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Lipid-vesicle-surface chromatography

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SUMMARY

Egg-yolk phospholipid vesicles (liposomes) containing stearylamine cations or phosphatidylserine anions, were formed and entrapped in agarose gel beads (Sepharose 6B) by a dialysis procedure. On a column of entrapped phospholipid–stearylamine (4:1) (cationic) vesicles, 0.36 mg of ferritin was bound per μmol lipids at 0.05 *M* ionic strength and pH 7. About 30% of the vesicle surface thus became covered with ferritin. Only 0.04 mg of citraconylated myoglobin was bound per μmol lipids, as myoglobin is much smaller than ferritin. Haeme groups were readily inserted into the lipid bilayers. An excess amount of bovine serum albumin (BSA) or ribonuclease A was applied to entrapped ionic vesicles and the bound proteins were eluted by increasing the ionic strength from 0.01 to 0.2 or 0.5 *M*. After three to five runs, 82–88% of the vesicles (the phospholipids) remained entrapped. The capacity of the cationic vesicle-column for BSA decreased more than did the amount of entrapped vesicles, which indicates a preferential loss of stearylamine. Ion-exchange experiments were done with human plasma and with BSA monomers and dimers on entrapped cationic vesicles. Plasma proteins could be separated. BSA dimers were eluted later than BSA monomers in a sodium chloride gradient and the separation was better than on DEAE-Sepharose. The contact area between the protein and the vesicle surface is important for the binding strength. Protein–vesicle surface interactions can be studied by chromatography on entrapped vesicles.

INTRODUCTION

Immobilized artificial membrane chromatography by use of synthetic chromatographic supports composed of a membrane-forming lipid, lecithin, mimicking a cell membrane lipid monolayer, has been reported recently by Pidgeon and Venkataram[1]. We have recently developed a procedure for dialysis entrapment of lipid vesicles and protein–lipid vesicles in gel beads without the use of hydrophobic ligands. Sufficiently large lipid vesicles become trapped in the gel bead pores in which they are formed[2]. Lipid vesicles (liposomes) are composed of amphiphiles, phospholipids, forming lipid bilayers that enclose one or more aqueous compartment(s)[3]. The entrapment of lipid vesicles in gel beads provides a possibility for chromatography of water-soluble

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proteins and hydrophilic peptides on the vesicle surfaces. This can be useful for studies of solute interactions with the lipid bilayers. The specific or non-specific interaction of biomolecules with cell membranes may afford special properties for the lipid-vesicle-surface chromatography. The main aim of this work was to show that the entrapped vesicles are accessible to proteins and are relatively stable on binding and elution of proteins with increasing ionic strength, and that chromatography is therefore possible with the vesicle surfaces as a stationary phase. Ion-exchange chromatography of plasma proteins and of BSA monomers and dimers on entrapped cationic vesicle columns was demonstrated.

EXPERIMENTAL

Materials

Sepharose 6B, Sepharose CL-6B, Sephadex G-25 M (PD-10) columns and horse spleen ferritin (gel filtration calibration protein) were purchased from Pharmacia LKB (Uppsala, Sweden). Bovine serum albumin (BSA) (A-7030), BSA monomers (A-1900) and dimers (A-9039), ribonuclease A (bovine pancreas, type III-A, R-5125), stearylamine and bovine brain extract (80–85% phosphatidylserine, B-1627) were purchased from Sigma (St. Louis, MO, U.S.A.). Citraconic anhydride (purum) was from Fluka (Buchs, Switzerland). Dialysis tubings were of the regenerated cellulose type with a diameter of 20 mm (cut-off M_r 12 000–14 000) from Viskasi (Chicago, IL, U.S.A.). All chemicals were of analytical-reagent grade unless stated otherwise. Human blood plasma was obtained from the blood bank at the University Hospital (Uppsala, Sweden) and was transferred to buffer A (ionic strength 0.22 *M*, pH 7.1; see below) by chromatography on a Sephadex G-25 column. Aliquots were stored at −70°C.

Buffers

Buffers A–D all contained the following components: 1 m*M* Na_2EDTA, 1 m*M* 2-mercaptoethanol and 0.1 m*M* D-glucose, combined with (A) 200 m*M* NaCl, 20 m*M* Tris–HCl (pH 7.1), (B) 20 m*M* NaCl, 20 m*M* Tris–HCl (pH 7.1), (C) 20 m*M* NaCl, 20 m*M* citric acid (pH 4) and (D) 200 m*M* NaCl, 20 m*M* citric acid (pH 4).

