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Screening effect of PEG on avidin binding to liposome surface receptors☆

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Abstract

This study investigates the screening effect of poly(ethylene glycol)-phospholipids (PE-PEG) on the interaction of avidin with PEGylated liposomes containing surface-bound biotin ligands. The influence of grafting density and lipopolymer chain length is examined. A simple fluorescence assay involving a receptor-mediated fluorescence increase of BODIPY-labeled avidin upon binding to biotinylated lipids is employed to study the screening effect of submicellar concentrations of 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylethanolamine-*N*-[poly(ethylene glycol)-2000] (PE-PEG^{2000}) and 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylethanolamine-*N*-[poly(ethylene glycol)-5000] (PE-PEG^{5000}) incorporated into 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine (DPPC) liposomes. The results show that incorporation of lipopolymers into DPPC lipid bilayers reduces binding of avidin to the biotinylated liposomes, and it is found that the screening effect of PE-PEG^{5000} is stronger than that for PE-PEG^{2000}. Thus, the results reveal that both the grafting density and the polymer length of the PE-PEG lipopolymers are of importance for the ability of water-soluble macromolecules to reach the surface of PEG liposomes. Furthermore, it is found that none of the lipopolymers completely prevents avidin from reaching the surface-bound biotin ligands.

Keywords: PEG liposomes; Steric stabilization; Receptor–ligand interaction; Lipopolymer; Drug targeting; Avidin; Biotin

In order for liposomes to be suitable as drug carriers, a long circulation time in the blood stream is required (Lasic and Martin, 1995; Lasic and Needham, 1995). Attachment of the flexible and water-soluble poly(ethylene glycol) (PEG) polymer to the liposome surface increases the blood circulation time significantly, and it is believed that PEG functions as a steric barrier that prevents proteins of the immune system (opsonins) from adsorbing onto the liposome surface (Blume and Cevc, 1990; Chonn et al., 1992). This hypothesis has been supported by experimental findings showing a decrease in the amount of

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proteins that associate with lipid bilayer surfaces when PEG lipopolymers are incorporated (Torchilin et al., 1994; Bedu-Addo et al., 1996; Noppl-Simson and Needham, 1996; Du et al., 1997). However, a further rational improvement of liposomal drug delivery formulations requires a deeper insight into the mechanisms involved in the screening effect of PEG towards macromolecules such as proteins and liposome degrading enzymes (Jørgensen et al., 1999a,b).

In this study, the ability of a 66 kDa receptor protein, avidin, to reach the surface of PEG-covered liposomes and subsequently to bind to surface-bound biotin ligands is examined. In order to quantify the amount of avidin that associates with the liposome surface ligands, avidin was labeled with the fluorescent probe BODIPY Fl (Molecular Probes, Inc., Eugene, OR). The fluorescence of this probe increases significantly upon biotin binding (Emans et al., 1995), and by measuring the fluorescence when avidin-BODIPY Fl is titrated with biotinylated liposomes the avidin binding can be followed. In relation to this experimental assay, it should be pointed out that avidin binds to biotin essentially irreversibly (dissociation constant, $K_d \approx 10^{-15}$; Pugliese et al., 1993). Consequently, it is possible to separately study how PEG affects the rate of association without having to take the dissociation into account.

Appropriate amounts of 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine (DPPC), 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylethanolamine-*N*-[poly(ethylene glycol)-2000] (PE-PEG^{2000}), 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylethanolamine-*N*-[poly(ethylene glycol)-5000] (PE-PEG^{5000}), and biotin-X-dihexadecanoyl-phosphatidylethanolamine (biotin-X-DHPE) stock solutions in $CHCl_3$:CH_3OH 1:1 (v/v) were mixed in a test tube and the solvent was evaporated. The lipids were from Avanti Polar Lipids, Inc. (Birmingham, AL). Biotin-X-DHPE was from Molecular Probes, Inc. The dry lipid films were suspended in 10 mM HEPES buffer (pH 7.5) containing 50 mM KCl and 1 mM NaN_3, and kept at 51°C for at least 90 min. During this period, they were vigorously shaken every 15 min. Subsequently, in order to create unilamellar liposomes, the lipid suspensions were extruded ten times through two stacked 0.1 μm polycarbonate filters at an extrusion pressure of 20 bar (Hope et al., 1985).

