Self-assembling of Associates of Amino Acids and Dipeptide Derivatives of [60]Fullerene in Aqueous Solution: a Study by Scanning Electron Microscopy

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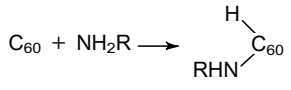
Amino acid and dipeptide derivatives of [60]fullerene form associates in aqueous solution.

Recently we reported a method allowing the preparation of mono(amino acid or dipeptide) derivatives of [60]fullerene,[1] in which an amino acid moiety is added to fullerene. The method has been employed for the synthesis of a number of fullerene amino acids and fullerene dipeptides (Scheme 1).



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1 R = CH_2COOH
2 R = L-CHMeCOOH
3 R = D,L-$CH(CH_2OH)COOH$
4 R = L-CHMeCO-L-NHCHMeCOOH
5 R = CH_2CO-L-NHCH($CHMe_2$)COOH
6 R = $CH_2CONHCH_2COOH$
7 R = CH_2CO-D,L-NHCHMeCOOH

Scheme 1

Unexpectedly, glycinefullerene **1**, L-alaninefullerene **2**, serinefullerene **3**, alanylalaninefullerene **4**, glycylvalinefullerene **5**, glycylglycynefullerene **6** and glycylalaninefullerene **7** turned out to be soluble in water. For example, the solubilities of derivatives **2** and **4** in water are 1.5 and 6 mg ml^{-1}, respectively. To understand this phenomenon, we used the method of electron microscopy. The study was carried out with a Hitachi S-2500 scanning electron microscope (resolution 3.5 nm) operating in a secondary electron mode. The samples for scanning electron microscopy (SEM) were prepared by means of the procedure employed in transmission electron microscopy for biological macromolecules[2] with alterations made to meet the requirements of samples in SEM.

A drop of the solution was applied to a freshly cut surface of mica; this appears to be 'atomically smooth' and thus excludes artifacts occurring on preparation of the samples. After being dried in air the sample was covered with a thin layer of gold (20 nm) to create a conducting surface.

The study showed that in aqueous solutions all compounds form large anisometric particles, associates, varying in size from 1 to 10 μm. The shape of the associates for most of the samples is oval. The largest associates were observed in the case of derivative **2**, with the major axis varying within 4–10 μm limits. Along with the large associates, small oval particles 0.5–1.5 μm in diameter were also observed.

The major axis of the derivative **4** associates was found to be mainly 0.5–1.0 μm, the large particles occurring only occasionally (2 μm) (Fig. 1). The 0.7–3.0 μm associates are characteristic of aqueous solutions of derivative **5**. Derivative **2** associates possess a shape of a rolled up stretched form, and Fig. 2 shows them rolling up. This is evidence that associates are flexible and capable of rolling up.

However, it was essential to prove that associates were present in the solution but were not formed on drying. Thus, we employed a cryogenic technique which allows us to avoid the effect of surface tension forces in the course of removing a solvent from the samples.

Preparation of the samples was carried out using a conventional procedure described earlier.[3,4] A thin layer of

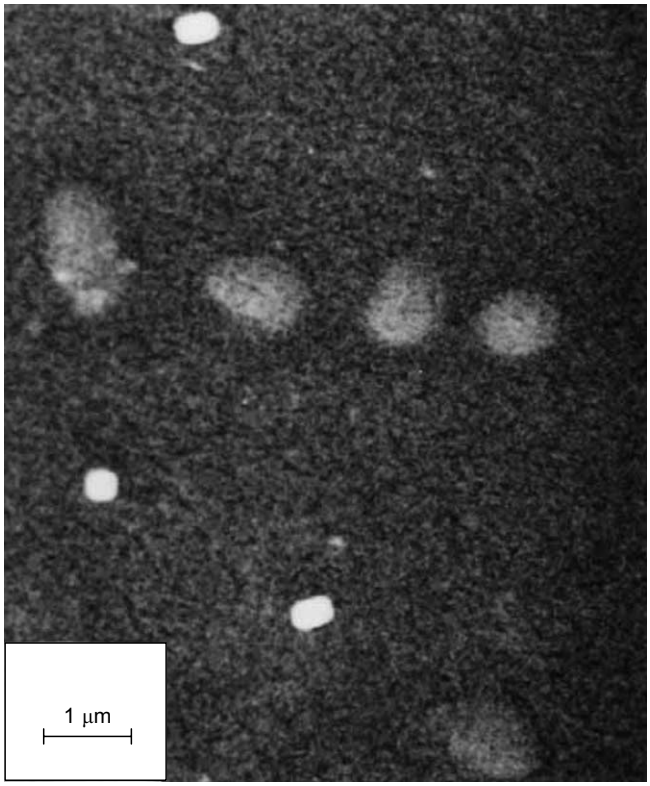

Fig. 1 Electron micrograph of fullerene-L-alanyl-L-alanine **4** micelles.

