

Immobilization of Liposomes on Hydrophobically Modified Polymer Gel Particles in Batch Mode Interaction

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Abstract

Immobilization of liposomal phospholipids onto Sephacryl S-1000 gels that were chemically conjugated with hydrophobic alkyl moieties, octyl, dodecyl and hexadecyl, was examined in batch mode interaction. Compared with the octyl gel, the dodecyl and the hexadecyl gels were found to immobilize the 3-4 times more phospholipids with the less hydrophobic moieties. The encapsulation of a water-soluble marker, with other evidences, suggests that the majority of the immobilized phospholipids maintained liposomal morphology. As the lipid of the interacting liposomes, egg yolk phosphatidylcholine (eggPC), 1,2-dimyristoylphosphatidylcholine (DMPC) and a mixture of DMPC and 1,2-dimyristamido-1,2-deoxyphosphatidylcholine were examined. At 22 °C, DMPC liposomes showed higher extent of immobilization than at 37 °C but not eggPC liposomes, suggesting that the phase of liposomal membrane could have influence on the immobilization. Exchange between the immobilized liposomes and free ones was found be small less than 3%. The gel that had been first interacted with liposomes to apparent saturation could further immobilize the newly added liposomes. The rate of this second immobilization was similar to that of the slow adsorption process; the both could be based on the same mechanism, possibly involving rearrangement of the immobilized liposomes on the gel as proposed by Lundahl. As had been observed in the flow mode, the immobilization had preference for smaller liposomes. In application of the system in batch mode, the size distributions of the immobilized liposomes and of those left in the supernatant may differ from that of the originally added liposomes.

Keywords: Liposome immobilization; Cross-linked polymer gel; Hydrophobic anchor; Batch mode interaction; Lipid adsorption.

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Acknowledgements

References

1. Introduction

Liposomes [1,2] have been an indispensable lipid bilayer membrane model and widely used in various studies that relate to biomembranes. A typical preparation of liposomes [2] is obtained as a colloidal suspension in an aqueous medium. However, on occasion, it is desirable to have liposomes not freely suspended. For example, in a study of an intermembrane phenomenon, one would frequently encounter a need to separate two different types of liposomes or distinguish liposomes from similar membrane vesicles. In some cases, ultracentrifugation or other separation methods may be applicable. However, those procedures are usually expensive and/or time-consuming. A more convenient method to achieve the separation is desirable.

We have been working on a study of relocation of cell membrane proteins from biological cells to liposomes [3]. The study involves interaction of liposomes with proteovesicles, which quite resemble the interacting liposomes in the size and other physicochemical characteristics that could be used for the separation of the two. To make the fast and convenient separation of the two types of the vesicles possible, we started to consider immobilizing one of the vesicles on an appropriate substrate of a larger size. The immobilized vesicles should be easily separated from the rest, possibly by simple filtration.

Liposome chromatography developed by Lundahl et al. [4,5] then came to our attention. There, to utilize liposomal bilayer membrane as a chromatographic stationary phase, Yang et al. [6] immobilized liposomes on cross-linked polymer gel beads that were hydrophobically modified with octyl moieties. The validity of the system has been demonstrated in their various chromatographic applications [4-6]. The system seemed to possibly fulfill our needs. And further, we see more possibilities in the system as a versatile tool of adsorption or immobilization of liposomes and lipid aggregates. This "liposome adsorbent" or "liposome-gel conjugate" may be useful in various applications including ours but not limited to chromatography.

As an adsorbent or a conjugate, the system would be used often in a batch mode. However, since the major interest of Lundahl and the coworkers is in its use for the chromatographic application, their study of the behavior of the system was confined in a flow system with column-packed liposome-immobilizing gel particles. Although some of the characteristics determined with the flow system may also be applicable, direct examination of the behavior of the immobilization system in a batch mode is essential to use the system as a versatile liposome adsorbent or liposome-gel conjugate.

This article describes the immobilization behavior in a batch mode of the hydrophobically modified Sephacryl S-1000 system originally studied in a flow mode by Yang et al. [6]. We extended the study to include preparation and characterization of two new hydrophobically modified Sephacryl S-1000 gels that bear longer alkyl moieties. Further, effect of lipid composition and phase on the immobilization and behavior of the resulting liposome-gel conjugate in further interaction with fresh liposomes were also investigated.