Lipid/cholate solutions

A 100 m*M* phospholipid solution was prepared by dispersing egg-yolk phospholipids (70% phosphatidylcholine, 21% phosphatidylethanolamine[4]) in 125 m*M* cholate, 200 m*M* NaCl, 2 m*M* dithioerythritol, 1 m*M* Na_2EDTA, 0.1 m*M* D-glucose and 20 m*M* Tris–HCl (pH 7.1). The suspension was stirred for 35 min at 22°C. Other procedures were as described previously[4].

A phospholipid–stearylamine solution was prepared by mixing stearylamine, the above 100 m*M* phospholipid solution, 750 m*M* cholate and buffer A to final concentrations of 10 m*M* stearylamine, 40 m*M* lipids and 62.5 m*M* cholate. The pH was adjusted to 8 and the mixture was stirred under nitrogen at 22°C until the stearylamine was dissolved.

Bovine brain extract (80–85% phosphatidylserine) was dissolved with cholate to final concentrations of 40 m*M* lipids (*ca.* 33 m*M* phosphatidylserine) and 100 m*M* cholate in 5 m*M* Tris–HCl (pH 7.1) containing 1 m*M* 2-mercaptoethanol and 1 m*M* Na_2EDTA. This solution is denoted phosphatidylserine solution below. The corresponding vesicles are denoted phosphatidylserine vesicles (anionic vesicles).

Methods

Entrapment. Egg-yolk phospholipid vesicles were entrapped in agarose gel beads by dialysis of the lipid solution that had been mixed with the gel beads[2]. Briefly, 3 ml of the phospholipid–stearylamine or phosphatidylserine solution (see *Materials*) was mixed with agarose gel beads by pumping the solution into a Sepharose 6B column (packed volume 2.5 ml). The gel was then transferred from the column into a dialysis cell (see Fig. 1 in ref. 2) and dialysed against 4 × 500 ml of buffer A (see *Materials*) for 2.5 days at 22°C. After dialysis the gel beads were washed three times with buffer A by centrifugation at 150 *g* for 5 min. The gel was repacked into a column and further washed by chromatography with five column volumes of buffer A. All entrapments and experiments with entrapped vesicles were done at room temperature.

Stability of entrapped vesicles. Excess amounts of BSA, 9.6 mg in 3 ml of starting buffer (10 m*M* Tris–HCl, pH 8.5, containing 0.1 m*M* Na_2EDTA and 0.2 m*M* 2-mercaptoethanol) were applied to a phospholipid–stearylamine (4:1) (cationic) vesicle–Sepharose 6B column (2.8 × 1 cm I.D.). This column was connected to a UV monitor (UV-2; Pharmacia LKB) and a recorder. The column was equilibrated with the starting buffer before protein application, and the bound proteins were eluted by increasing the ionic strength. This chromatographic experiment was repeated on the same column three to five times over 1.5–2 days at room temperature.

All fractions upon application, rinsing, elution and equilibration were collected in weighed tubes (to determine the fraction mass) and the amounts of the released phospholipids were determined by phosphorus analyses according to the method of Bartlett[5]. The entrapped phospholipids remaining in the column after the repeated chromatographic experiments was solubilized with 100 m*M* cholate for phosphorus analysis. The amount of phospholipids retained in the corresponding chromatographic run No. N, expressed as a percentage of the initial amount of entrapped lipids, was calculated as $100(E-R_{N-1})/E$, where E is the initial amount of entrapped phospholipids, which was calculated as the sum of the amounts of lipids released throughout the repeated chromatographic experiments and the amount of lipids solubilized with 100 m*M* cholate, and R_{N-1} is the phospholipids released in the preceding chromatographic experiment(s), $N-1$. For entrapped phosphatidylserine (anionic) vesicles, 8 mg of ribonuclease A in starting buffer (0.01 *M* NaCl in 5 m*M* Tris–HCl, pH 7.1) were applied to the vesicle–Sepharose 6B column (2.8 × 1 cm I.D.). Other experimental conditions were the same as for the entrapped cationic vesicles.

