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Review

Chromatography on cells and biomolecular assemblies

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Abstract

Red cells, biomembrane vesicles, proteoliposomes and liposomes non-covalently immobilized in gel particles or beads have been used as stationary phases for biomembrane affinity analyses and ion-exchange chromatographic separation. Lipid monolayers coupled to silica beads have been utilized for membrane protein purification in detergent solution and plant cell walls for group separation of macromolecules according to size and charge. Further methodological studies are essential to implement general practical application. © 1997 Elsevier Science B.V.

Keywords: Reviews; Cells; Proteins; Glucose

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1. Introduction

The purpose of the present review is to focus attention on the use of cells and biomolecular assemblies as stationary phases for chromatographic separations of macromolecules and small biomolecules on the basis of size, charge or bioaffinity and analyses by, for example, quantitative affinity chromatographic techniques.

As early as 1966 chromatography of D-glucose and L-glucose on red cell membranes was studied [1,2], but retardation of D-glucose was misinterpreted to reveal binding to the membranes, whereas reevaluation in 1970 suggested that 'the sugar-complexing entities on the column continue to operate as "carriers" mediating sugar access into membrane-enclosed compartments' [3], which is the first example of transport retention chromatography (TRC).

Recently, human red cells were immobilized for chromatographic activity analysis [4,5] of the human red cell glucose transporter, Glut1, that supplies the cell with D-glucose [6–8]. D-glucose interacts with Glut1 by hydrogen bonding and, thereby, passes through the transmembrane protein, whereas L-glucose does not. This selectivity between the enantiomers was utilized in TRC [9] on red cells, and the specific interactions between glucose, glucose-transport inhibitors and Glut1 were analyzed by quantitative biomembrane affinity chromatography (BAC). TRC was also applied to Glut1 proteoliposomes [9,10] and BAC to red cell membrane vesicles and Glut1 proteoliposomes [5,11–15]. Gentle steric immobilization by freeze–thaw fusion of the vesicles or proteoliposomes in the beads [9,16–18] and the sheltered environment in cavities of the gel allowed Glut1 to retain native properties in a stable reconstituted system during long periods of time [12,13]. The membrane proteins are imbedded in lipid bilayers, and the membranes, in turn, are remarkably stable in the beads, particularly in the dextran-coated cavities of Superdex 200 agarose beads (Pharmacia Biotech, Uppsala). These and similar systems will hopefully allow quick and accurate analyses of important biospecific interactions and reveal how membrane protein activities are affected by the temperature, the surrounding lipids, components in the aqueous medium, etc.

Lipid bilayers can also be used for separation purposes. Proteins have been separated by ion-exchange chromatography on charged liposomes entrapped in gel beads [19–21] and by temperature-controlled phase transition chromatography on lipid bilayers coated on silica gel [22]. Interaction between water-soluble peptides and liposome surfaces [23] and partitioning of amphiphilic drugs between the mobile aqueous phase and liposomal lipid bilayers were reflected by the chromatographic retention of the solutes on liposomes in ml-scale columns [24,25] and capillaries [26] or by decreased mobility upon capillary electrophoresis with semi-stationary suspended liposomes [27]. Drug partitioning data obtained in the above-mentioned systems [24–27] may allow predictions concerning transmembrane drug absorption through epithelial cell layers. The latter has recently been reviewed [25] and will not be discussed here.

Lipid-analogue monolayers, called immobilized artificial membranes (IAM), have found chromatographic application in the purification of detergent-solubilized membrane proteins, for example, enzymes with phospholipids as substrate [28–32], and for analysis of drug partitioning into the IAMs [33]. These monolayers consist of phospholipid analogues covalently coupled to silica beads conferring properties intermediate between those of hydrophobic-ligand phases and of immobilized lipid bilayers. Enzyme reactors have been prepared by adsorption of microsomal vesicles onto these monolayers [34,35], which may be of interest for future chromatographic use of such vesicles.

The red cells and biomolecular assemblies required a carrier gel, whereas suspension-cultured plant cells [36] or cell walls [37] were rigid enough to be packed into columns, without use of a supporting material, for analyses of the wall porosity by chromatography similar to gel filtration. Cell-wall microcapsules have been used for size group fractionation of samples containing cells, proteins, nucleic acids or small compounds [38–44].

