Controlled Detachment of Immobilized Liposomes on Polymer Gel Support

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Immobilization of small liposomes on polymer gel beads and controlled detachment of the liposomes from the support was described. Liposomes were immobilized on the gel with hydrophobic anchors that contained disulfide linkage. The liposomes were then detached by reductive cleavage of the linkage with dithiothreitol. The major part of the fluorescein-conjugated dextran encapsulated in the liposomes was retained throughout the immobilization-detachment process.

Liposomes, vesicles of lipid bilayer membrane, are frameworks for various novel chemical systems. For instance, they have been studied as sophisticated drug carriers or used as biomembrane models in researches of biochemically important molecules such as proteins. Even more sophisticated chemical systems which have their bases on liposomes have been proposed.[1]

Previously, immobilization of small liposomes (typically 20–200 nm) on polymer gel beads of a larger size (approximately 100 μm) was studied with its use as chromatographic materials in scope.[2–4] Naturally, in those studies, the immobilization of liposomes was intended to be permanent.

In the present study, we tried to develop an immobilization system that can release the immobilized liposomes when it is desired. For such "detachable" immobilization, polymer gel beads of 40–100 μm that bore hydrophobic alkyl moieties via disulfide linkages were examined as the support material. With the hydrophobic moieties as the anchor to the bilayer membrane, liposomes are expected to be immobilized on the gel.[2] By reductive cleavage of the disulfide linkages, the immobilized liposomes may be released from the support (Figure 1). Such a system, for example, should benefit construction of liposome-based chemical systems which frequently involves separation of small liposomes from the bulk aqueous phase. Gel filtration and ultracentrifugation have been most frequently used in such separation although those conventional methods require significant time and cost.

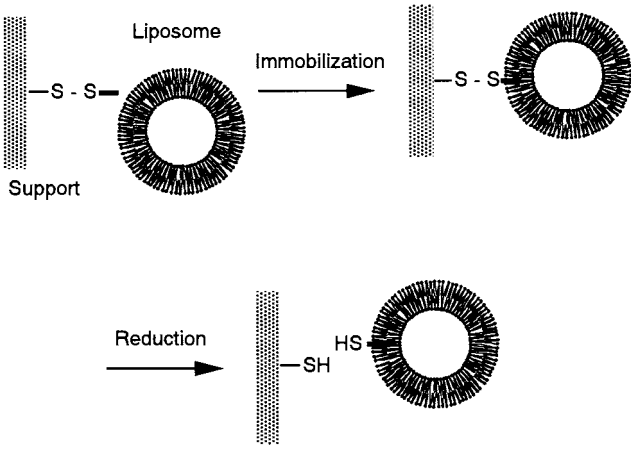

Figure 1. Immobilization and detachment of liposomes on a support with cleavable hydrophobic anchors. Immobilized liposomes are liberated by reduction of disulfide linkage.

The polymer gel beads for the immobilization of liposomes were prepared as shown in Figure 2. Thiol moieties were introduced to Sephacryl S-1000 gel (Pharmacia Biotech, Uppsala, Sweden) by following the procedure previously described for Sepharose 6B.[5] Typically, S-1000 gel (3 g, swelled in water) was treated with a mixture of the epoxide **1** (4.5 mL) and sodium borohydride (14 mg) dissolved in a 0.6 M aqueous sodium hydroxide (5 mL) to react one end of **1** with the hydroxyl group on the gel. Then the epoxy moieties on the gel were converted to Bunte salts by reaction with sodium thiosulfate (1 M, 9 mL). Reduction of the resulting gel with excess dithiothreitol yielded a gel bearing thiol moieties. The amount of the thiol moieties on the prepared thiol gel was determined using the method described by Grassetti and the coworker.[6] Up to 79 μmol of the thiol moieties was found per gram of the dried gel. The thiol gel was further treated with 2,2'-dipyridyl disulfide to protect the thiol moieties as pyridyldithio groups from uncontrolled oxidation during the storage.

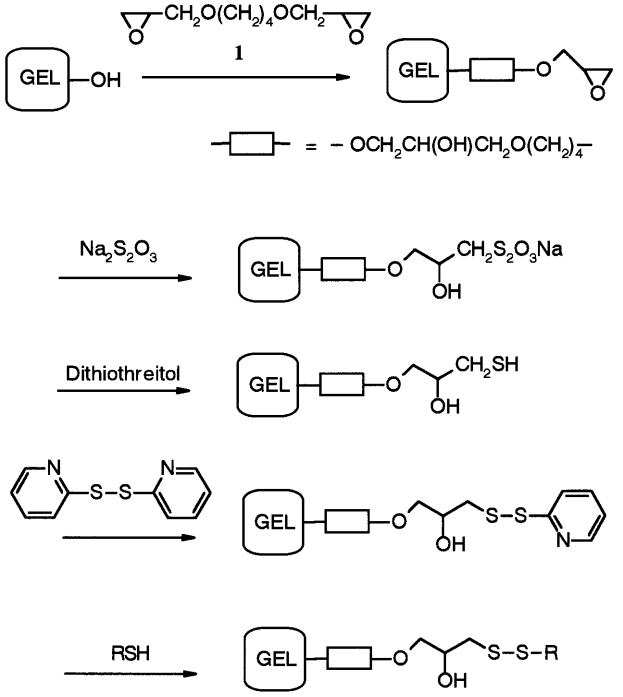

Figure 2. Modification of Sephacryl S-1000 gel.