Citraconylation of myoglobin. Horse-heart myoglobin was citraconylated by the procedure described by Lundahl[6] using 10 mmol of citraconic anhydride per mmol of lysine or per gram of myoglobin. This procedure replaces the positive charges of the N-terminal amino group and the lysine ε-amino groups with carboxyl groups. The citraconylated myoglobin (CMG) was transferred to buffer B by chromatography on Sephadex G-25. In some experiments CMG was dialysed against 4 × 500 ml of buffer B for 2 days at 7°C. Isoelectric focusing of CMG and dialysed CMG showed 3–5 bands in the pH range 4–5.3.

Electrophoresis. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) with a linear gradient of acrylamide concentration from 8 to 25% and silver staining of the gel was done essentially as described in ref. 7. Isoelectric focusing was done on ready-made polyacrylamide gels containing carrier ampholytes with a pH range of 3–10 (LKB Ampholine PAGplates; Pharmacia LKB) at 2000 V for 3000 Vh. The focusing gel was stained with Coomassie Brilliant Blue R-250.

TABLE I

CAPACITY OF CATIONIC VESICLES FOR BINDING OF FERRITIN

16 mg of ferritin in 5 ml of buffer B (ionic strength 0.05 *M*, pH 7.1) were applied in buffer B to 0.5-ml tandem columns of Sepharose 6B, neutral lipid vesicle–Sepharose 6B and cationic lipid vesicle–Sepharose 6B. Elutions were done for each column separately. Flow-rate, 6 ml/h.

Column	*Amount of lipids*[a] *(μmol)*	*Eluted ferritin*			
		Buffer A (ionic strength 0.22 M) (mg)	*100 mM cholate (mg)*	*Sum (mg)*	*μg protein / μmol lipid*
Sepharose 6B	–	0.004	0.003	0.007	–
Neutral vesicle[b]–Sepharose 6B	5.9	0.01	0.005	0.015	2.5
Cationic vesicle[c]–Sepharose 6B	5.6	1.65	0.35	2.00	360

[a] The amount of lipids in the entrapped cationic vesicles is expressed as the sum of the amounts of phospholipids and stearylamine. The latter amount was calculated according to the ratio of the stearylamine added to the phospholipids.

[b] Egg-yolk phospholipid vesicles.

[c] Egg-yolk phospholipid–stearylamine vesicles (molar ratio 4:1).

Protein amounts. Protein amounts were determined by automated total amino acid analysis with 24-h hydrolysis in 6 *M* hydrochloric acid.

RESULTS

Surface accessibility of entrapped vesicles

For studies of the interaction of proteins with entrapped vesicles the vesicle surfaces must be easily accessible. Protein interactions with the entrapping gel matrix should be minimal. To investigate this, we chose a large spherical protein, ferritin (M_r 440 000, K_{av} 0.33 on Sepharose CL-6B, isoelectric point 4.3–4.4[8]) and applied an excess amount of this protein at an ionic strength of 0.05 *M* to three tandem columns containing Sepharose 6B, egg-yolk phospholipid (neutral) vesicle–Sepharose 6B and cationic vesicle–Sepharose 6B. The ferritin passed through the first two columns and bound extensively (0.36 mg/μmol lipid; see Table I) in the cationic vesicle–gel column, which became dark brown. Approximately 30% of the outer surfaces of the vesicles became covered with ferritin molecules, as estimated by use of the cross-sectional area of ferritin (*ca.* 11 300 Å^2), of egg-yolk phospholipid molecules [*ca.* 70 Å^2 (ref. 9)] and of stearylamine (*ca.* 35 Å^2). More than 80% of the adsorbed ferritin could be released by increasing the ionic strength to 0.22 *M*.

Binding of a small, highly negatively charged protein to entrapped vesicles

On the application of citraconylated myoglobin (CMG) to entrapped neutral and cationic vesicle–Sepharose 6B columns, both columns became dark red. The lipids and the bound material were eluted with cholate. The eluate contained a large amount of haeme groups, as determined by measuring the absorbance of the haeme groups at

TABLE II

BINDING OF CITRACONYLATED MYOGLOBIN (CMG) AND HAEME GROUPS TO NEUTRAL AND CATIONIC LIPID VESICLE–GEL COLUMNS (1 × 0.7 cm I.D.) WITH AMOUNTS OF ENTRAPPED LIPIDS[a] OF 5.3 AND 6.0 μmol, RESPECTIVELY

Samples A and A', 5 mg/ml of CMG in 4.5 ml and 5.5 ml of buffer B, respectively. Sample B, 3.2 mg/ml of dialysed CMG in 2.5 ml of buffer B. Flow-rate, 6 ml/h.