Fluorescence measurements were carried out using an SLM 8100 spectrofluorometer (SLM-Aminco, Rochester, NY). Two milliliters of avidin-BODIPY Fl (Molecular Probes) (10 μg/ml) were titrated with 2 mM DPPC liposomes containing 0.5 mol% biotin-X-DHPE and various amounts of PE-PEG^{2000} and PE-PEG^{5000}. Ten microliters of the liposome suspensions were added after 300, 800, and 1300 s. BODIPY Fl was excited at 505 nm and emission recorded at 512 nm. The temperature in the cuvette was maintained at 24.5°C. The fluorescence was recorded for a total of 1800 s and the data points shown in Fig. 1 were evaluated as the average intensity of the 1700–1800 s interval. Moreover, the fluorescence intensities were normalized by dividing all values by the initial intensity.

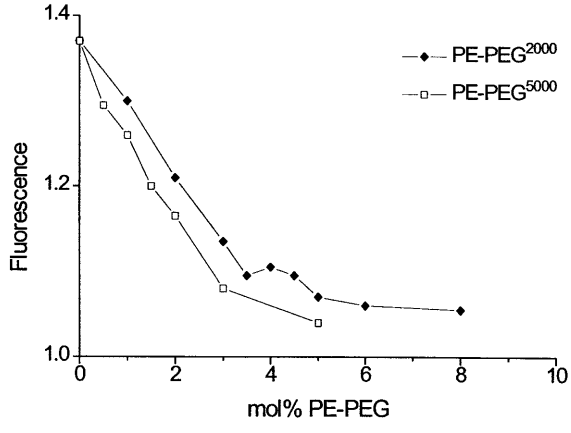

Fig. 1. Fluorescence of avidin-BODIPY Fl after the addition of biotinylated liposomes containing various amounts of PE-PEG^{2000} and PE-PEG^{5000}. High fluorescence intensities correspond to large amounts of bound avidin. It is observed that both PE-PEG^{2000} and PE-PEG^{5000} diminish the fluorescence corresponding to a lower amount of avidin bound to the biotin ligands. For equal concentrations of the PE-PEG lipopolymers, PE-PEG^{5000} provides a slightly better screening effect than does PE-PEG^{2000}. Even at the largest grafting densities, none of the lipopolymers completely inhibit binding, as indicated by the fact that the normalized fluorescence intensities remain greater than 1.

The increase in avidin-BODIPY Fl fluorescence upon biotin binding was utilized to monitor binding of avidin to biotinylated liposomes containing various amounts of PE-PEG. Fig. 1 shows the increase in fluorescence that was observed for liposomes containing different amounts of PE-PEG^{2000} and PE-PEG^{5000}. For both lipopolymers, a distinct decrease in avidin binding is observed when the lipopolymer is incorporated, and it is noted that PE-PEG^{5000} offers a better surface protection than PE-PEG^{2000} when the polymers are present in equal concentrations. Interestingly, for PE-PEG^{2000}, the effect from increasing the polymer concentration seems to diminish at concentrations larger than 3.5% PE-PEG^{2000}, indicating that the screening effect of each polymer chain is larger at low grafting densities. This observation is in good agreement with theory, which predicts that PEG^{2000} polymers undergo a mushroom to brush conformational transition at 3.5–4 mol% PE-PEG^{2000} (Kenworthy et al., 1995; Baekmark et al., 1997). Thus, the results imply that, when the liposome surface is completely covered with PEG^{2000} in the mushroom conformation, only a slight improvement of the screening effect can be achieved for higher lipopolymer concentrations. In contrast, the data for PE-PEG^{5000} shows no indication of the mushroom to brush transition, which is predicted to take place at a lipopolymer concentration of approximately 1.5 mol% PE-PEG^{5000} (Kenworthy et al., 1995). Finally, it should be noticed that neither PE-PEG^{2000} nor PE-PEG^{5000} is capable of completely blocking the approach of avidin to the liposome surface. This is an important result in relation to receptor-based targeting of drug-delivery liposomes, as it shows that ligands are not completely shielded by PEG.