2. Experimental

2.1. Materials

Phosphatidylcholine extracted and purified from egg yolk (eggPC) was obtained from NOF Corporation (Tokyo, Japan). 4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES) and 1,2-dimyristamido-1,2-deoxyphosphatidylcholine was supplied by DOJINDO Laboratories (Kumamoto, Japan). Fluorescein isocyanate-conjugated dextran (FITC-dextran; average molecular weight 40,500), 1,2-dimyristoylphosphatidylcholine and 1,4-butanediol diglycidyl ether were products of Sigma-Aldrich (St. Louis, MI). Sodium dodecyl sulfate (SDS) and Clear-sol I were products of Nakalai Tesque (Kyoto, Japan). Sephacryl S-1000HR and [1-¹⁴C]cholesteryl oleate were obtained from Amersham Biosciences (Piscataway, NJ). 1-Octanethiol, 1-dodecanethiol, 1-hexadecanethiol, sodium borohydride and Phospholipid C-Test Wako were products of Wako Pure Chemicals (Osaka, Japan).

2.2. Chemical modification of Sephacryl S-1000HR gel

Sephacryl S-1000HR gel was chemically modified in accordance with the procedure published by Yang et al. [6]. For the introduction of octyl moieties to the gel, approximately 70 ml (wet bed volume) of swelled Sephacryl S-1000HR was washed using 2 liters each of 25% aqueous ethanol, 50% aqueous ethanol and Milli-Q grade water in this sequence, and the bulk water was removed by suction. The washed gel thus obtained was treated with a mixture of 50 g (250 mmol) of 1,4-butanediol diglycidyl ether and 140 mg (3.7 mmol) of sodium borohydride dissolved in 50 ml of a 0.6M aqueous sodium hydroxide solution. The gel was then reacted with 3.4 g (23 mmol) of 1-octanethiol mixed with 50 ml of ethanol in a solution of 750 mg (20 mmol) sodium borohydride in 50 ml of 1M aqueous sodium hydroxide. The modified gel thus obtained was stored in a 1 mg/ml aqueous sodium azide solution at 4 °C. For gels bearing dodecyl or hexadecyl moieties, 3.4 g (17 mmol) of 1-dodecanethiol or 4.6g (18 mmol) of 1-hexadecanethiol was used in place of 1-octanethiol. The carbon and the sulfur contained in the modified gels were assayed by the Center for Elemental Analyses in Kyoto University.

2.3. Preparation of liposomes

Liposomes prepared by the extrusion procedure [7] were used throughout this study unless otherwise noted. Typically, 100 mg of phosphatidylcholine was dissolved in 7 ml of chloroform in a round bottom flask. Then a thin lipid film was formed on the flask wall by gently removing the organic solvent under reduced pressure with a rotary evaporator, and the film was further dried in a vacuum chamber overnight. The film was swelled in 7 ml of a 10 mM HEPES buffer (pH 7.4; containing 150 mM sodium chloride), and the swelled lipid was removed from the flask wall and suspended by gently swirling with small glass beads. The crude liposome suspension thus obtained was extruded (Lipex Extruder, Vancouver, British Columbia, Canada) through a series of polycarbonate membrane filters (5 times each through 1000, 600 and 400 nm pores and 10 times each through 200 and 100 nm in this order). The phospholipid concentration of the liposome suspension thus obtained was determined and adjusted to 5 mM by diluting the HEPES buffer. Throughout the present study, phospholipids were assayed using a kit (Phospholipid C-Test Wako) and a spectrophotometer (Hitachi 650-10S, Tokyo, Japan). The average diameter of liposomes was determined by dynamic light scattering measurement (Photal DLS-700, Osaka, Japan).

To obtain liposomes encapsulating FITC-dextran, FITC-dextran was dissolved in the HEPES buffer (FITC-dextran concentration 10 mg/ml) and was used as the swelling solution in the preparation. The FITC-dextran that was not encapsulated during the preparation was removed by gel filtration through a Sepharose 4B column (diameter 18 mm and length 400 mm; eluted with the HEPES buffer). The amount of FITC-dextran was estimated based on the fluorescence intensity of a sample measured by a fluorospectrophotometer (excitation at 495 nm and emission at 520 nm; Hitachi U-3400, Tokyo, Japan). In the measurement, liposomes or phospholipid-gel conjugates was treated with 1.0% aqueous sodium dodecyl sulfate. Typical efficiency of the FITC-dextran encapsulation was 2-3 %.

2.4. Interaction of liposomes with hydrophobically modified gels

Typically, 4.0 ml of liposome suspension (phospholipid concentration 5.0 mM) was mixed with 0.50 ml (wet bed volume) of hydrophobized Sephacryl S-1000HR gel, and the mixture was incubated at 37 °C with gentle shaking. For the assay of the phospholipids, 0.060 ml of the

liposome-gel suspension was taken, and the gel was separated from the supernatant by passing through a small column with a cotton filter. The column was washed with 5 ml of the HEPES buffer, and the washing was combined with the filtrate, and the phospholipid content in the supernatant was determined. Then, to remove phospholipids adsorbed onto the gel, the column was eluted with 3 ml of a buffer that came with the phospholipids assay kit and containing a detergent. The resulting elute was used in the assay of the phospholipids adsorbed on the gel.