The hydrophobic anchors were introduced to the gel by replacing the pyridylthio groups with alkylthio groups. In a typical procedure, the pyridylthio-protected gel was treated with an alkanethiol dissolved in 30% aqueous ethanol at 5 °C for 72 h. The reaction released 2-pyridinethiol, which existed exclusively as its tautomer 2-pyridinethione and showed absorption at 343 nm.[6] The amount of the alkyl moieties on the gel was estimated based on the absorbance. The reaction with 1-octanethiol yielded C_8 gel, which contained 47 μmol of octyl moieties per gram of the dried gel. With 1-hexadecanethiol, C_{16} gel with 22 μmol/g of the hexadecyl moieties was obtained.

The alkylthio gel thus prepared was interacted with liposomes. Liposomes were prepared from purified phosphatidylcholines of egg yolk (eggPC; from Avanti Polar-Lipid, Alabaster, AL, U.S.A.) in 0.1 M HEPES buffer (pH 7.5) by the extrusion procedure.[7] Upon coincubation of the liposome suspension (lipid concentration 5 mM, 3 mL) with the C_8 gel (containing 1.9 μmol of the octyl moieties) at 37 °C, the phospholipid started to disappear from the bulk aqueous phase, and the corresponding amount of the phospholipid was found on the gel. The adsorption reached saturation in 24 h, and the amount of the phospholipid adsorbed on the gel was 4.6 μmol, corresponding to 31% of the liposomal lipid present in the system (Table 1). With a larger amount of the gel, more liposomal lipid was adsorbed on the gel. When the amount of C_8 gel was increased three times, 62% of the lipid in the system was found on the gel. Meanwhile the ratio of the adsorbed lipid molecules to the octyl moieties decreased from 2.46 to 1.65. No spontaneous release of the adsorbed phospholipid from the gel was detected when the lipid-bearing C_8 gel was incubated in the buffer at 37 °C for 24 h.

Table 1. Adsorption of liposomal phospholipid to modified Sephacryl S-1000 gel[a]

Gel	Lipid used per alkyl moiety	Immobilized Lipid / %	Lipid immobilized per alkyl moiety
C_8	8	31	2.46
	4	56	2.25
	2.7	62	1.65
C_{16}	8	33	2.63

[a]Lipid concentration 5 mM; 37 °C after 24 h.

When the pyridylthio-protected gel or the free-thiol gel was used in place of the C_8 gel, less than 1% of the phospholipid was attached to the gel under comparable conditions. This result is consistent with immobilization of the liposomes by the alkyl moieties anchoring to the liposomal membrane. The adsorption of the phospholipid to the C_{16} gel was also examined. However, no significant difference from the C_8 gel was observed in the amount of the immobilized phospholipid.

The adsorption process was also examined using liposomes in which fluorescein-conjugated dextran (FITC-dextran; approximate MW of 45,000; obtained from Sigma, St. Louis, MO, U.S.A.) was encapsulated. After the adsorption, at least 70% of the FITC-dextran was remained encapsulated, indicating that the majority of the immobilized liposomes kept their structural integrity intact throughout the adsorption.

In the next step, detachment of the immobilized liposomes from the gel was tested. The C_8 gel that bore the liposomes was treated with an excess amount of dithiothreitol (10 times to the alkylthio moieties). Upon the treatment, phospholipid appeared in the aqueous bulk phase, indicating the release of the adsorbed liposomal lipid from the gel. After 20 h, 58% of the immobilized lipid was released (Table 2). Approximately 90% of the release occurred within 5 h. In the experiment using the liposomes encapsulating FITC-dextran, at least 65% of the FITC-dextran released from the gel by the dithiothreitol treatment was found encapsulated in the liposomes. The result indicates that the major part of the liposomes maintained their structural integrity also in the detachment process.

Table 2. Release of immobilized liposomal phospholipid from modified Sephacryl S-1000 gel[a]

Gel	Immobilized lipid / μmol	Dithiothreitol per alkyl moiety	Lipid released / %
C_8	9.28	4	15
		20	58
C_{16}	3.29	4	23
		20	52

[a]37 °C after 20 hr.

Further treatment of the gel with fresh dithiothreitol additionally released only 2% of the phospholipid. Apparently, the liposomes remained on the gel was immobilized in a manner different from the released liposomes and could not be detached by dithiothreitol.

The present study demonstrated immobilization of small liposomes on a larger polymer gel support and the following controlled detachment of the immobilized liposomes. The procedure may also applicable to other small membrane vesicles, for instance, obtained from biological cells. Our study concerning further improvement of the immobilization and the detachment processes including possible usage of other types of the linkage, is presently ongoing.

References and Notes

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