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Interaction between cells and poly(ethylene glycol)-lipid conjugates

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ABSTRACT

Eight types of poly(ethylene glycol)-lipid(PEG-lipids) carrying different lipid tails were synthesized. These PEG-lipids were labeled with fluorescein isothiocyanate (FITC-PEG-lipids) to examine their interaction with cells and to quantitatively determine amounts of PEG-lipids bound on the cell surface. FITC-PEG-lipids spontaneously anchored to the cell membrane within 15 min without loss of cell viability. The type of lipid had very little effect on the anchoring rates, while an increase in the hydrophobicity of the lipid portion of the PEG-lipids slowed their dissociation rates. Densities of FITC-PEG-lipids on the cell surface ranged from 1×10^{-3} to 1×10^{-2} molecules/nm^2, depending on the kinds of lipids employed. The relationship between the stability of the lipids on the cell membrane and the hydrophobicity of the lipid moieties will give a basis for the selection of a hydrophobic moiety in PEG-lipid conjugates for use in specific applications.

1. Introduction

The cell surface is complex in structure and composed of various molecules, including lipids, proteins, and carbohydrates. Cell–cell and cell-extracellular matrices interactions through these molecules play important roles in embryo development and tissue morphogenesis, and also in cell and tissue functions in adults. Previous investigations have modified the cell surface in an effort to control these interactions with biomolecules and to examine their potential in biomedical applications, such as nucleic acid or protein delivery to cells [1,2], cell delivery to a particular organ [3], and tissue engineering to promote the regeneration of tissues [4]. The methods to modify the cell surface can be grouped into three categories: covalent conjugation; electrostatic interaction; and hydrophobic interaction between amphiphilic molecules with the lipid bilayer of the cell membrane [5,6]. Each of these methods has merits and demerits. The covalent bond formation with membrane proteins or with sugar moieties of the membrane proteins should be stable [7,8], but this bond might deteriorate or modify the functions of membrane proteins. In electrostatic interactions [9], polycations can be immobilized on the cell surface by simply adding a solution of the polycation to a cell suspension, because most of the cell surface is negatively charged due to the presence of sialic acids. Most polycations, however, are highly cytotoxic, resulting in cell death. Amphiphilic conjugates have also been examined for cell surface modification [10–12]. The hydrophobic part of the amphiphilic conjugate anchors into the lipid bilayer of the cell membrane through hydrophobic interaction. The hydrophobic interaction between the hydrophobic region of amphiphilic conjugate and the lipid bilayer of the membrane is relatively weak. Although amphiphilic conjugates are not predicted to disturb cell functions, they are released from the cell surface easily.

We have previously employed amphiphilic conjugates, single-stranded DNA-poly(ethylene glycol)-phospholipids (ssDNA-PEG-lipids), to modify the cell surface [6,13,14]. The lipid moiety is efficiently inserted into the lipid bilayer of the cell membrane through the hydrophobic interaction and thus the ssDNA-PEG-lipid, which is immobilized on the cell membrane, can be used for various biomedical applications. Proteins, urokinase, and liposomes containing anticoagulant have been immobilized on islets of Langerhans (islets) to increase their blood compatibility [15,16], and the islets have been micro-encapsulated with other cells [17,18]. Additionally, cells have been immobilized in a site-specific way on two-dimensional patterns using the conjugates [14,19]. We observed that the stability of the ssDNA-PEG-lipid on the cell membrane was highly dependent on the hydrophobicity of lipid moieties. We proposed that the properties of ssDNA-PEG-lipids

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could be adjusted for specific purposes by changing the hydrophobicity of the lipids.

In this study, we synthesized eight kinds of PEG-lipid conjugates carrying fluorescein isothiocyanate (FITC-PEG-lipids) and analyzed their interactions with cells. Our goal was to elucidate the relationship between the hydrophobicity of the lipids and their stability on the cell membrane. Clearer knowledge of this lipid/cell membrane interaction will provide a basis to select the best hydrophobic moiety in ssDNA-PEG-lipids to optimize the use of these conjugates in specific applications.

2. Material and methods

2.1. Materials

Boc-protected-amino-PEG-carbonate-NHS (Boc-PEG-NHS, Mw 5000), methoxy-PEG-carbonate-NHS (MeO-PEG-NHS, Mw 5000), 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine (DMPE), 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine (DPPE), 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine (DSPE), and 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE) were purchased from NOF Corporation (Tokyo, Japan). 1-Tetradecanoyl-*sn*-glycero-3-phosphoethanolamine (lysoPE (m = 12)), 1-hexadecanoyl-*sn*-glycero-3-phosphoethanolamine (lysoPE (m = 14)), and 1-octadecanoyl-*sn*-glycero-3-phosphoethanolamine (lysoPE (m = 16)) were purchased from Avanti Polar Lipids, Inc. (Alabaster, AL, USA). Fluorescein isothiocyanate (FITC) was purchased from Dojindo Laboratories (Kumamoto, Japan). Dichloromethane, chloroform, diethyl ether, toluene, *N,N′*-dimethylformamide (DMF), ethanol, triethylamine (TEA), and tetrahydrofuran (THF) were purchased from Nacalai Tesuque (Kyoto, Japan). 1,6-Diphenyl-1,3,5-hexatriene (DPH), trifluoroacetic acid (TFA), ethylenediamine were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Dulbecco's phosphate buffered saline (PBS) was purchased from Nissui Pharmaceutical, Co., Ltd. (Tokyo, Japan). Cholesteryl chloroformate was obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). All of these reagents were used as obtained.