2.5. Interaction of liposome-immobilizing gels with fresh liposomes

Radio-labeled liposomes were prepared from a mixture of 35 mg DMPC and approximately 100kBq of [1-¹⁴C]cholesteryl oleate. To 5.0 ml of the radio-labeled liposomes (phospholipid concentration 5.0 mM) was added 0.50 ml (wet bed volume) of octyl-gel, and the mixture was incubated with gentle shaking at 37 °C for 5 days. The gel was separated and washed three times with 7 ml each of the HEPES buffer using centrifugation (400 x g). The liposome-gel conjugate thus obtained was coincubated with 4.0 ml of non-labeled DMPC liposomes (phospholipid concentration 5.0 mM) at 37 °C. During the coincubation, 0.200 ml of the gel suspension was occasionally taken, and the supernatant was separated from the gel particles by passing through a 450 nm membrane filter. To 0.100 ml of the supernatant thus obtained was added 10.0 ml of Clear-sol I, and the radioactivity in the mixture was determined using a liquid scintillation counter (Aloka LSC-1000, Tokyo, Japan). The rest of the supernatant was used in the phospholipid assay.

2.6. Interaction of hydrophobically modified gels with liposomes of different sizes

For preparation of small liposomes, the crude liposome suspension obtained as described above was treated with a probe-type ultrasonic irradiator (Tomy Seiko UR-200, Tokyo, Japan). The irradiated sample was centrifuged (32,000 x g, 4 °C) for 13 hours, and extruded through polycarbonate membrane filter 10 times (pore size 50 nm). The phospholipid concentration was determined and adjusted to 5.0 mM by diluting with the HEPES buffer. The average diameter of the small liposomes was determined by dynamic light scattering measurement.

In a typical interaction experiment, 6.0 ml of liposome suspension (phospholipid concentration 5.0 mM) was coincubated with 0.50 ml (wet bed volume) of octyl-gel at 37 °C. The

gel was then separated and washed with the buffer three times using centrifugation (2,000 x g 10 min), and further incubated with 6.0 ml of the second liposome suspension (phospholipid concentration 5.0 mM).

3. Result and Discussion

3.1. Chemical modification of Sephacryl S-1000HR gel

Lundahl et al. examined the introduction of hydrophobic alkyl moieties to various types of polymer gel particles [6,8]. Among the gels tested, Sephacryl S-1000 showed high phospholipid adsorption capacity, large exclusion volume and stability to the chemical modification, and thus can be expected to provide a suitable liposome immobilization system for our purpose.

Figure 1 shows the scheme of the chemical modification. The amount of the alkyl moieties introduced to a gel was estimated based on the change in the carbon and the sulfur contents of the gel. The elemental analysis of the diglycidyl ether derivative of Sephacryl S-1000HR gel found 45.97% of carbon and 1.10% of sulfur. Although the structure of the Sephacryl gel published by the manufacturer contains no sulfur atoms [9], the presence of this unaccounted sulfur in the gel was also noted by Yang et al. [6]. The reaction of the gel with 1-octanethiol resulted in the change of the carbon and the sulfur contents to 47.09% and 1.69%, respectively. Assuming that the change is solely due to the addition of 1-octanethiol to the epoxy moieties on the gel, the amount of the introduced octyl moieties was calculated as 0.190 mmol per gram of the dried hydrophobized Sephacryl gel. The value is in good agreement with one reported by Yang (0.220 mmol) [6].

Similarly, hydrophobized Sephacryl gels bearing longer alkyl moieties, dodecyl and hexadecyl, were also prepared. The amounts of the introduced alkyl moieties for those gels were 0.168 and 0.116 mmol per gram of the dried gel for the dodecyl and the hexadecyl, respectively.

3.2. Interaction of liposomes with hydrophobically modified gels

Upon coincubation of an eggPC liposome suspension and the octyl gel, the liposomal phosphatidylcholine began to disappear from the supernatant. At the same time, the corresponding amount of phosphatidylcholine was found adsorbed on the gel. The time course of the adsorption is shown in Figure 2. The adsorption involves at least two processes distinguishable in their rates. The faster adsorption was dominant in the first hour, and the slower one further continued. At 6 hours, the amount of the adsorbed phospholipid was 0.029 mmol per ml of the octyl gel (wet bed volume) for liposomes of approximately 150 nm in the average

diameter, and the value is consistent with the result reported by Yang et al. with their flow system of column-packed octyl gel; 0.020 mmol for liposomes of 200 nm diameter and 0.037 mmol for ones of 100 nm [6]. After 50-100 hours, the adsorption seemed to reach the saturation. Further examination of the interaction with various amounts and concentrations of liposomes (data not shown) confirmed that the adsorption reaches apparent completion with 0.040 mmol of adsorbed phospholipids per ml of the octyl gel (wet-bed volume).

