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Nano-sized Gene Delivery System Based on Cationic Amphiphiles

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We have developed a scheme of synthesis of aliphatic derivatives of tri- and tetrapeptides with different length of hydrocarbon chains in the hydrophobic domain. These compounds have been used to form liposomes, which physico-chemical properties were examined.

Keywords: Liposomes, Lipotripeptides, Lipotetrapeptides, Gene delivery, Transfection, The critical micelle concentration.

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INTRODUCTION

The distribution of liposomes in the body depends on the composition of the liposomal membrane, their size, charge and other chemical and physical parameters of the vesicles. Small bilayer liposomes unlike large particles in case of intravenously, intraperitoneal or intramuscular injection can penetrate more quickly into the bloodstream, which points to the limited ability of the latter to pass through the membrane of the capillaries and blood vessels [1].

Aliphatic derivatives of tri and tetra peptides produce biodegradable and biocompatible particles which are stable during storage without loss of biological activity. In some studies was found that larger size of the polar groups produce smaller diameter of liposomes based on amphiphiles in an aqueous medium [2]. Furthermore, the introduction of additional amino groups facilitates increasing the surface charge of the particles.

MATERIALS AND METHODS

We used N,N′-Dicyclohexylcarbodiimide (Lancaster, UK), Di-tert-butyl dicarbonate (Lancaster, UK), 1-Hydroxybenzotriazole hydrate (Lancaster, UK), L-ornithine (Diaem, Japan), L-lysine (Diaem, Japan), L-glutamic acid (Fluka, Switzerland), Fmoc-Lys(Boc)-OH (Sigma-Aldrich, Germany).

^{1}H-NMR spectra were recorded in $CDCl_3$ on a pulsed NMR spectrometer Bruker WM-400 (Germany), with an operating frequency of 400 MHz. Internal standard – hexamethyldisiloxane.

IR spectra were recorded on Bruker EQUINOX-55 (Germany).

Mass spectra were obtained by using a MALDI TOF mass spectrometer Ultraflex (Bruker Daltonics GmbH, Germany) with 2,5-dihydroxybenzoic acid, dissolved in 30 % acetonitrile containing 0.1 % TFA as a matrix. Desorption of the samples was carried out by irradiation of nitrogen laser (wavelength 337 nm), an operating frequency is 50 Hz. Spectra were recorded in the reflective mode of positively charged ions in the range of m / z 0,5-5 kDa. For each spectrum were summarized 1000 laser pulses (100 pulses with 10 different stains of points). Analysis of the MS data was performed by program Flex Analyses 3.0 (Bruker Daltonics).

RESULTS AND DISCUSSION

We have developed a scheme of synthesis of aliphatic derivatives of tri-and tetrapeptides with different length of the hydrocarbon chains in the hydrophobic domain.

Synthesis of the target compounds include the following steps: preparation an esterified L-glutamic acid derivatives by residues of fatty alcohols, activating Fmoc, Boc or Boc, Boc-protected L-ornithine and L-lysine to form a peptide bond between the components, removing the protecting groups from the resulting dipeptide derivatives, activation of the carboxyl groups of amino acids and peptide bond formation giving aliphatic tri-and tetrapeptides. The structures of all compounds were confirmed by ^{1}H-NMR, IR, mass spectra.

In order to determine the critical micelle concentration of synthesized compounds we measured the optical density in the range of concentrations of 10^{-2}-10^{-6} mol/l.

The point of inflection on the graph of optical density/concentration of amphiphile in water corresponds to the critical micelle concentration. At a lower concentration amphiphiles exist as a true solution or a disordered dispersion which can't be used for gene delivery. The curve for compounds with short-chain hydrocarbon moieties has three inflection points, due to various related structural transitions: from micelles dispersion to conventional spherical micelles, then to disk micelles and then to lamellar vesicles. We found that lipotetrapeptides have lower critical micelle concentration than previously synthesized lipodipeptides (10^{-5} and 10^{-4}, respectively). This property allows using lower concentrations of compounds for constructing gene delivery systems, which leading to reduced toxicity.

The particle size distribution was determined by particle size analyzer LSTM 13320 (Beckman Coulter, USA) according to the method of photonic-correlation spectroscopy based on the principle of dynamic light scattering. For example, for $(Orn)_3Glu(C14)_2$ the most common size (97 % of the total particles) after sonication was 60 nm.

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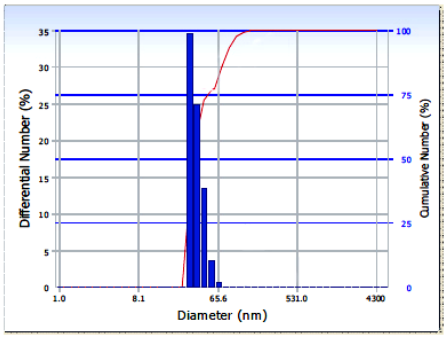

Fig. 1 – The particle size distribution for $(Orn)_3Glu(C14)_2$

Also we determined dzeta-potential for obtained particles, it has a high value (over 41 mV). This parameter is responsible for the electrostatic repulsion of the particles, which leads to long-term stability of lipid dispersions at room temperature. The resulting liposomal particles were stable for 6 weeks (optical density of dispersions did not changed by more than 5 % for this period).

REFERENCES

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During testing the efficiency of transfection for synthesized compounds, we also found the following relationship - efficiency increases with increasing in length of the hydrocarbon chain of the hydrophobic domain.

CONCLUSION

Thus, we compared several characteristics of aliphatic derivatives of tri- and tetrapeptides with the previously synthesized lipodipeptides. It was shown that the novel cationic amphiphiles have several advantages, including smaller critical micelle concentration, smaller particle size, longer storage at room temperature, a higher percentage of transfected cells. So cationic amphiphiles based on amino acids with an increased positively charged polar side are promising vectors for gene delivery.

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