Sample	*Column*	*Amount of bound CMG*		*Amount of bound haeme group (nmol/μmol lipid[a])*
		μg/μmol lipid[a]	*nmol/μmol lipid*	
A	Neutral vesicle[b]–Sepharose 6B	5.5	0.31	7.5
A'	Cationic vesicle[c]–Sepharose 6B	9.6	0.55	14.5
B	Cationic vesicle[c]–Sepharose 6B	39	2.2	<1.5

[a] Amount of entrapped lipids including stearylamine as in Table I.
[b] Egg-yolk phospholipid vesicles.
[c] Egg-yolk phospholipid–stearylamine vesicles (molar ratio 4:1).

412 nm, but only a small amount of protein. The amounts of haeme groups and CMG were about twice as high on the cationic as on the neutral vesicles (Table II). Haeme groups were released from the CMG and became inserted into the lipid bilayer of the entrapped vesicles on binding of the CMG to the vesicle surfaces. Dialysis removed haeme groups from the CMG solution. Accordingly, only small amounts of haeme groups became adsorbed when dialysed CMG was applied to the columns, and the cationic vesicles bound a relatively large amount of citraconylated apomyoglobin (Table II), about 0.23 mg on the 0.5-ml column. The main conclusion is that the haeme groups readily become inserted in the lipid bilayer. Their carboxyl groups decrease the vesicle charge. Therefore, CMG bound reasonably well to the cationic vesicles only when the amount of haeme groups was decreased.

Stability of entrapped vesicles

We have previously shown that entrapped vesicles are retained through several washing steps[2]. The question posed here is whether the entrapped vesicles are stable on changes in osmotic pressure for chromatography of proteins. Fig. 1A illustrates the retention of the phospholipids of lipid vesicles entrapped in gel beads on repeated cycles of salt gradient elution. The vesicles that became too small relative to the pores in which they were entrapped were eluted in the first chromatographic run. After the first run, the retention of phospholipid vesicles in subsequent chromatographic experiments decreased only slightly. Most of the remaining vesicles were stably entrapped. In BSA chromatography on entrapped cationic vesicles the retentions of the vesicles were 88% on five repeated cycles of the chromatographic experiment and 82% on three cycles with increasing ionic strength from 0.01 to 0.21 *M* (○ in Fig. 1A) and from 0.01 to 0.51 *M* (△ in Fig. 1A). The retention was 88% for ribonuclease A on entrapped anionic vesicles (□ in Fig. 1A). As expected, the entrapped vesicles were

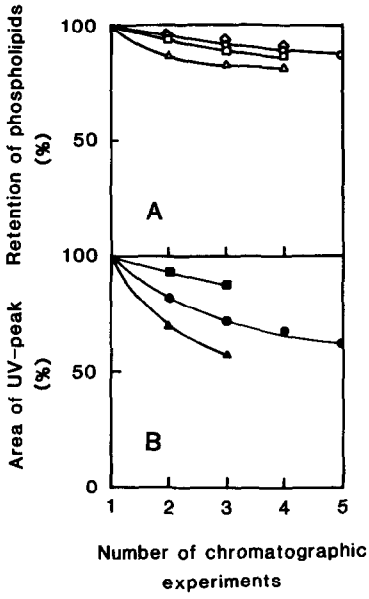

Fig. 1. (A) Stability of entrapped vesicle columns. Retention of entrapped phospholipid vesicles in consecutive chromatographic runs. Chromatography of BSA on the entrapped cationic vesicles (○, △) and of ribonuclease A on the entrapped anionic vesicles (□), or on the cationic vesicles without application of proteins (◇), NaCl gradient elutions: (○) 0–0.2 *M* NaCl; (△ and ◇) 0–0.5 *M* NaCl; (□) 0.01–0.5 *M* NaCl. For the experimental procedures see *Stability of entrapped vesicles*. (B) Relative area of UV peaks for protein elutions. The filled symbols correspond to the open symbols in (A). The first peak of elution before leakage of phospholipids occurred was expressed as 100% in relation to the second, the third, and so on. Each curve in (A) and (B) corresponds to experiments on a single column.