The interaction of liposomes with the hydrophobized gels bearing the longer alkyl moieties was also examined. The dodecyl and hexadecyl gels showed much higher extent of the phospholipid adsorption than the octyl gel. Under the comparable conditions, the dodecyl gel adsorbed 0.100 mmol of the phospholipids per ml of the gel (wet-bed volume), and the hexadecyl gel, 0.120 mmol in 100 hours. The result indicates that the hydrophobic alkyl moieties on the gels play a crucial role in the adsorption and suggests that the immobilization of the phospholipids should be based on the hydrophobic interaction. Assuming that 0.063 g of dried Sephacryl gel would swell to 1.0 ml [6], the number of the phospholipid molecules immobilized per hydrophobic moiety can be calculated as 3.3, 9.4 and 16 for octyl, dodecyl and hexadecyl moieties, respectively.

Although the amounts of the immobilized phospholipid varied, the time courses of the immobilization share a common profile among the three different hydrophobized gels. Approximately 50-60% of the immobilization occurred as the fast process in the first 1 hour, which was followed by the rest 40-50% of the slow immobilization.

The integrity of liposome structure upon the immobilization was examined using liposomes with encapsulated FITC-dextran. In the first few hours of the immobilization, significant leakage of the encapsulated FITC-dextran was observed. With octyl gel, 40% of the FITC-dextran was released from the gel to the bulk aqueous phase in this period. In the cases of gels with the longer hydrophobic moieties, the release was slightly more extensive; approximately 50% for dodecyl or hexadecyl gel. The results indicate that the immobilization causes disturbance to the liposomal membrane. The insertion of the hydrophobic moieties into the liposomal bilayer membrane should perturb the packing of the liposomal phospholipid molecules. Meanwhile, the extensive leakage was only observed in the early stage of the immobilization. After 10 hours, there was no more significant release of the encapsulated FITC-dextran.

The retention of at least 50-60% of the encapsulated FITC-dextran on the gel suggests that majority of the liposomes should be immobilized still maintaining the morphology and integrity as liposomes. Although with the present system, there has been no direct evidence that

the adsorbed phospholipid molecules exist on the gel in the form of liposomes, our recent investigation of a closely related liposome immobilization system [10] revealed that immobilized liposomes can be detached from the gel as liposomes. Considering that the two systems differ only in the linkage structure of the hydrophobic moieties to the gel (thioether in the present system and disulfide in the other), one may conclude that the majority of the adsorbed phospholipids should exist on the gel in the form of liposomes.

3.3. *Immobilization and lipid composition of liposomes*

The immobilization was further examined with liposomes of different phosphatidylcholine compositions, DMPC and a mixture of DDPC and DMPC. The hydrophobic long chains in DMPC are shorter than those in eggPC, and DDPC [11] possesses two amide linkages in place of the two ester bonds linking the hydrophobic long chains to the glycerol backbone of DMPC. Although there is no difference in the length of the hydrophobic moieties between DDPC and DMPC, the amide structure provides DDPC with more hydrophilic nature than DMPC as demonstrated in its relatively fast lipid transfer between lipid bilayer membranes [12]. The transition temperature of DDPC to its liquid crystalline phase is 25.6 °C [13], which is slightly higher than DMPC (24.0 °C) [14]. The phase behavior of a DMPC/DDPC mixed system has also been examined previously, and no phase separation was detected in a differential scanning calorimetric study around the present DDPC content [11].

At 37 °C, the immobilization was rather insensitive to the phospholipid composition of the interacting liposomes. No noticeable difference from eggPC liposomes both in the rate and the extent of the adsorption was observed for either DMPC or DDPC/DMPC liposome (approximately 0.040 mmol per gram of swelled octyl gel). When the adsorption behavior was examined at 22 °C, no significant difference was seen in the case of eggPC liposomes from 37 °C. However, DMPC liposomes showed more extensive adsorption at the lower temperature (approximately 0.050 mmol per gram of swelled octyl gel). This result suggests that the phase of the phospholipid bilayer membrane should have influence on the adsorption. At 22 °C, which is slightly lower than the main phase transition temperature of DMPC bilayer membrane, the membrane is not in the liquid crystalline phase as is at 37 °C. Meanwhile bilayer membrane of eggPC is in its liquid crystalline phase at the both temperature. The presence of membrane defects at a temperature close to the phase transition temperature has been suggested by a

permeation study [15], and such membrane defects may allow easier intrusion of the hydrophobic anchors into liposomal bilayer membrane.