At present, the above methods are best suited for research laboratories, but analytical applications are likely to find their way into the biomedical and pharmaceutical industries. Pilot-scale preparative separations will require further development aimed at

preparing cheap and stable stationary materials for applications that cannot be met by conventional means.

2. Preparation of stationary phases

2.1. Immobilization of human red cells in gel particles

Gel particles were prepared by a method similar to that described previously for preparation of continuous beds [26,45]. In brief, piperazine diacrylamide and methacrylamide were polymerized in the presence of N-allyldimethylamine to form a positively charged gel [4]. The gel was dispersed mechanically to form irregular particles containing cavities or channels which increased the surface area available for immobilization of, for example, red cells [4]. Upon mixing with the particulate gel, the red cells were adsorbed mainly by ionic interaction. Other weak interactions contributed to the binding, as indicated by the fact that a moderate amount of cells were bound also in the absence of the cationic ligands. This material constitutes a unique stationary chromatographic phase.

2.2. Immobilization of red cell membrane vesicles and (proteo)liposomes in gel beads, with a proposed gel bead structure

Gel beads suitable for separation of protein molecules, such as agarose beads, were used for steric immobilization of red cell membrane vesicles [12], proteoliposomes or liposomes by freeze–thawing a mixture of suspended biomembranes and dried gel beads to induce fusion and entrapment [5,11–13,15–18], or by dialysis-preparation of (proteo)liposomes in beads [17–21,46,47]. Non-covalent immobilization was also achieved by adsorption of (proteo)liposomes to hydrophobic ligands [5,9,17,18,20,21,48]. A recent review [14] covers these techniques and describes a novel method in which biotinylated phosphatidylethanolamine was included in the bilayers for binding to avidin ligands coupled to the gel. A characteristic of these methods is that liposomes somewhat larger than excluded proteins enter the gel beads, presumably because of the flexibility of the bilayers above the phase transition temperature.[1] Furthermore, if liposomes are fused and entrapped by freeze-thawing to become spherical and uniformly-sized, they appear to be much larger than the 'separation pores'. Internal volume data for egg phospholipid liposomes immobilized by freeze–thawing in Superdex 200 gel beads [50] give a liposome diameter of approximately 50 nm [51], whereas the largest pores in these beads are less than 10 nm in diameter, as estimated from the Stokes' radius of excluded globular proteins with a molecular mass of about $600 \cdot 10^3$ rel. mol. mass units [49]. We therefore propose that the liposomes become immobilized in gel cavities or network rooms [16] (Fig. 1) such as those that can be observed in scanning electron micrographs [52,53]. The porosity or empty space in an agarose gel bead can be described as consisting of a conglomerate of cavities limited by the agarose bundles and connected by unevenly distributed short pores of various size and form, making the cavity volume available for entrapment of liposomes or vesicles of sizes considerably larger than the maximal protein-separating pore size. This structure is related to a network model of the bead material [54], but differs from the cone-shaped cavity model [55].

2.3. Coupling of lipid analogues to form IAM monolayers in silica beads

Lipid monolayers of dimyristoylphosphatidylcholine (DMPC) were originally prepared by reacting a cyclic anhydride, dodecanedicarboxylic acid anhydride, with monomyristoyl lysolecithin to obtain DMPC that was carboxylated at one of the myristoyl chain ends. This derivative was modified by reaction with carbonyldiimidazole. The DMPC-imidazole was then coupled by a peptide bond to silica gel beads derivatized with propylamine groups to obtain a

[1] For example, liposomes of 25-nm diameter showed a K_{av} value of 0.58 upon gel permeation on Sepharose 4B (when adsorption was eliminated by inclusion of liposomes in the eluent; Lundahl and Greijer, unpublished data), whereas a globular protein of about 18-nm Stokes' radius ($4000 \cdot 10^3$ rel. mol. mass units [49]) is excluded from this gel.