the solution applied to the mica surface was frozen in liquid N_2 at a rate of 100 °C s^{-1}. This was followed by sublimation of ice at −100 °C and then at −80 °C for 15 h. The rate of freezing was monitored by means of a micro thermocouple connected to a storing oscillograph in a similar fashion as described in ref. 5. Only at a high rate of freezing followed by a low temperature lyophilic evaporation of the solvent (freeze-drying) was it possible to exclude artifacts from the cryogenic preparation of the samples. Fig. 3 presents the thermogram of freezing for a drop of the compound **2** solution.

Figs. 4 and 5 show that under such conditions, **2** forms associates 2–6 μm in size. Upon application of the cryogenic procedure, along with associates, aggregates of the latter were also observed that consisted mostly of two particles, with larger ones consisting of three to five particles occurring occasionally.

The capacity of the above amino acid or peptide derivatives of fullerene for self-organization, *i.e.* to form associates, arises from the presence of both hydrophilic (amino acid or dipeptide) and hydrophobic (fullerene) moieties within them. One can suggest that in aqueous solutions fullerene derivatives undergo association in such a way that hydrophilic amino acid or peptide fragments become located on the associate surface while the hydrophobic fullerene moities are directed inside the associates. Probably, the shell of the associates is a bilayer (as in liposomes) in which the hydrophilic fragments of the molecules are directed

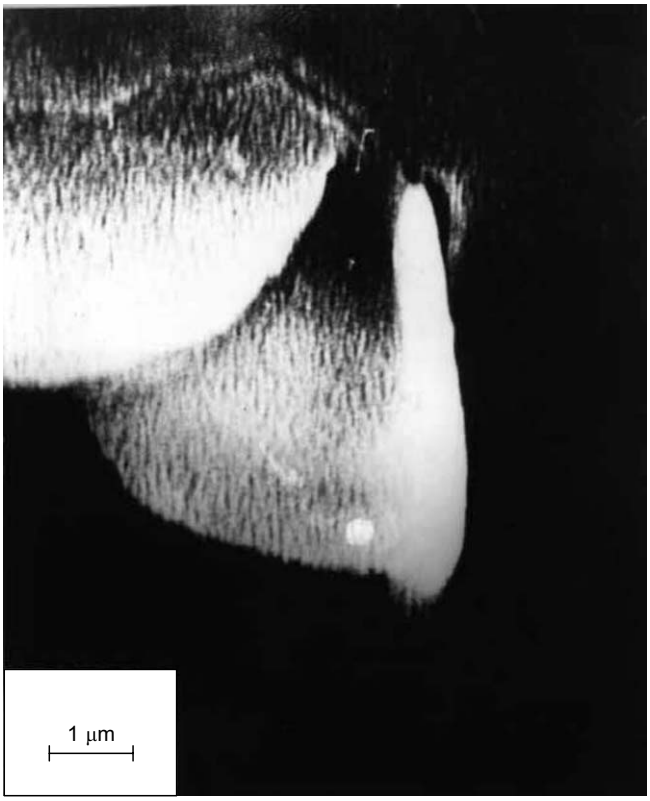

Fig. 2 Electron micrographs of fullerene-L-alanine **2** micelles; rolling up the micelles.

both outwards and inwards. The associate itself can be filled with a solvent or can be a conglomerate of amino acid or peptide derivatives of fullerene containing either a small amount of a solvent or, perhaps, none at all.

A change in the surface of the associates, shrivelling the shells, observed after freeze-drying (Fig. 4) is in accord with the hypothesis that associates contain the solvent (water) inside.