3.4. *Interaction of liposome-immobilizing gels with fresh liposomes*

After 5 days of the interaction of octyl gel with DMPC liposomes, the gel was separated and washed, and a fresh batch of DMPC liposomes was added to the gel. To distinguish those liposomes first immobilized onto the gel from the newly added ones, the latter were radio-labeled with [1-¹⁴C]cholesteryl oleate. Upon the coincubation, the radioactivity in the supernatant started to disappear as shown in Figure 3A. At the same time, the corresponding amount of phospholipids was also moved out from the supernatant (data not shown). Cholesteryl oleate is considered to be a non-exchangeable marker of lipid membrane [16], and also, the ratio of the radioactivity to the phospholipids in the supernatant remained almost constant (96% of the initial ratio after 72 hours), indicating that the most of the marker and the phospholipids were not relocated independently; rather they moved out from the supernatant together in the form of liposomes.

Although the first interaction was supposed to almost saturate the gel with the phospholipids, there still seemed to be room for additional adsorption of liposomes. From the data in Figure 3A, one can assume that approximately 29% of the newly added liposomes moved onto the gel in 72 hours. The rate of this "extra-adsorption" was similar to that of the slow adsorption process, suggesting that the two adsorptions could be based on the same mechanism. Lundahl et al. [4] explained that the slow immobilization by blocking of the entrances of the gel pores with the fast-immobilized liposomes and subsequent rate-limiting shift of the blocking liposomes to the further inside of the pores to make room for the late-coming liposomes. Although this hypothesis also explains the present results well, it may not be necessary to assume the access restriction by the pores. Alternatively, the immobilization of a single liposome requires a certain minimum number of the anchors [10], and after the initial fast absorption, there should be scattering on the gel small spaces with unused hydrophobic anchors in which the number of the anchors is less than the minimum. Then slow moving or rolling of the immobilized liposomes could merge the spaces to one with a sufficient number of the anchors to allow a liposome to be newly immobilized.

In the next experiment, octyl gel was first interacted with radio-labeled DMPC liposomes

to apparent saturation and then coincubated with non-labeled DMPC liposomes. In this case, the radioactivity released from the gel into the supernatant in the second interaction was only approximately 3% (Figure 3B). This release can be due to exchange between the immobilized liposome and the free one and/or relocation of the radioactive marker between the two liposomes. In any case, one may conclude that the detachment of the immobilized liposomes from the gel should be small. In addition, no release of the radioactivity from the gel was detected upon coincubation with a HEPES buffer containing no liposomes for 72 hours. The immobilization was firm and stable without interacting liposomes.

3.5. *Immobilization and the size of liposomes*

The extra-adsorption observed above was further investigated by using liposomes of two different sizes. The hydrophobized gel was first interacted with liposomes of a large size (average diameter 156 nm) for 72 hours to hold 0.040 mmol of the phospholipid per gram of the swelled gel and, after washing, further interacted with liposomes of a small size (average diameter 39 nm). Figure 4 shows the concentration of the phospholipids in the free liposome fraction. Upon the addition of the small liposomes, the phospholipids of the liposomes disappeared quickly resulting in the adsorption of approximately 0.048 mmol of the phospholipids in 28 hours.

For comparison, small liposomes were first immobilized on fresh octyl gel, and then further interacted with the large liposomes. In this case, the first adsorption of the small liposomes was quite extensive. In 11 hours, 0.055 mmol of the phospholipid of the small liposomes was adsorbed. The adsorption of the phospholipid of the large liposomes can be estimated as approximately 0.030 mmol in the same period of time. However, the following second adsorption of the large liposomes was rather poor; only 0.015 mmol of the phospholipid was adsorbed in 5 hours. To a fresh octyl gel, 0.025-0.030 mmol of the large liposomes should be immobilized.

Further evidence of the preferred adsorption of smaller liposomes was found in the average diameter of the interacted liposomes determined by dynamic light scattering. In the experiments, large liposomes were interacted either with unmodified Sephacryl S-1000HR or the octyl-gel. With the unmodified gel, there was no significant change in the average diameter of the liposomes before and after the interaction (102 nm and 104 nm, respectively). In contrast, the interaction with the octyl gel increased the average diameter of the liposomes from 103 nm to 128

nm. The more extensive adsorption of the relatively smaller liposomes in the interacted liposomes should have shifted the size distribution to the larger.

Yang et al. [6] also observed the preferred adsorption of smaller liposome with their flow system and reported higher adsorption capacity, 0.120 mmol phospholipid per ml of swelled octyl gel, for "small" liposomes (diameter 40 nm estimated from the internal volume) compared with 0.037 mmol for "medium" liposomes (100 nm diameter) and 0.020 mmol for "large" ones (200 nm). The preference is probably because smaller liposomes are sterically less hindered and have better access to the internal space of the gel pores. Smaller liposomes may also require less hydrophobic anchors for the immobilization per liposome and therefore more readily occupy the immobilization sites on the gel than larger ones.