more stably confined to the pores when the salt concentration of the eluent was decreased to that at which the vesicles were formed. The effect of osmotic pressure alone (◇ in Fig. 1A) on the leakage of the entrapped vesicles was less than the combined effects of binding of protein and subsequent elution by increasing the ionic strength (○, □ and △ in Fig. 1A). This phenomenon was further studied by use of a model protein, lysozyme, for binding on the surface of entrapped phosphatidylserine vesicles[10]. For the entrapped anionic vesicles the decrease in the area of the UV peaks corresponding to eluted proteins was approximately in proportion to the decrease in the corresponding retention of the entrapped vesicles (□ and ■ in Fig. 1A and B, respectively). However, the area for the entrapped cationic vesicles decreased more steeply (▲ and ● in Fig. 1B) than did the corresponding retention of the vesicles (△ and ○ in Fig. 1A). It seems that stearylamine incorporated into the vesicle bilayer was released to some extent on the binding and release of BSA from the cationic vesicles, which reduced the charge density on the vesicle surface. It should be noted that the areas of the UV peaks mainly represent "protein-binding capacity". However, the vesicles that are released during protein elutions give light scattering at 280 nm, which also shows up as an apparent absorption, particularly in the case of the first chromatographic run with a non-negligible loss of lipids.

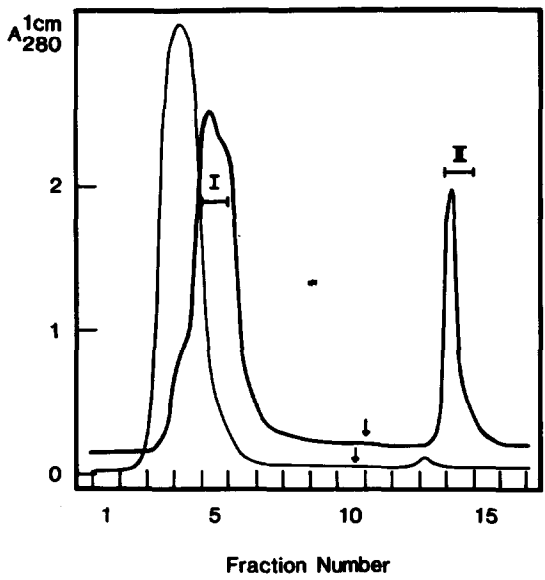

Fig. 2. Chromatography of human plasma on a Sepharose 6B column with entrapped cationic lipid vesicles (thick line) or without lipid vesicles (thin line). The column was equilibrated with buffer B (ionic strength 0.05 *M*, pH 7) before and after application, and bound proteins were eluted with buffer C (ionic strength 0.06 *M*, pH 4). The arrows indicate the start of the elution with buffer C. Sample, human blood plasma (see *Materials*) diluted 10-fold with distilled water; sample volume, 2 ml; column dimensions, 3.2 × 1 cm I.D. (bed volume 2.5 ml); flow-rate, 3 ml/h; fraction volume, 0.5 ml; amount of entrapped phospholipids in the column, 15 μmol.

Chromatography of plasma proteins on entrapped cationic vesicles

On chromatography of human plasma proteins on an entrapped cationic vesicle–Sepharose 6B column, at ionic strength 0.05 *M* and pH 7, we found that many proteins in the M_r range from 16 000 to *ca.* 300 000, or proteins with subunits in this range, bound to the column and could be eluted (peak II, thick line, in Fig. 2; lane II in Fig. 3) at pH 4 (buffer C, ionic strength 0.06 *M*). Albumin and other proteins passed straight through the column (peak I, thick line, in Fig. 2 and lane I in Fig. 3). The leakage of phospholipids on elution with buffer C was 1%. A similar chromatographic experiment in which the bound plasma proteins were eluted by increasing the ionic strength from 0.05 to 0.24 *M* and decreasing the pH from 7 to 4 showed a chromatographic pattern (not shown) and non-bound and bound proteins (lanes a and b in Fig. 3) similar to that in Fig. 2 (thick line) and to lanes I and II in Fig. 3, respectively. The loss of the vesicles was 10% in this instance. It is clear that the entrapped cationic vesicles were more stable on protein elution with decrease in pH alone than together with increase in ionic strength. After storage of the column at 7°C for 3 days the leakage of phospholipids was 1.5% on elution with a decrease in pH alone. Chromatography of plasma after removal of the entrapped vesicles with 100 m*M* cholate showed mainly a single peak corresponding to non-bound materials. The peak was minimal on elution with buffer C (Fig. 2, thin line).