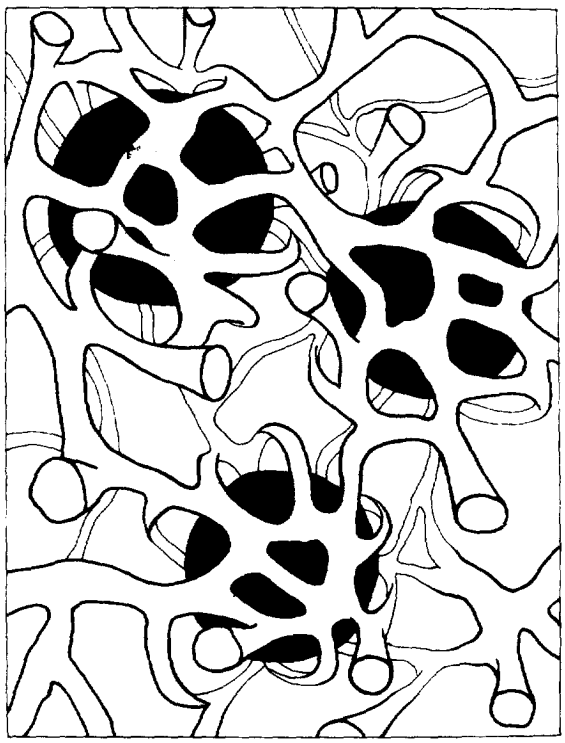

Fig. 1. Schematic illustration of a porous gel bead containing cavities with entrapped liposomes (Section 2.2). Drawing: H. Tylza.

densely packed 1-myristoyl-2-(13-carboxyltridecanoyl)-*sn*-phosphatidylcholine monolayer with the headgroups facing out from the matrix [56]. Non-reacted amine groups were first blocked with decanoic symmetric anhydrides and subsequently with propionic symmetric anhydrides until the surface became ninhydrin-negative [30,31]. The procedure has been extended to produce mixed-ligand IAM surfaces that mimic phosphatidylcholine combined with phosphatidylethanolamine, phosphatidylserine, phosphatidylglycerol and phosphatidic acid [30,31,57]. For detailed descriptions of the many synthesized variants, see Refs. [31,32].

2.4. Preparation of plant cells and plant cell walls for packing into columns

Suspension-cultured *Chenopodium rubrum* L. cells were packed into columns for chromatographic analysis [36]. Cell walls were prepared from the *C. rubrum* cells by repeated sonication and washing for packing into chromatographic beds for separations utilizing the relatively large cell wall pores [36]. Cultured *C. album* cells formed multicellular clusters which were lipid-extracted and treated with pancreatic enzymes to obtain cell wall aggregates for similar use [38,41]. The cell-wall composition of various preparations is described extensively in [43], as are the effects of ethanol extraction, proteolysis and sodium carbonate treatment on the size of the cell-wall pores in [42].

3. Methodology

3.1. Transport retention chromatography (TRC)

Cells or proteoliposomes that are immobilized in a chromatographic column allow separation of solutes that can pass the membrane from those that cannot. Ideally, the difference in elution volume will be equal to the inner volume of the immobilized structures. This may be achieved for a solute that is transported across the membrane with the aid of a membrane protein, versus another solute that is excluded [4,9,10,21]. If the permeability barrier is imperfect, or if equilibrium does not prevail, the separation will decrease. The separation principle resembles that on which the chromatographic dialysis separation on plant-cell microcapsules is based [44], but the selectivity is entirely different. With suitable cells or reconstituted systems, TRC can separate a bioactive, transported enantiomer from an inactive, non-transported form of the substance.

3.2. Biomembrane affinity chromatography (BAC)

BAC experiments have been done in two modes, frontal and zonal analysis. In both modes a stationary phase of immobilized biomembrane structures (cells, membrane vesicles or proteoliposomes) has been used. A solute with specific affinity for a membrane protein of interest is applied to the column and will be retarded to a certain extent depending on the binding strength, non-specific interactions, the amount of protein and the solute concentration. The chromatographic methodologies and theories for

quantitative evaluation of data from obtained elution profiles have been described in detail elsewhere [11–13,58–61].

For frontal affinity chromatographic analysis [12,13,58,60,61], a large sample volume is applied to obtain an elution profile showing a front and a plateau region the height of which corresponds to the concentration [B] of an applied interactant B. Equation (8) in [60] applies:

$$V = V_{min} + P_t/([B] + K_{BP}^{-1}) \quad (1)$$

The elution volume, V, of the front thus equals the sum of the elution volume V_{min}, i.e., the non-specific elution volume obtained when the specific interaction is completely suppressed, and the elution volume corresponding to the specific interaction. P_t is the amount of immobilized operative binding sites and K_{BP} is the association constant for the interaction. By inclusion of another competitive interactant, X, in the eluent, the corresponding association constant for the interaction between X and the protein can be determined. Non-linear regression analysis may be used with advantage.