In application of the present immobilization system in a batch mode, it may be necessary to be aware of the preferred adsorption of smaller liposomes. One could not always assume that the size distributions of the immobilized liposomes or of those left in the supernatant be same as that of the originally interacted liposomes.

Conclusion

The present study revealed the sufficient detail of the liposome immobilization behavior of the hydrophobized gels used in a batch mode. The results should help application of the system as an adsorbent or a conjugate substrate of liposomes or phospholipid aggregates in various studies. For example, as such a study that requires to conveniently distinguish liposomes from proteovesicles, our study of intermembrane relocation of acetylcholinesterase is presently ongoing based on this system.

Acknowledgements

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Appendices

The following additional plots are provided as the supplementary materials.

Fig.S1. Retention of encapsulated FITC-dextran.

Fig.S2. Adsorption of liposomal DMPC and DDPC/DMPC.

Fig.S3. Amount of phospholipids in free liposome fraction interacting with liposome-gel conjugates.

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Figure Captions

Fig. 1. Chemical modification of Sephacryl S-1000 gel.

Fig. 2. Immobilization of liposomal phospholipids onto hydrophobically modified gels. EggPC liposomes were incubated with Sephacryl S-1000 gels bearing octyl (circle), dodecyl (square) and hexadecyl (triangle) moieties at 37 °C.

Fig. 3. Radioactivity in free liposome fraction interacting with liposome-gel conjugates. DMPC liposome suspension (circle) or HEPES buffer (triangle) was coincubated with octyl gel already immobilizing approximately 0.040 mmol DMPC per gram of the swelled gel. A: Free liposomes labeled with [1-¹⁴C]cholesteryl oleate. B: Immobilized liposomes radio-labeled.

Fig. 4. Phospholipid adsorption and size of liposomes. Octyl gel was first interacted with EggPC liposomes (6.0 ml, phospholipid concentrations 5.0 mM) and then after washing, further interacted with liposomes of a different size (the same volume and concentration). The arrows indicate the point the liposomes were changed. A: First interacted with large liposomes (average diameter 156 nm) and then with small ones (average diameter 39 nm). B: Small liposomes and then large ones.

Fig.S1. Retention of encapsulated FITC-dextran. EggPC liposomes encapsulating fluorescein isocyanate-conjugated dextran (FITC-dextran; average molecular weight 40,500) were interacted with Sephacryl S-1000 gels bearing octyl (circles), dodecyl (square) and hexadecyl (triangle) moieties at 37 °C.

Fig.S2. Adsorption of liposomal DMPC and DDPC/DMPC. Liposomes were incubated with Sephacryl S-1000 gels bearing octyl (circles), dodecyl (square) and hexadecyl (triangle) moieties at 37 °C.

Fig.S3. Amount of phospholipids in free liposome fraction interacting with liposome-gel conjugates. DMPC liposome suspension was coincubated with octyl gel that were already immobilizing approximately 0.040 mmol DMPC per gram of the swelled gel. The free DMPC liposomes were labeled with [1-¹⁴C]cholesteryl oleate, and the relocation of the radioactive marker was shown in Figure 3A.

