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Properties and transfection activity of cationic dimeric amphiphiles based on amino acids

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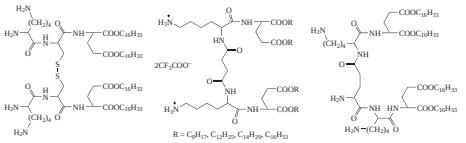
The physico-chemical and transfection properties of units self-formed by new cationic dimeric amphiphiles based on amino acids in water were characterized.

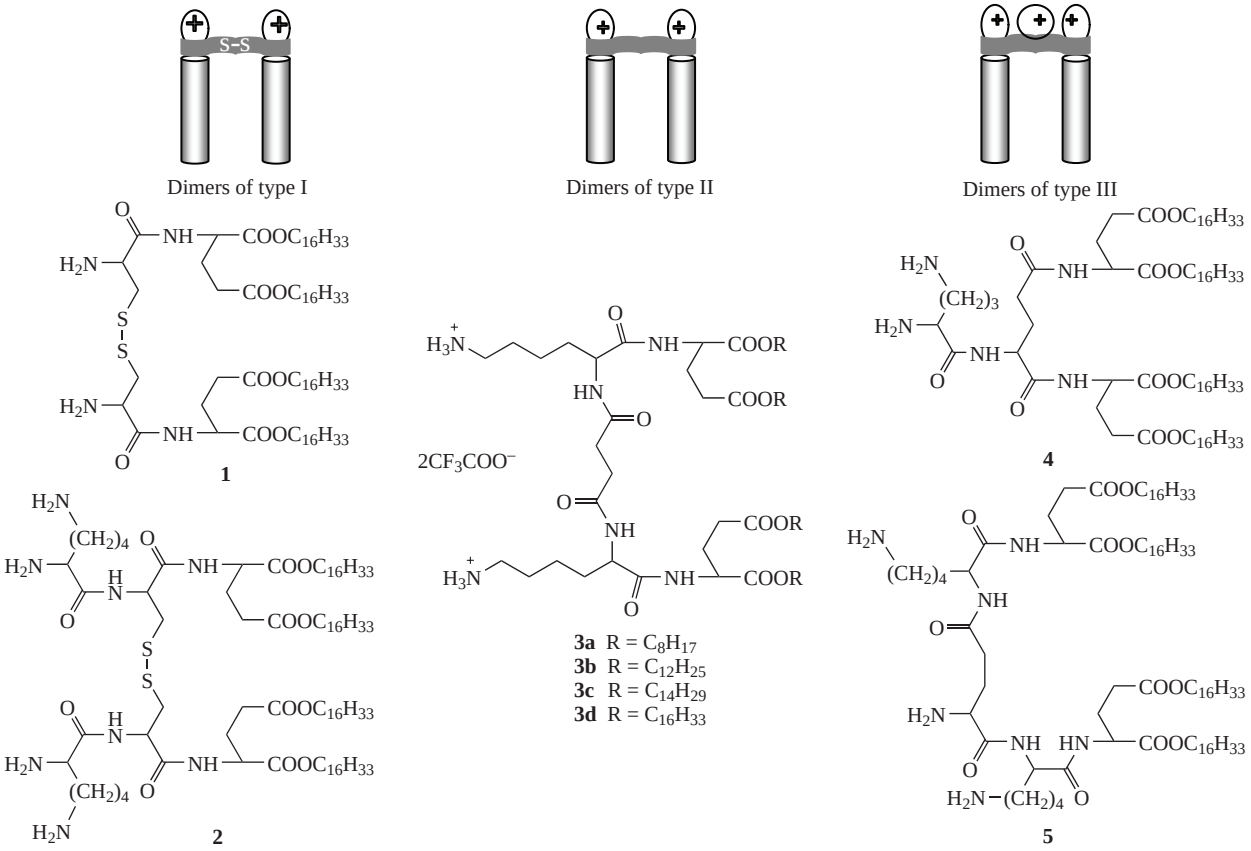


The design of systems for the transport of biologically active compounds to target cells is actively developed as applied to effective and safe therapeutic drugs.[1–4] Liposomes are promising delivery vectors for biologically active compounds, which are already used in clinical practice.[5] Cationic liposomes are effective transfection agents, which are used for delivering nucleic acids into cells.[6,7] High density of a positive charge on the surface of liposomes provides more effective transfection. Dimeric compounds with polar head bearing several ionized amino groups can form bilayer particles in water and deliver nucleic acids into the cells.[8,9]

In order to obtain cationic liposomes with a high specific positive charge, the synthesis of modified dimeric amphiphiles was carried out. These are L-cystine derivatives **1** and compounds **2** with two L-ornithine residues (dimers of type I), succinic acid derivatives **3a–d** with different lengths of hydrophobic parts (dimers of type II) and derivatives with L-glutamic acid residues in spacer fragments with one (**4**) or two (**5**) L-ornithine residues (dimers of type III).[10,11]

The average diameter of units was established using photon correlation spectroscopy. For compounds **1**, **2**, **3d**, **4** and **5**, the average diameters are 100, 150, 230, 210 and 140 nm, respectively.