In a similar way, interactions with the immobilized protein can be studied by use of zonal analysis [11,59], whereby small-volume samples of interactant are applied while the second interactant X is included in the eluent at a series of concentrations. Quantitative analysis in the zonal mode generally requires less sample and is faster than frontal analysis, but suffers from drawbacks associated with approximations that have to be applied in the evaluation [62].

3.3. Immobilized artificial membrane (IAM) chromatography

The experimental approach to membrane protein purification on IAMs is largely empirical, since IAM surfaces have both hydrophobic and polar groups and resemble, in part, reversed-phase, hydrophobic-interaction and ion-exchange phases [31]. When the separations were performed with detergent(s) in the eluent, as was frequently the case in membrane-protein purification, the stationary phase was composed of both the immobilized lipid analogues and detergent molecules partitioning into the monolayer. Some applications take advantage of protein affinity for the monolayers [29–32]. In contrast to entrapped membrane structures, covalently immobilized IAMs are characterized by high stability against the effects of non-aqueous solvents and detergents, but cannot properly accommodate integral membrane proteins.

3.4. Cell wall chromatography

Chromatography on plant cell walls resembles dialysis techniques, but shows a high separation efficiency. The pore size of the deproteinized hemicellulose–cellulose walls for a material prepared in a given way typically fell between 4 and 6 nm, and could be increased by controlled decay of the pectin (polygalacturonan) of the cell walls by heating in alkaline solution [42,43]. A method for characterization of the size-permeation limits of cell walls has been described [63]. The cell-wall chromatographic method strictly separates excluded molecules that are eluted at a volume corresponding to about 40% of the gel bed volume from molecules that enter the cell-wall vesicles and are eluted near the total bed volume [42]. The separation is influenced by an ion-exclusion effect, since the cell wall pores are lined with carboxylate groups [38–44].

4. Chromatographic applications

4.1. Chromatography on red cells

Human red cells adsorbed to gel particles were employed for analysis of the activity of Glut1 in the native red cell membrane [4]. TRC (Section 3.1) partially separated D-glucose from L-glucose, since D-glucose was transported by Glut1 whereas the membrane was impermeable to L-glucose [4] (Fig. 2). The difference in elution volume was much larger than in corresponding experiments with proteoliposomes immobilized on hydrophobic ligands [9,10], probably owing to leakage of the sugars through the liposomal lipid bilayers, whereas the internal volume of the membrane compartments and the number of Glut1 molecules were about the same in the compared systems. The red cell TRC elution profiles were reproducible over a period of 10 days, provided

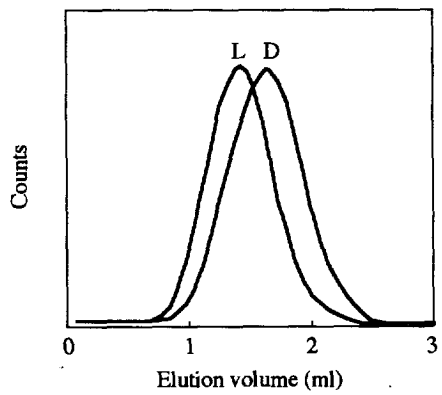

Fig. 2. TRC of D-[^{14}C]glucose (D) and L-[^{3}H]glucose (L) on human red cells adsorbed to polymer particles. In an experiment with cytochalasin B (2 μM) in the eluent, the stereoselective retardation of D-glucose was completely inhibited (not shown). Bed dimensions: 10 mm (diam.)×14 mm. Temperature: 23°C. Eluent: isotonic phosphate-buffered saline (pH 7.4) containing 5 mM KCl and 1 mM ATP. Flow-rate: 0.1 ml min^{-1}. Sample volume: 300 μl. (Reprinted from [4], with kind permission).

that the cells were supplied continuously with an eluent of suitable composition.

Quantitative affinity chromatography on the red cell column showed that D-glucose and the competitive inhibitor cytochalasin B had high affinities for Glut1. These affinities became weaker as the original composition of the lipid surroundings of Glut1 was changed upon stripping and reconstitution [4,5,12,13] (Table 1), which emphasizes the importance of neighboring membrane components for the Glut1 activity. In contrast the affinity of Glut1 for the competitive inhibitor forskolin was seemingly not affected by the Glut1 environment.

