account the hydrophobicity of **1**, it can be assumed that the associate is characterized by the existence of both pores and holes and a low content of the solvent in the particle.

The amount of the solvent in the micelle is low, because in the opposite case (when the amount of water in the micelle is high), the freeze-drying method should result in the appearance of artifacts as a network structure owing to the crystallization and recrystallization of ice in the course of preparative procedures.[4,6] No network structure was observed when cryomethods were used for all the water-soluble amino acid and peptide derivatives of fullerene studied.

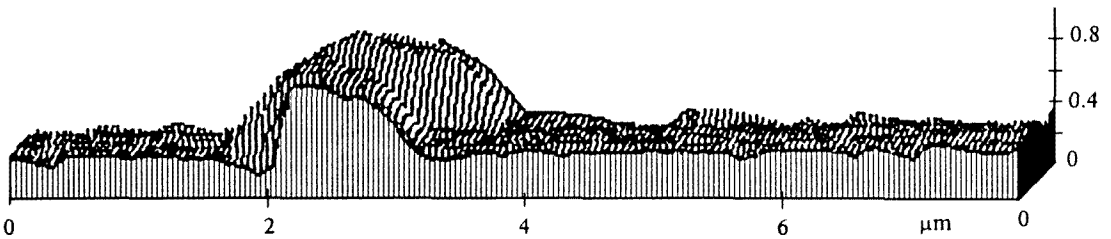

Fig. 2. Three-dimensional image of the associate obtained by an RTP-1 tunneling scanning microscope (freeze-drying method).

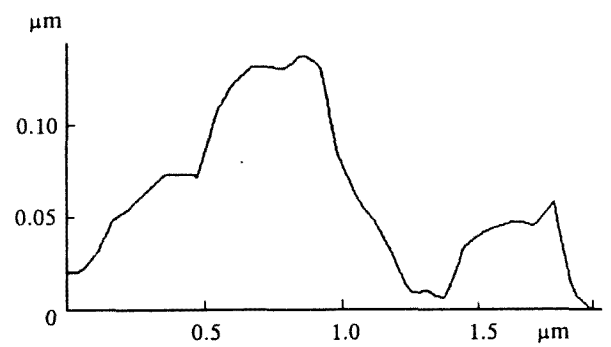

Fig. 3. Profilograms of associates consisting of two particles (drying in air).

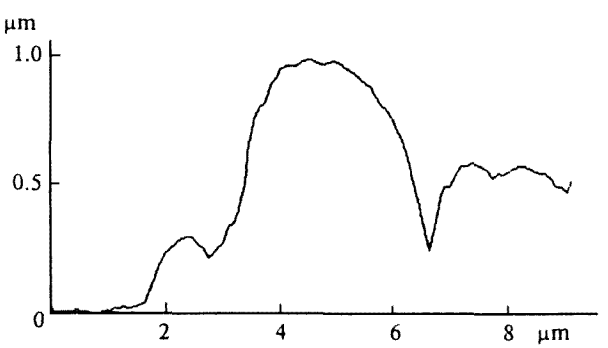

Fig. 4. Profilograms of associates (freeze-drying method).

Based on the data obtained, we believe that micelles of fullerene derivative of alanine are flattened anisodiametric particles 0.5 to 10 μm long containing insignificant amounts of water. The thickness of micelles is related to their lengths, and their edges are thinner than the central regions.

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References

1. M. E. Vol'pin, E. M. Belavtcheva, V. S. Romanova, A. I. Lapschin, L. I. Aref'eva, and Z. N. Parnes, *Mendeleev Commun.*, 1995, 129.
2. V. K. Adamchuk, A. V. Ermakov, and S. I. Fedoseenko, *Elektron. Prom-st' [Electronic Industry]*, 1991, No. 3, 14 (in Russian).
3. T. Nei, in *Principles and Techniques of Scanning Electron Microscopy. Biological Applications*, Ed. M. A. Hayat, New York, 1974, **2**, 113.
4. E. M. Belavtseva, L. G. Radchenko, E. F. Titova, and G. F. Shalygin, *Biofizika*, 1980, **25**, 4 [*Sov. Biophys.*, 1980, **25** (Engl. Transl.)].
5. C. T. Hall, *J. Biophys. Biochem. Cytol*, 1956, **2**, 625.
6. E. M. Belavtseva, T. A. Kabanova, and L. G. Radchenko, *Biofizika*, 1983, **28**, 958 [*Sov. Biophys.*, 1983, **28** (Engl. Transl.)].
7. T. F. Anderson, *Am. Naturalist*, 1952, **86**, 91.
8. M. S. Ostrikov, T. P. Dukhina, and N. Levis, *Zh. Nauch. Prikl. Fotogr. Kinematogr. [J. Scient. Appl. Photogr. Cinematogr.]*, 1964, **9**, 254 (in Russian).

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