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Influence of molecular weight of PEG chain on interaction between streptavidin and biotin–PEG-conjugated phospholipids studied with QCM-D

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ABSTRACT

Poly(ethylene glycol)-conjugated phospholipid (PEG-lipid) derivatives spontaneously incorporate into lipid bilayer membranes, thus, they are useful for immobilizing bioactive substances onto cell surfaces. Here, we investigated how the density and molecular weight of PEG molecules influenced immobilization and cellular uptake of a bioactive substance. We analyzed how three biotin–PEG-lipids (1k, 5k, and 40k, with PEG molecular weights: 1 kD, 5 kD, and 40 kD, respectively) interacted with streptavidin on a surface attached to a quartz crystal microbalance with dissipation (QCM-D). We found that the volume excluded by 1k PEG could not effectively prevent adsorption of bovine serum albumin (BSA). In contrast, 5k PEG chains could completely prevent protein adsorption. However, due to strong static repulsion, 40k PEG chains could not be packed at high density. Thus, BSA migrated between PEG chains, and adsorption was not effectively prevented. When streptavidin was added, it bound to multiple neighboring sites on 1k and 5k biotin–PEG-lipids, which reduced chain viscoelasticity. In contrast, streptavidins bound at a one-to-one stoichiometry with the 40k biotin–PEG-lipids, which increased chain viscoelasticity. However, differences in PEG viscoelasticity and PEG molecular weights did not influence cellular uptake of immobilized streptavidin. Therefore, these are not important factors in designing polymers that prevent cellular endocytosis.

Statement of significance

Poly(ethylene glycol)-conjugated phospholipid (PEG-lipid) derivatives have been widely used to modify not only liposome surface, but also the surfaces of cells and pancreatic islets for cell transplantation. Since the entire cell surface can be modified with PEG-lipid through hydrophobic interactions, it is a promising approach for improving graft survival in clinical settings. Although the surface modification is protective in the early stages of transplantation, it is important to understand the factors that influence on the cellular uptake. In this study, we examined the influence of the surface density and molecular weights of PEG-lipids on the cellular uptake by QCM-D and cellular experiments. It was found that the differences in viscoelasticity of PEG chain did not affect on the cellular uptake.

1. Introduction

Poly(ethylene glycol)-conjugated phospholipid (PEG-lipid) derivatives have been widely used to modify the surfaces of liposomes [1–6]. This PEG-lipid surface modification played important roles in reducing opsonization, preventing aggregation, and providing steric hindrance to avoid capture by reticuloendothelial system (RES) cells, which clear liposomes from the body [1,5]. Therefore, modification with PEG-lipids could significantly prolong liposome lifetimes *in vivo*. We previously used PEG-lipid derivatives to modify the surfaces of cells and pancreatic islets for transplantation [7–13]. The entire cell surface can be modified with PEG-lipid derivatives, which spontaneously incorporate into lipid bilayer membranes through hydrophobic interactions. PEG-lipid derivatives can also be used to immobilize various bioactive substances on the cell surface that can regulate coagulation systems and immune reactions. For example, immobilized molecules that deter complement activation [13–16] represent a promising

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approach for improving graft survival after intraportal islet transplantation in clinical settings.

One major problem in clinical islet transplantation for treating type 1 diabetes is that survival rates are lower for transplanted islets than for a transplanted pancreas. Currently, the islet suspension is infused into the liver of the recipient patient through the portal vein. This approach has been accepted as a safe, effective therapy. Islets are carried by the blood stream to the liver, where they become entrapped inside hepatic blood vessels. However, during infusion, the surfaces of transplanted islets are exposed to host blood; this exposure activates host defense mechanisms, including blood coagulation and the complement response, which subsequently induces inflammation [17]. This series of reactions is termed an instant, blood-mediated inflammatory reaction (IBMIR) [18–20]. The IBMIR destroys the donor islets immediately after infusion into the liver. This issue remains unresolved in clinical islet transplantation applications. Therefore, immobilizing bioactive substances onto donor islet surfaces with PEG-lipid derivatives is a promising approach for overcoming these issues [8,9]. For example, we previously showed that islets could be protected by immobilizing fibrinolytic urokinase on the cell surface. Those islets showed improved survival after transplantation into the livers of diabetic mice [14]. Also, when the soluble domain of complement receptor 1 (sCR1) was immobilized on the surface, islets were protected from attack by activated complement factors [15]. Thus, we previously demonstrated that local protection with bioactive substance immobilization could potentially improve graft survival.

However, immobilized bioactive substances are eventually taken up by cells through endocytosis. Although we know that bioactive substances are protective in the early stages of transplantation, it is important to understand the factors that influence their uptake. We reasoned that the uptake of immobilized substances might be influenced by the surface density and molecular weights of PEG-lipids. However, it is difficult to analyze cell surface events in real time.

In this study, we analyzed the interactions between PEG-lipid derivatives and bioactive proteins with a quartz crystal microbalance with dissipation (QCM-D). This instrument can detect interactions in real time without the need for labeling. Here, we examined the interaction between different biotin–PEG-lipids and streptavidin, a protein with four biotin binding sites. We used streptavidin as a model for bioactive substances. The reaction of streptavidin and biotin has been used for investigation of lysis reaction in complement activation on cell membrane and cellular internalization because streptavidin is multivalent and the valence can be easily modulated by addition of biotin [21,22]. We synthesized three different biotin–PEG-lipids, with 1k (1 kD), 5k (5 kD), and 40k (40 kD) PEG chains. These biotin–PEG-lipids were incorporated into a self-assembled monolayer (SAM) surface, which served as a model lipid bilayer membrane. Then, after blocking non-specific binding sites, streptavidin was added to bind the biotin moieties. We measured the change in mass (Δf) and the dissipation factor (ΔD) to determine how different PEG chain molecular weights might influence these interactions. In addition, living cells were modified with biotin-pretreated streptavidin, which retained only one site for binding to the biotin–PEG-lipids. Then, we modified cultured cells with the same PEG lipids and analyzed them with confocal laser scanning microscopy to determine the influence of PEG chain length on streptavidin uptake.