Table 1
Dissociation constants (K_d) for the interactions between human red cell Glut1, D-glucose, forskolin and cytochalasin B

Object	K_d		
	Glucose (mM)	Forskolin (μM)	Cytochalasin B (nM)
Red cells	6.8[a]	1.8[a]	33[b]
Red cell membranes[c]	17[d]	1.7[b]	52[b]
Glut1 proteoliposomes[e]	46[d]	1.8[b]	72[b]

pH 7.4, 23°C, I=0.15–0.17. Determinations by frontal affinity chromatography.
[a] Value from [4].
[b] Value from [5].
[c] Depleted of peripheral proteins [64].
[d] Average of values from [5,13].
[e] Glut1 was prepared from red cell membranes depleted of peripheral proteins and reconstituted with egg phospholipids [18].

The fact that red cells adsorbed in the gel particles showed satisfactory chromatographic results indicates that other cells or vesicles may similarly be immobilized for chromatographic purposes. However, the task is demanding, since the carrier material must be suitable for immobilization, compatible with the activities of the cell components that are to be studied, rigid enough for the purpose of chromatography at a reasonably high flow-rate, and allow rapid equilibration of solutes between the mobile and stationary phase.

4.2. Chromatography on red cell membrane vesicles and proteoliposomes

Quantitative affinity chromatography was done on cytoskeleton-depleted red cell membrane vesicles or Glut1 proteoliposomes immobilized by freeze–thawing [5,11–13,15–18] or by use of the biotin–avidin system [14] for analyses of interactions between Glut1, D-glucose and the inhibitors cytochalasin B and forskolin (Table 1). The striking observation was made that the immobilized transport protein retained its cytochalasin B-binding and glucose affinity at a nearly constant level over time periods of 1–3 months at room temperature (Fig. 3) [12,13]. Such long-term stability of a reconstituted mammalian membrane protein is encouraging in attempts at using membrane proteins for separation and analysis, in enzyme reactors, biosensor systems and other biotechnological systems and for crystallization. Furthermore, the freeze–thaw immobilization procedure used recently allowed a relatively high linear flow-rate, about 5 cm min^{-1} [15], with little loss of entrapped material, which is important for practical use.

An example of separation of the above solutes on a column with immobilized membrane vesicles is shown in Fig. 4, demonstrating that BAC may be applied for preparative purposes.

For the general application of BAC, further investigation of the various immobilization techniques would be useful. The procedures of freeze–thawing,

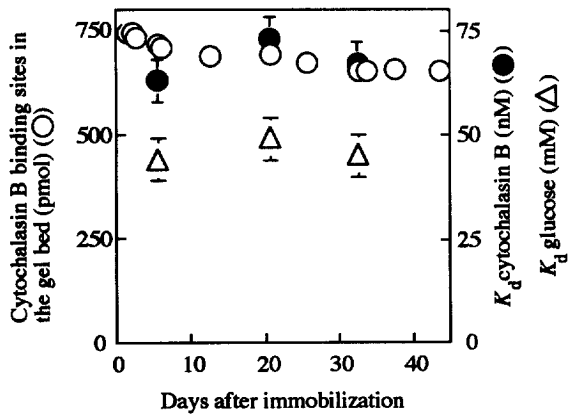

Fig. 3. Stability of immobilized Glut1 at room temperature. The given amount of binding sites and K_d values were determined by frontal BAC (see Section 3.2). Glut1 was purified from human red cell membranes depleted of peripheral proteins, and was reconstituted with egg phospholipids by gel-filtration detergent depletion [20,21]. The proteoliposomes were sterically immobilized in Superdex 200 prep grade gel beads by a freeze–thaw procedure (see Section 2.2). Bed dimensions: 5 mm (diam.)×48 mm. Eluent: 150 m*M* NaCl, 10 m*M* Tris–HCl, 1 m*M* EDTA (pH 7.4). Data from Fig. 3 of [13] and unpublished data from the same series of experiments.