2.2. Synthesis of PEG-lipids and labeling with FITC

NH_2-PEG-lipids were synthesized as previously reported [20]. Briefly, Boc-PEG-NHS (180 mg, 36 μmol) and each phosphatidylethanolamine (molar ratio = 1.2:1) were dissolved into 3 mL of dehydrated dichloromethane, and TEA (5 μL) was added to the solution. The reaction mixture was stirred overnight at room temperature (RT). Then, TFA (2 mL) was added and stirred for 30 min at 4 °C to remove the Boc group. The reaction mixture was poured into iced diethyl ether to precipitate the crude product. After filtration, the precipitate was dried under reduced pressure for 1 h and then the precipitate was extracted into a round bottom flask with chloroform. After evaporation, the solid was dissolved with 3 mL of benzene and freeze-dried. The NH_2-PEG-lipid was obtained as a white powder.

For FITC labeling, each NH_2-PEG-lipids (80 mg, 13 μmol) and FITC (molar ratio = 1:4) were dissolved into dehydrated DMF (3 mL). The reaction mixture was shielded from light and stirred overnight at RT. The reaction product was collected as a precipitate and purified as described above. The FITC-PEG-lipid was obtained as a yellow powder. The FITC-PEG-lipids were dissolved in PBS and the solution was further fractionated into solutions containing polymeric product or low molecular weight substances by gel filtration using a Sephadex G-25 column. A series of reaction was checked by ^{1}H NMR and MALDI-TOF-MS (Figs. S1–S4).

MeO-PEG-NHS (180 mg, 36 μmol) and each phosphatidylethanolamine (molar ratio = 1.2:1) were dissolved into 3 mL of dehydrated dichloromethane solution and stirred overnight at RT. After precipitation and purification as described above, MeO-PEG-lipid was obtained as a white powder.

2.3. Synthesis of FITC-PEG-cholesterol

N^1-Cholesteryloxycarbonyl-1,2-diaminoethane (NH_2-Cholesterol) was synthesized according to the method reported previously [21]. Briefly, cholesteryl chloroformate (200 mg, 445 μmol) in dichloromethane (20 mL) was added dropwise to 15 mL of ethylenediamine with vigorous stirring at 4 °C. The reaction mixture was stirred at RT for 18 h. After the addition of 100 mL of water, the mixed solution was extracted three times with dichloromethane. The combined organic layers were washed with saturated sodium bicarbonate solution, followed by drying over Na_2SO_4. After evaporation, the resultant residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 4:1, with a small amount of TEA). NH_2-Cholesterol was obtained as a white solid (155 mg, 78%).

FITC-PEG-Cholesterol was synthesized under similar conditions as the synthesis of FITC-PEG-lipids. Boc-PEG-NHS (100 mg, 20 μmol), NH_2-Cholesterol (20 mg, 42 μmol) and TEA (5 μL) were dissolved in dehydrated dichloromethane (5 mL) and stirred for 2 days at RT. After evaporation, TFA (0.5 mL) was added and stirred for 30 min at RT to remove the Boc group. The resulting crude products were reprecipitated in chilled diethylether. The precipitate, NH_2-PEG-Cholesterol, was added to the solution of FITC (5 mg, 13 μmol) in DMF (20 mL) with TEA (5 μL) and stirred for 12 h at RT. After removal of solvent, the residue was purified by gel permeation chromatography (Sephadex G-25) to isolate FITC-PEG-Cholesterol from other small molecules including FITC.

2.4. Cell culture

CCRF-CEM cell line (human acute lymphoblastic leukemia, T-cell), HEK293 cell line (human embryonic kidney), HeLa cell line (human cervical carcinoma), were obtained from the Health Science Research Resources Bank (Osaka, Japan). P3X63-Ag8.653 cell line (mouse myeloma) was obtained from Riken Cell Bank (Tsukuba, Japan). CCRF-CEM cells and P3X63-Ag8.653 cells were suspended and cultured in RPMI1640 medium (Invitrogen) supplemented with 10% fetal bovine serum (FBS; Invitrogen), 100 U/mL penicillin, and 0.1 mg/mL streptomycin (Nacalai Tesuque), at 37 °C and 5% CO_2. HEK293 and HeLa cells were maintained in Eagle's minimum essential medium (Invitrogen) supplemented with 10% FBS, 100 U/mL penicillin, and 0.1 mg/mL streptomycin, at 37 °C and 5% CO_2. HEK293 and HeLa cells were harvested by the addition of a 0.25% trypsin/1 mM EDTA solution (Nacalai Tesque).

2.5. Cell surface modification by FITC-PEG-lipids

Cell suspension (10^6 cells) and FITC-PEG-lipids (100–500 ng) were mixed in PBS (Total volume: 1 mL) and incubated for predetermined times at 37 °C for cell surface modification with FITC-PEG-lipids. The cells were collected by centrifugation at 180 × g for 5 min at 25 °C. The cells were resuspended in 10 mL of PBS and were collected by centrifugation. This washing procedure was repeated twice. After modification with FITC-PEG-lipids, the cells were observed by confocal laser scanning microscopy (FV10i, Olympus, Tokyo, Japan). The fluorescence intensities of modified cells were examined by flow cytometry (Guava EasyCyte mini, Merck Millipore, Billerica, MA, USA).