2. Materials and methods

2.1. Materials

We purchased 1,2-dipalmitoyl-*sn*-glycerol-3-phosphatidylethanolamine (DPPE) from NOF Corporation (Tokyo, Japan). The three polyethylene glycol (PEG) moieties were: α-3-[(3-maleimido-1-oxopropyl)aminopropyl-ω-(succinimidyloxy carboxy)] (NHS-PEG (40k)-Mal, MW: 40,000 Da) and (*N*-hydroxysuccinimide 5-pentanoate) ether 2-(biotinylamino)ethane (biotin–PEG (5k)-NHS, MW: 5000 Da), both purchased from NOF Corporation, and succinimidyl-[(*N*-maleimidopropionamido)-tetracosaethylene glycol] ester (NHS-PEG (1k)-Mal, MW: 1000 Da), purchased from Thermo Scientific (Rockford, IL, USA). The protein desalting spin column was also purchased from Thermo Scientific. Diethylether, dichloromethane, chloroform, methanol, ethanol, triethylamine, and D-biotin were purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). Dulbecco's phosphate-buffered saline (PBS; pH 7.4), RPMI-1640 medium, fetal bovine serum (FBS), penicillin, streptomycin, and trypan blue solution were purchased from Invitrogen Co. (Carlsbad, CA, USA). Glass-bottom culture dishes were purchased from MatTek Co. (Ashland, MA, USA). Bovine serum albumin (BSA), 1-dodecanethiol, and chloroform-d were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). Biotin-glycine-cysteine (biotin-GC) was synthesized by BEX Co., Ltd (Tokyo, Japan). Streptavidin was purchased from Nacalai Tesque (Kyoto, Japan). Alexa Fluor488 streptavidin (Alexa488-streptavidin) was purchased from Life Technologies Co. (Carlsbad, CA, USA).

2.2. Synthesis of biotin–PEG (1k, 5k, 40k)–DPPEs

Biotin–PEG(5k)-NHS (180 mg) was combined with DPPE (20 mg), triethylamine (50 μL), and dichloromethane (4 mL), and stirred for 48 h at room temperature (RT). Precipitation with diethyl ether yielded biotin–PEG(5k)–DPPE as a white powder (165 mg; 80% yield). Biotin–PEG(5k)–DPPE was used for subsequent experiments without further purification.

Two sizes of Mal-PEG(1k, 40k)-NHS (50, 100 mg) were combined with DPPE (33 and 1.7 mg, respectively), triethylamine (50 μL), and dichloromethane (4 mL). These solutions were stirred for 4 days at 40 °C and for 7 days at room temperature (RT), respectively. Precipitations with diethyl ether yielded Mal-PEG(1k, 40k)–DPPE as pink and white powders, respectively (58 mg; 70% yield and 92 mg; 90% yield, respectively).

Then, Mal-PEG(40k)–DPPE (27 mg) was mixed with biotin-GC (0.5 mg) in PBS (1.4 mL) and incubated at 40 °C for 24 h. The mixture was purified in a spin column equilibrated with PBS to produce biotin–PEG(40k)–DPPE.

Mal-PEG(1k)–DPPE (3.2 mg) was mixed with biotin-GC (1 mg) in methanol (1.4 mL) and incubated at RT for 24 h. The mixture was purified in a spin column equilibrated with PBS to produce biotin–PEG(1k)–DPPE.

^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): Biotin–PEG (5k)–DPPE: ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): 0.88 (t, 6H, —CH_3), 1.25 (br, 48H, —CH_2—, DPPE), 2.31 (q, 4H, —O—C (=O)—CH_2—C, DPPE), 3.65 (br, 500H, PEG), 4.14 (m, 2H, C—CH (—C)—N, biotin). Mal-PEG (1k)–DPPE: 0.86 (t, 6H, —CH_3), 1.24 (br, 48H, —CH_2—, DPPE), 2.30 (q, 4H, O—C (=O)—CH_2—C, DPPE), 3.63 (br, 87H, PEG), 6.71 (s, 1.8H, —HC=CH—, maleimide). Mal-PEG (40k)–DPPE: 0.85 (t, 6H, —CH_3), 1.22 (br, 48H, —CH_2—, DPPE), 2.30 (q, 4H, O—C (=O)—CH_2—C, DPPE), 3.62 (br, 4470H, PEG), 6.71 (s, 1.8H, —HC=CH—, maleimide).

2.3. Analysis of interactions between streptavidin and biotin–PEG–DPPEs

QCM-D measurements were performed with a Q-senseE4 (Biolin Scientific AB, Stockholm, Sweden). This instrument simultaneously monitors the shifts in oscillator frequency (Δf) and energy dissipation (ΔD) on a 4.95 MHz, gold-coated, QCM-D sensor, at different overtones [23]. The oscillator frequency decreases when any substance is adsorbed on the sensor surface. Thus, the amount of adsorbed material on a given surface can be measured based on