Interestingly, in all cases the application of water saturated with benzene as a solvent brought about an enlargement of

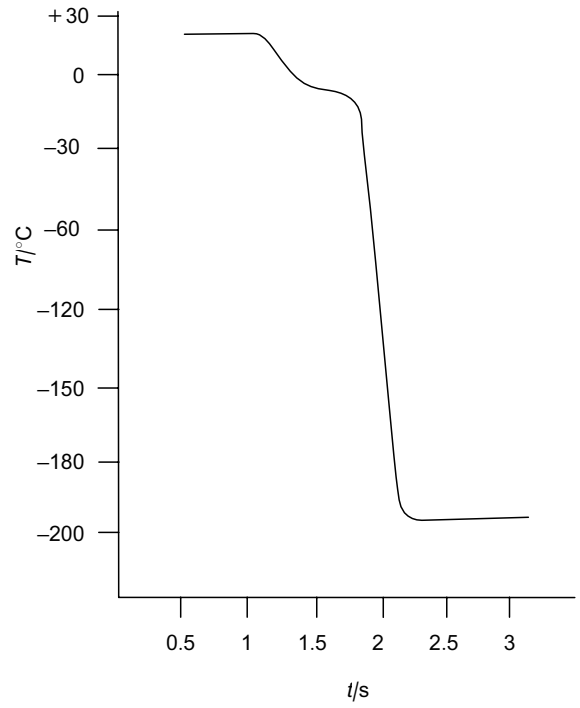

Fig. 3 Thermogram of freezing for a drop of fullerene-L-alanine solution.

Fig. 4 Electron micrograph of micelles of fullerene-L-alanine **2** after 'freeze-drying'.

the particles, the latter becoming more uniform in size. For example, the major axis of associates for derivatives **2** and **5** turned out to be 7–9 μm (instead of 0.5–10 μm) and 1.5–3 μm (instead of 0.7–3 μm), respectively (Fig. 6). We believe these data support the hypothesis that associates could be filled with a solvent.

Thus, we have shown that all the compounds studied form associates in concentrated aqueous solutions, *i.e.* these solutions are colloidal ones. However, as shown by means of the ultracentrifugical method,[6] dilution of saturated solutions

Fig. 5 Electron micrograph of micelles of fullerene-L-alanine **2** after 'freeze-drying'.

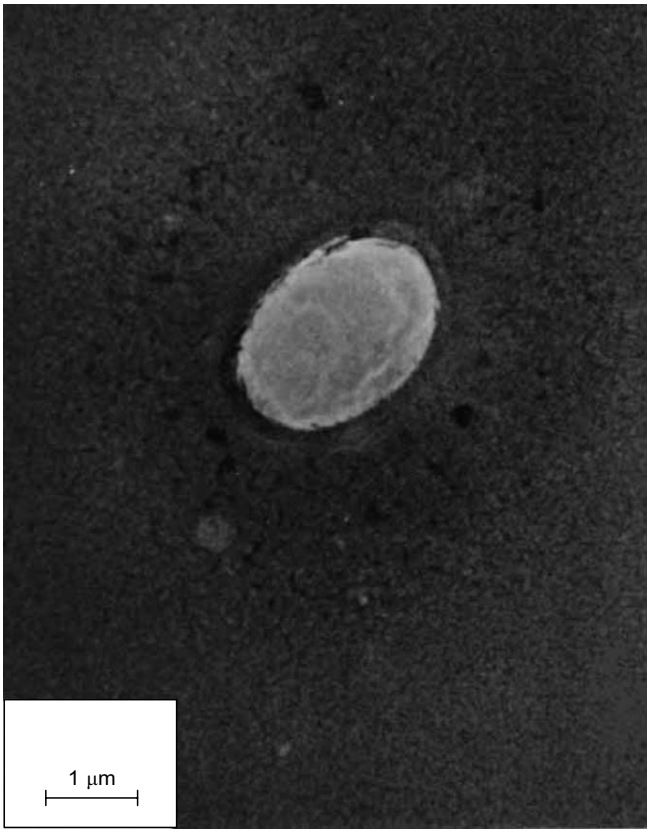

Fig. 6 Electron micrograph of micelles of fullereneglycyl-L-valine **5** in water saturated with benzene.

of the above compounds results in rapid decrease in size of the micelles, and on sufficient dilution, true solutions are formed.

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