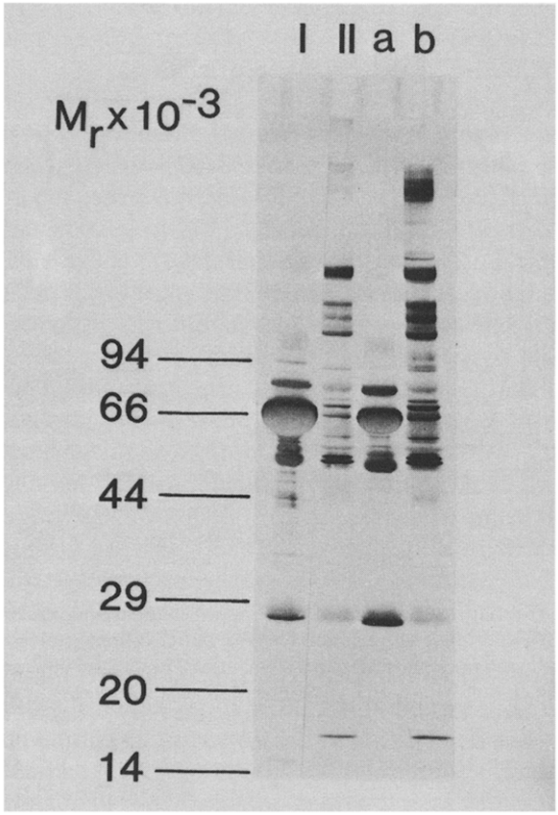

Fig. 3. SDS-PAGE of the non-bound and bound proteins. Sample volume, 35 μl. Lanes I and II, the non-bound and bound plasma proteins indicated by bars in Fig. 2, thick line. Lanes a and b show the same non-bound and bound proteins, respectively, in another similar fractionation for which the elution was done with buffer D (ionic strength 0.24 *M*, pH 4).

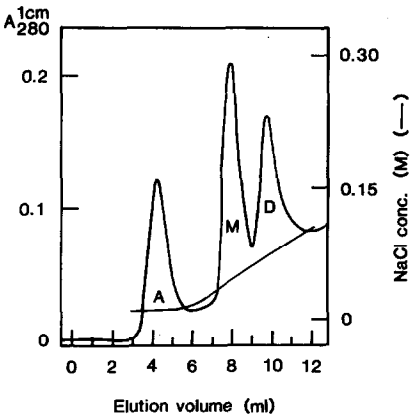

Fig. 4. Chromatography of a mixture of BSA monomers (M) and dimers (D) on a cationic vesicle–Sepharose 6B column (2.9 × 1 cm I.D.). Sample, 0.2 mg of each component in 0.2 ml of starting buffer (10 m*M* Tris–HCl, pH 8.5, containing 0.1 m*M* EDTA and 0.2 m*M* mercaptoethanol). The proteins were eluted with a gradient of NaCl in the starting buffer. Flow-rate, 1 ml/h. A, Unidentified non-proteinaceous material.

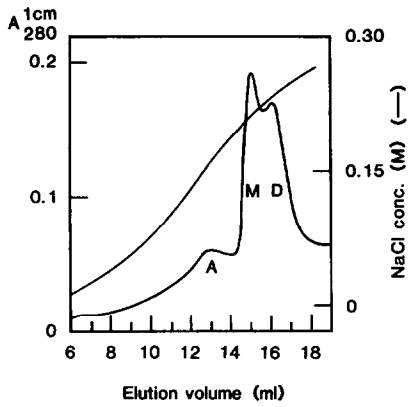

Fig. 5. Chromatography of BSA monomers and dimers on a DEAE-Sepharose 6B column (3.0 × 1 cm I.D.). The experiment was done as in Fig. 4. Notations as in Fig. 4.