An important characteristic of colloid dispersions is storage stability. It was shown that the injection of a spacer between two polar head groups increased the stability of liposomes based on the above compounds, compared to lipodipeptides, which were studied earlier.[12] Furthermore, the high ζ-potential (>50 mV) of



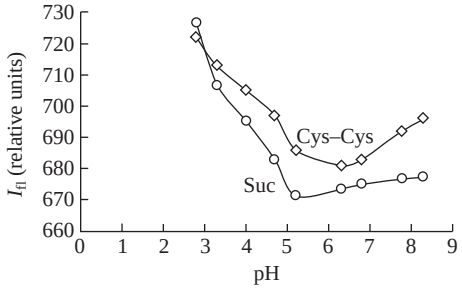


Figure 1 Fluorescence intensity of liposomes based on amphiphiles of types I (Cys–Cys) and II (Suc) containing uranine A.

liposomes causes electrostatic repulsion of the particles that allows one to save the integrity and size of the units.

The pH sensitivity of liposomes was studied using the dispersions of synthesized lipodipeptides in a phosphate buffer (pH 8.0) with uranine A (sodium fluorescein, a soluble diagnostic drug used in ophthalmology). Non-injected dye was removed by gel filtration. By adding 0.2 M hydrochloric acid, the pH of solution was changed with an increase in the fluorescence intensity of uranine A. A decrease in pH to 5.0–5.5 for dimeres of types I and II was insignificant. A further decrease in pH caused a sharp increase in fluorescence intensity due to bilayer destabilization and dye flowing from the inner volume of liposomes (Figure 1).

Complexes with DNA formed by bis-amphiphiles are stable in a bloodstream (pH is alkalescent) and destructed only within a cell (pH is acescent), realizing biologically active compounds; consequently, the pH sensitivity of these compounds is a very important property of transfection mediators.

Based on synthesized compounds, it is possible to obtain a liposomal form of doxorubicin. The inclusion of an antitumor drug was carried out by simple mixing, passive gradient loading with a freeze–thaw method, and by active ammonium sulfate gradient loading. Free doxorubicin was isolated from liposomal form by dialysis. It was found that the simple mixing method provides about 90% of included drug, and two other methods – more than 95%.

We studied physico-chemical properties of lipoplexes (lipopeptide complexes with DNA). For the synthesized compounds, the ratio of components with a maximum degree of nucleic acid binding into complex was determined. In the experiments on displacement of ethidium bromide by amphiphiles from complex with DNA for dimers of type I the maximum complex formation was observed at ratios of 3:1 (N/P) for compound **3d**, 4:1 (N/P) for compound **1** and 8:1 (N/P) for compound **8**.

The ability of lipoplexes to permeate into a cell was examined.[†] High level of transfection was observed with dimers of type I based on bis(dihexadecyl-L-glutamyl-L-lysyl)cysteine and bis-(ditetradecyl-L-glutamyl-L-lysyl)cysteine. About 60% of cells produced GFP protein. Dimers of types II and III showed the same efficiency only in a mixture with cholesterol in a mass ratio of 1:1 (Figure 2). Addition of cholesterol diminishes the toxicity of transfection agent and significantly decreases its cost.

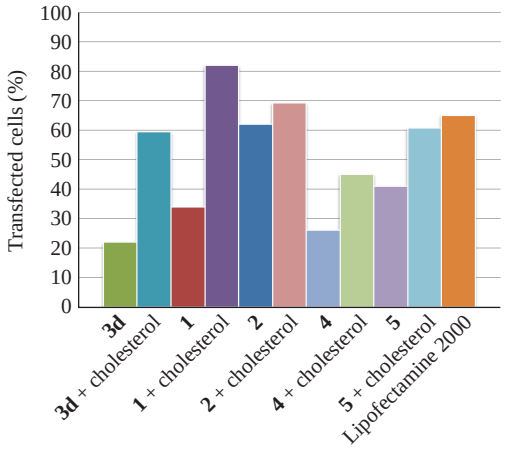


Figure 2 Transfection efficiency of HEK293T cell line.

Thus, the results obtained allow us to conclude that bis-amphiphiles can be used as promising transport systems for antitumor drugs and genetic materials.

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[†] *The study of transfection efficiency of lipopeptides.* Human embryonic kidney cells HEK293T were used. Cells were grown in monolayer in plastic Petri dishes at 37 °C, 5% CO_2. The medium DMEM (PanEco) with 300 mg dm^{-3} glutamine (PanEco), 80 mg dm^{-3} gentamicin (PanEco) and 10% fetal calf serum (PanEco) were used for cultivation. The day before transfection, cells were seeded in 24-well plate at 1×105 cells/well in a volume of 0.7 ml per well of DMEM medium with 300 mg dm^{-3} glutamine, 80 mg dm^{-3} gentamicin and 10% fetal calf serum. A few hours prior to transfection, medium was replaced with DMEM with 300 mg dm^{-3} glutamine and 10% fetal calf serum. Transfection mixture was prepared in DMEM: 1 μg plasmid pEGPP-n1, carrying a reporter gene GFP was added to 50 μl of DMEM, transfection agent in 50 μl of DMEM was used in ratios plasmid/lipopeptide mg/mg: 1:2, 1:4, 1:8, 1:16, 1:32, thus the volume of the transfection mixture was 100 μl. A mixture of 2 μl of commercial transfection reagent Lipofectamine 2000 (Invitrogen) and 1 g pEGPP-n1 at the same total volume of 100 μl DMEM was used as positive control. The mixture was stirred and incubated at room temperature for 30 min and added dropwise to the wells with HEK293T. Cells were incubated at 37 °C, 5% CO_2. After 4 h, the medium was exchanged for a new one. Transfection efficiency was assessed at 12, 24, 48, 72 h of incubation with a fluorescence microscope and by flow cytometry (Cytometer FACScan, BectonDickinson) by the amount of GFP^+-cells.