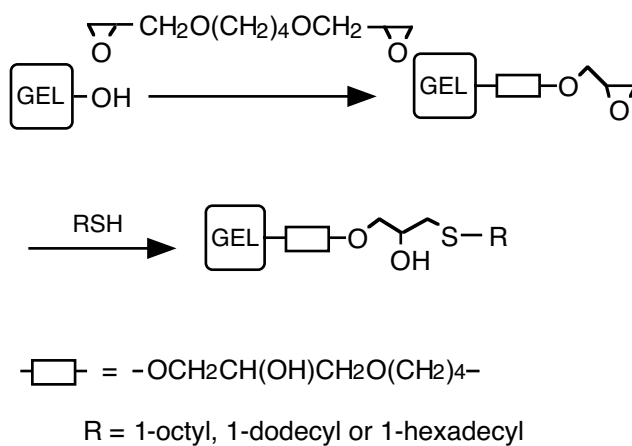


Figure 1

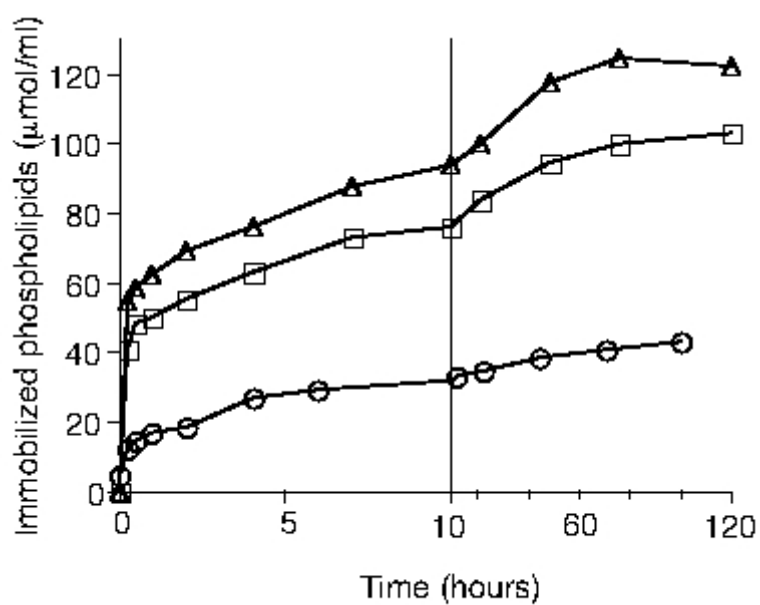


Figure 2

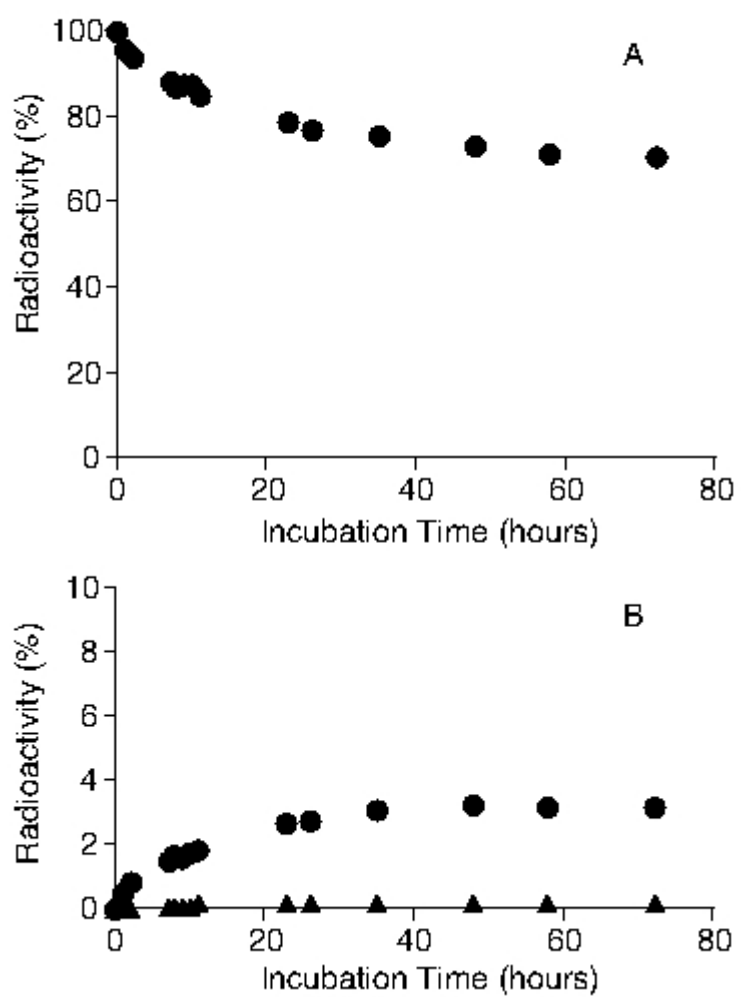


Figure 3

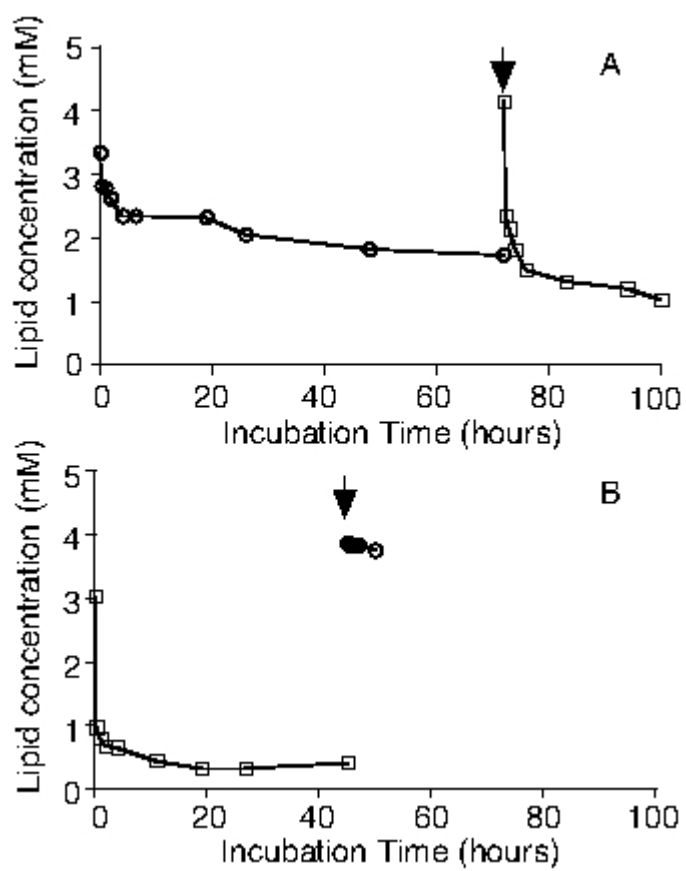