Chromatography of bovine serum albumin on entrapped cationic vesicles

BSA monomers and dimers became well separated on entrapped cationic vesicles at the low flow-rate of 1 ml/h (Fig. 4). The identity of the components was verified by "native" electrophoresis (not shown). Separation could also be achieved at a higher flow-rate, 10 ml/h (not shown). Chromatography on DEAE-Sepharose 6B gave only a slight separation at a flow-rate of 1 ml/h (Fig. 5). At 10 ml/h the monomer and dimer did not separate (not shown). The monomer and dimer were eluted at low ionic strengh (0.048 and 0.075 *M*, respectively) from entrapped cationic vesicle–Sepharose 6B. As expected, the elution of the monomer and dimer on DEAE-Sepharose 6B required a higher ionic strength (0.213 and 0.237 *M*, respectively). The fact that the binding strength of BSA monomer and dimer was much lower on the cationic vesicle–gel than on the DEAE gel is due to the two-dimensional interactions between protein molecules and the bilayer surface of entrapped vesicles, and to the relatively low charge density of the stearylamine cations on the surface. The average distance between the stearylamine molecules can be estimated as 18 Å.

DISCUSSION

The results indicate that the adsorption of proteins on the surfaces of charged entrapped vesicles is dependent on the vesicle charge density and on the protein size in addition to charge. The protein size (and shape) affects the contact area with the vesicle surface. Protein desorption from the vesicle surfaces was achieved below 0.1 *M* ionic strength. The electrostatic interactions between the charged vesicles and proteins were thus relatively weak, in agreement with results by Maretzki *et al.*[11] concerning the binding of proteins to membrane surfaces.

We have found that 30% of the outer surface of the entrapped cationic vesicle became covered by ferritin molecules. Ferritin is composed of several subunits with a total net negative charge of at least −130 at pH 7 (estimated from the amino acid composition). Owing to the strong electrostatic repulsion between adjacent ferritin molecules and the relative low ligand density of the stearylamine (20%) on the vesicle surfaces, close packing of the ferritin is impossible.

Citraconylation increases the number of negative charges present on the proteins at neutral pH[12,13]. However, much smaller amounts of citraconylated myoglobin (CMG, Table II) were bound than of ferritin (Table I) on the entrapped cationic vesicles. The vesicle bilayers contained approximately one stearylamine molecule per 320 Å^2, which corresponds to an average distance between the cations of 18 Å [the distance between the anions of the Mono S ion exchanger is 2.3–2.5 Å (F. Regnier, personal communication)]. About four stearylamine molecules may come into contact with one myoglobin molecule, whereas approximately twice as many stearylamine molecules might interact with one ferritin molecule. For CMG the binding will therefore be less efficient than for ferritin, in agreement with our results. It is possible to obtain a more efficient binding for small proteins by use of a high ligand density of the charge groups on the vesicle surface.

Insertion of haeme groups into the vesicle bilayers on the interaction of haemoglobin with phosphatidylserine vesicles has been reported by Shviro *et al.*[14]. We observed a similar phenomenon on chromatography of CMG on both entrapped cationic and neutral lipid vesicles (Table II). The released haeme groups became incorporated into the lipid bilayer of the entrapped vesicles and were therefore separated from the non-bound protein. This is an experimental example of the non-specific interaction of biomolecules with lipid membranes on chromatography, as mentioned by Pidgeon and Venkataram[1].

Quantitative binding studies may be done with large, flat lipid bilayer surfaces on planar supports, as in the elegant procedures described by McConnell *et al.*[15] and Tamm[16]. Our results confirm that the surfaces of vesicle entrapped in agarose gel beads are largely accessible to macromolecules. Therefore, entrapped vesicles can also be used for lipid bilayer–macromolecule interaction studies.

The regular two-dimensional array of charged groups on the vesicle surface and the low binding strength between proteins and the vesicle surfaces may give a high resolution with low salt gradient elution. Antibodies and other cell-specific proteins can be immobilized on the entrapped vesicle surfaces[17,18] for affinity chromatography.

Unfortunately, there were small losses of the entrapped vesicles of *ca.* 1–15% (data from test under Results and from Fig. 1A) on the chromatography of proteins under the different experimental conditions. Obviously, this decreased the column capacity for protein binding (see Fig. 1B). It was found that protein elution with a low pH (4.0) or with a gradient of low final ionic strength decreased the loss of the entrapped vesicles. In addition, a decrease in sample load may be required as the protein binding and release also affect the leakage (see Results). Polymerized vesicles prepared by covalently cross-linking the individual phospholipid molecules[19] enhance their resistance to osmotic shock[20], to ethanol and to sodium dodecyl sulphate[21]. Therefore, by use of such polymerized vesicles entrapped in gel beads their stability on protein chromatography may be improved.

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