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Self-organising aggregates of lipodipeptides in an aqueous medium and their complexes with DNA

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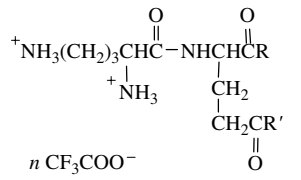
The forms of phase organization of lipodipeptides in an aqueous medium have been determined by electron microscopy.

We have previously reported the synthesis, membrane-forming properties and *in vitro* and *in vivo* transfection activity of dicationic amphiphiles based on L-glutamic acid.[1,2]



1 R = R′ = $O(CH_2)_{15}Me$, $n = 2$
2 R = R′ = $O(CH_2)_7Me$, $n = 2$
3 R = $O(CH_2)_{15}Me$, R′ = O^-, $n = 1$

In order to expand the library of cationic amphiphiles, reveal the relationship between the structure and activity of this class of compounds and develop medical transport systems on their basis, we have suggested new modifications (**1–3**) of the structures reported previously.

Critical aggregation concentrations have been determined for the amphiphiles and electronic micrographs of their aqueous dispersions have been obtained.

The plot of absorbance as a function of the logarithm of concentration for compound **1** has a characteristic shape with an inflexion point [Figure 1(*a*)], which is typical of surface-active compounds.

In an aqueous medium, compound **1** based on L-Glu dihexadecyl ester forms spherical liposomes with a mean diameter of 100 nm [Figure 1(*b*)].†

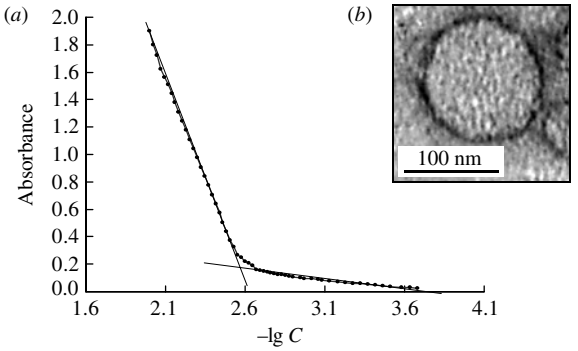


Figure 1 (*a*) Plot of absorbance *vs*. concentration. (*b*) Image of aqueous dispersions obtained by means of electron microscopy with negative staining with uranyl acetate for compound **1**.

† The micrographs were obtained by Dr. Sci. (Biol.) V. I. Popenko in the electron microscopy laboratory at V. A. Engelhardt Institute of Molecular Biology of the Russian Academy of Sciences.

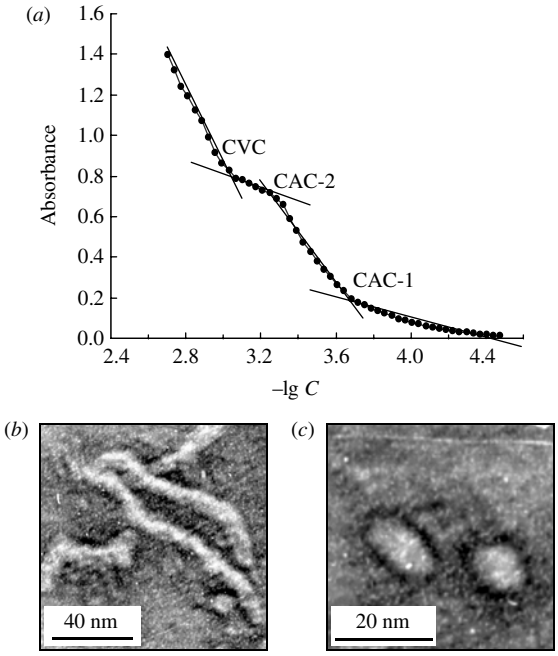


Figure 2 (*a*) Plot of absorbance *vs*. concentration. Electron micrographs of aqueous dispersions of compound **2** (*b*) before and (*c*) after ultrasonic treatment (the concentration is 9×10^{-4} mol dm^{-3}).

The plot of absorbance as a function of the logarithm of concentration for amphiphile **2** with two short-chain hydrocarbon tails has three inflexion points; this is an unusual shape for surface-active compounds [Figure 2(*a*)]. This can be due to various structural transitions in an aqueous medium: from dispersion to micelles (CAC-1), from ordinary globular micelles to rod-shaped micelles that are precursors of bilayer aggregates (CAC-2) and formation of lamellar vesicles (CVC). The indirect evidence of vesicle morphology was the possibility of inclusion of sodium fluorescein in the internal volume of aggregates.