Figure 4

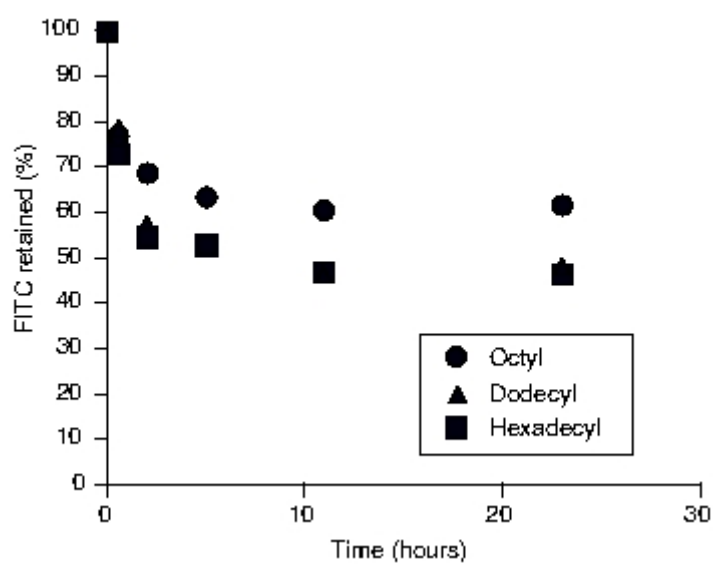


Figure S1

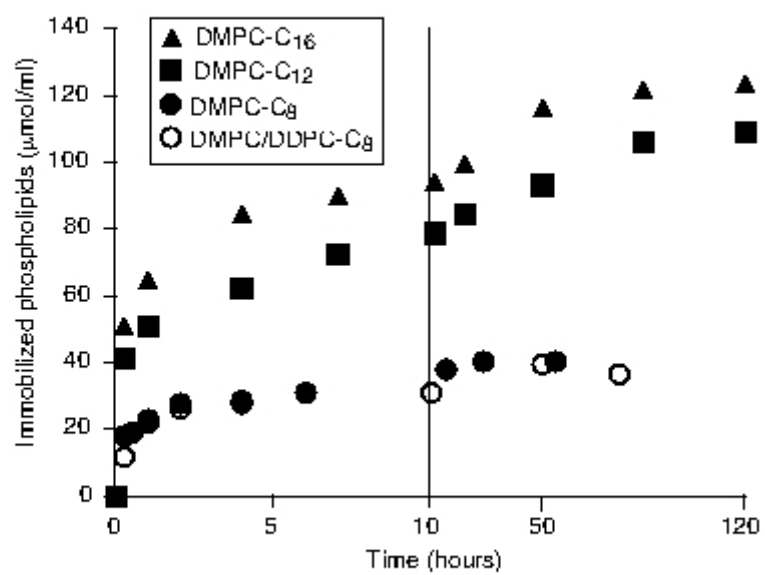


Figure S2

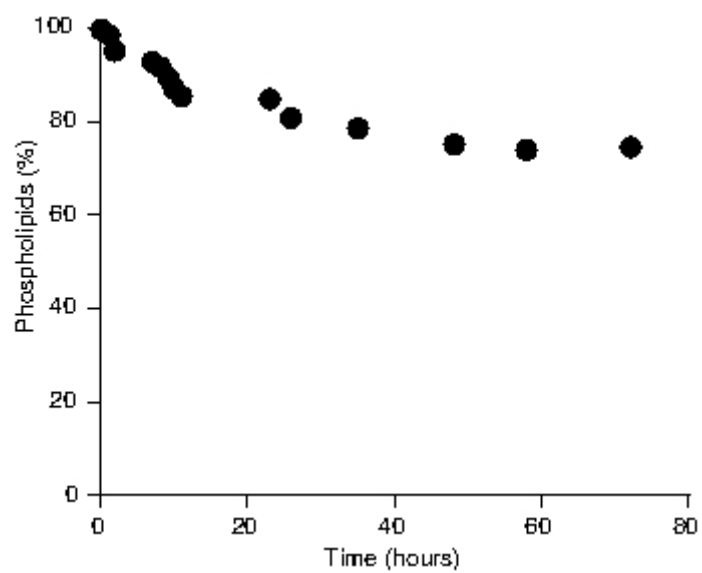


Figure S3