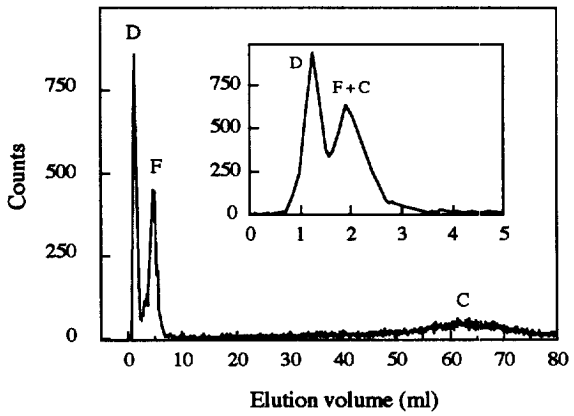

Fig. 4. Affinity separation of D-glucose (D), forskolin (F) and cytochalasin B (C) on Glut1 in human red cell membrane vesicles depleted of peripheral proteins and sterically immobilized in Superdex 200 (see Fig. 3) gel beads by freeze–thawing (see Section 2.2) (unpublished work by A. Lundqvist). Bed dimensions, temperature and eluent: see Fig. 3. Flow-rate: 1.0 ml min^{-1}. Sample mixture: 100 μl of 17 n*M* [^{3}H]glucose, 33 n*M* [^{3}H]forskolin and 100 n*M* [^{3}H]cytochalasin B. Elution volumes: 1.25, 4.70 and 63.5 ml. Inserted panel: the elution profile after inactivation of Glut1 by use of eluent supplemented with 5 m*M* $HgCl_2$. Only non-specific retardation remained; forskolin and cytochalasin B were eluted at 1.95 and 2.05 ml, respectively, as shown by separate runs. Glucose was eluted essentially as with the active Glut1. K_d values for the interactions with Glut1 are given in Table 1.

adsorption to hydrophobic ligands and affinity coupling by use of the biotin–avidin system must be considered in relation to the requirements for suitable pore and cavity sizes. Novel gel materials may be needed for entrapment or adsorption of vesicles and proteoliposomes containing large proteins or protein assemblies.

To further test the applicability of the methodology, our research group has successfully studied the interaction of the nucleoside transport inhibitor, nitrobenzylthioinosine, with the nucleoside transporter from red cells by use of the BAC technique (L. Haneskog, unpublished data).

Non-chromatographic applications of the immobilization methods are possible. For example, a proteoliposomal enzyme reactor [65] has been prepared by from lipids and protein by dialysis-detergent depletion in the presence of gel beads [46]. Membrane proteins can also be incorporated into lipid bilayers prepared by addition of lipids to a phosphatidylethanolamine monolayer covalently coupled via the headgroups to a carrier [66] and by adsorption of phospholipids to magnetite (Fe_3O_4) cores, whereby magnetoliposomes are formed consisting of lipid bilayers enwrapping the cores [67,68].

4.3. Chromatography on lipid bilayers or liposomes

Proteins were adsorbed onto the liposome surfaces and subsequently separated by salt gradient elution on charged liposomes formed and entrapped in gel beads upon detergent-depletion by dialysis [19–21,47]. Human serum proteins [19] as well as monomers and dimers of bovine serum albumin (Fig. 5) were resolved by this ion-exchange chromatographic technique. Both protein size and charge thus affected the result. Saturation of the liposome surfaces led to electroneutrality with densely packed protein molecules as counterions to the surface charges [20,21]. Both the net charge and the size of the adsorbed proteins appeared to be decisive factors owing to the low surface density of the charged

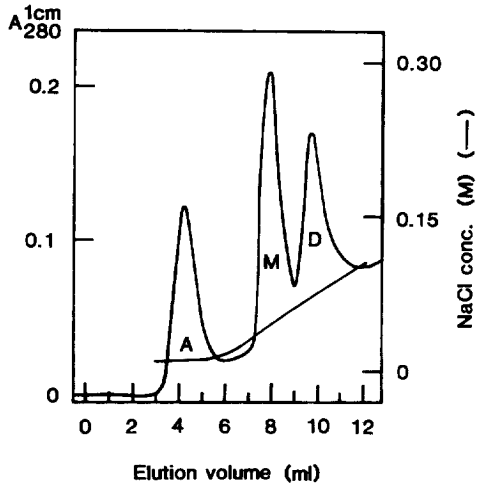

Fig. 5. Ion-exchange chromatographic separation of monomeric (M) and dimeric (D) bovine serum albumin on positively charged liposome surfaces. Zone A contained no protein. The liposomes were formed and entrapped in Sepharose 6B gel beads (Pharmacia) upon dialysis of a cholate solution of egg phospholipids and stearylamine mixed with the beads. Bed dimensions: 10 mm (diam.)×29 mm. Temperature: 23°C. Starting buffer (S): 10 m*M* Tris–HCl (pH 8.5), 0.1 m*M* EDTA, 0.2 m*M* mercaptoethanol. Sample: 0.2 mg of each component in 0.2 ml of buffer S. Elution: NaCl gradient in buffer S. (Reprinted from [19], 1990, with kind permission).