At concentrations between CAC-2 and CVC, the micrographs of aqueous dispersions of compound **2** show the existence of rod-shaped aggregates [Figure 2(*b*)], which are converted after ultrasonic treatment to give smaller structures resembling disc-shaped micelles [Figure 2(*c*)].

An unusual behaviour of lipid dispersions in water was also observed for compound **3** based on L-Glu α-hexadecyl ester. The structure of this amphiphile favours both the formation of an inner salt and intramolecular ionic interactions. Presumably, the compound can form primary, secondary and multilayer vesicles in water; the latter are usually called 'onions'[3] (Figure 3).

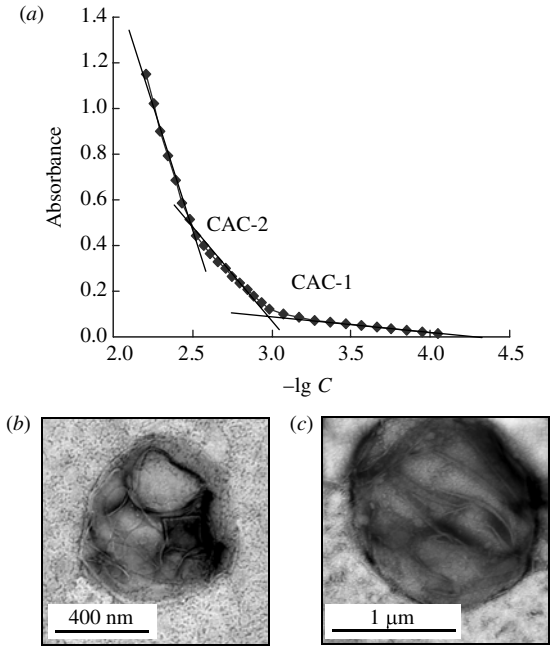


Figure 3 (*a*) Plot of absorbance *vs*. concentration. Electron micrographs of an aqueous dispersion of compound **3** at a concentration of (*b*) 6×10^{-3} or (*c*) ~10^{-2} mol dm^{-3}.

It was found that, in water, amphiphile **3** forms folded layered structures with a diameter of about 400 nm at a concentration of 6×10^{-3} mol dm^{-3} (between CAC-1 and CAC-2) [Figure 3(*b*)].

At concentrations above CAC-2 (~10^{-2} mol dm^{-3}), this compound forms very large aggregates (~2 μm) beyond the nanorange [Figure 3(*c*)], which cannot be used as transport systems.[4] The subsequent studies of amphiphile **3** were carried out in water at a concentration near CAC-1.

Using electron microscopy, a change in morphology was observed for aggregates formed due to complexation between cationic amphiphiles **1**, **3** and DNA (high molecular weight from bacteria, Sigma) in molar charge ratio of amphiphiles (expressed as amines, *N*) to DNA (expressed as phosphates, *P*), 1/1 (*N*/*P*). It was shown that the original spherical liposome shape of compound **1** in aqueous dispersions is converted to strands ('beads on a thread'[5]). Simultaneously, the sorting of particles by size is observed [Figure 4(*a*)]. The surface of lipopeptide **3** complexes with DNA appears to be a multilamellar structure, which is probably due to the layered shape of the original aggregate [Figure 4(*b*)].

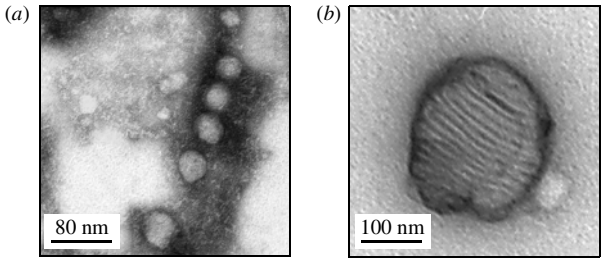


Figure 4 Images of aqueous dispersions of compounds (*a*) **1** and (*b*) **3** with DNA.

Thus, we revealed the possibility of formation of difference aggregates in an aqueous medium using certain structures of synthesised lipodipeptides.

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