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References

Baekmark, T.R., Pedersen, S., Jørgensen, K., Mouritsen, O.G., 1997. The effects of ethylene oxide containing lipopolymers and tri-block copolymers on lipid bilayers of dipalmitoylphosphatidylcholine. Biophys. J. 73, 1479–1491.

Bedu-Addo, F.K., Tang, P., Xu, Y., Huang, L., 1996. Interaction of polyethyleneglycol–phospholipid conjugates with cholesterol–phosphatidylcholine mixtures: sterically stabilized liposome formulations. Pharm. Res. 13, 718–724.

Blume, G., Cevc, G., 1990. Liposomes for the sustained drug release in vivo. Biochim. Biophys. Acta 1029, 91–97.

Chonn, A., Semple, S.C., Cullis, P.R., 1992. Association of blood proteins with large unilamellar liposomes in vivo. Relation to circulation lifetimes. J. Biol. Chem. 267, 18759–18765.

Du, H., Chandaroy, P., Hui, S.W., 1997. Grafted poly-(ethylene glycol) on lipid surfaces inhibits protein adsorption and cell adhesion. Biochim. Biophys. Acta 1326, 29–36.

Emans, N., Biwersi, J., Verkman, A.S., 1995. Imaging of endosome fusion in BHK fibroblasts based on a novel fluorimetric avidin–biotin binding assay. Biophys J. 69, 716–728.

Hope, M.J., Bally, M.B., Webb, G., Cullis, P.R., 1985. Production of large unilamellar vesicles by a rapid extrusion procedure. Characterization of size distribution, trapped volume and ability to maintain a membrane potential. Biochim. Biophys. Acta 812, 55–65.

Jørgensen, K., Kiebler, T., Hylander, I., Vermehren, C., 1999a. Interaction of a lipid membrane destabilizing enzyme with PEG-liposomes. Int. J. Pharm. 183, 21–24.

Jørgensen, K., Vermehren, C., Mouritsen, O.G., 1999b. Enhancement of phospholipase A_2 catalysed degradation of polymer grafted PEG-liposomes: effects of lipopolymer-concentration and chain length. Pharm. Res. 16, 1491–1493.

Kenworthy, A.K., Hristova, K., Needham, D., McIntosh, T.J., 1995. Range and magnitude of the steric pressure between bilayers containing phospholipids with covalently attached poly(ethylene glycol). Biophys. J. 68, 1921–1936.

Lasic, D.D., Martin, F., 1995. Stealth Liposomes. CRC Press, Boca Raton, FL.

Lasic, D.D., Needham, D., 1995. The 'Stealth' liposome: a prototypical biomaterial. Chem. Rev. 95, 2601–2628.

Noppl-Simson, D.A., Needham, D., 1996. Avidin–biotin interactions at vesicle surfaces: adsorption and binding, cross-bridge formation and lateral interactions. Biophys. J. 70, 1391–1401.

Pugliese, L., Coda, A., Malcovati, M., Bolognesi, M., 1993. Three-dimensional structure of the tetragonal crystal form of egg-white avidin in its functional complex with biotin at 2.7 Å resolution. J. Mol. Biol. 231, 698–710.

Torchilin, V.P., Omelyanenko, V.G., Paisov, M.I., Bogdanov, A.A., Jr, Trubetskoy, V.S., Herron, J.N., Gentry, C.A., 1994. Poly(ethylene glycol) on the liposome surface: on the mechanism of polymer-coated liposome longevity. Biochim. Biophys. Acta 1195, 11–20.