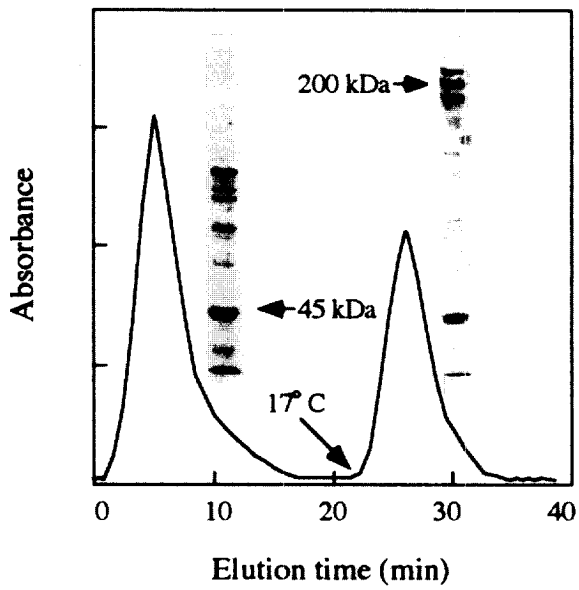

Fig. 6. Charge-selective phase transition chromatography of a human platelet lysate using temperature-controlled dimyristoylphosphatidylglycerol domain structures in dielaidoylphosphatidylcholine lipid bilayers in a silica gel (Transil-A, Nimbus Biotechnologie, Leipzig, Germany). Lysate proteins adsorbed at 4°C were released at 17°C. SDS-PAGE analyses of the zones are inserted. (The figure is redrawn from Fig. 4, top panel, of [22], with kind permission).

molecules. The fact that the stationary charged molecules can diffuse laterally in the lipid bilayers give this method special properties that deserve further study.

Lipid bilayer phase transitions could be temperature-controlled for protein separation on a bilayer of dielaidoylphosphatidylcholine supplemented with the anionic lipid dimyristoylphosphatidylglycerol on silica gel [22]. When proteins were applied below the transition temperature of the bilayer, negatively charged proteins passed through the gel bed, whereas positively charged ones became bound to domains of high negative charge density formed in the gel phase. The latter proteins were released at the onset of lateral diffusion in the fluid phase when the temperature was increased (Fig. 6). However, in this thermodynamic mode of ion-exchange chromatography also the separation reflected differences in protein size.

Immobilized neutral liposomes showed weak interactions with peptides upon chromatography. C-terminal Cys caused retardation (Fig. 7), as did the presence of hydrophobic side chains, to judge from the relationship between water-to-oil transfer free energy and chromatographic capacity factor [23]. Similar Cys-dependent peptide separation was achieved by isocratic IAM chromatography [56]. For micro-scale applications, electrophoretic analyses of peptide separation and drug partitioning have been done with liposomes as a pseudo-stationary phase (included in the running buffer) [27], and freshly prepared liposomes have been adsorbed by hydrophobic interaction in derivatized continuous beds for chromatographic analyses of drug partitioning [26]. Peptide studies should be feasible also with the latter technique.

Analysis of peptide–lipid bilayer and peptide–membrane protein interactions by utilizing the chromatographic or electrophoretic techniques could prove to be useful for characterization of peptide drugs and other peptides with biological activities, and for comparison with interaction analyses on

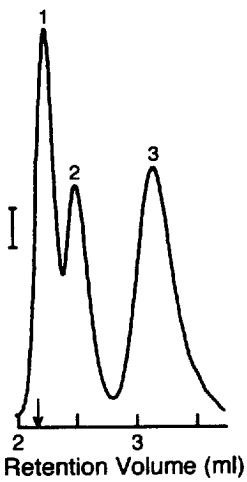

Fig. 7. Separation of the peptides YGSTWPG (1), YGSTWPGC (2), and YGSTWPGCC (3) on egg phosphatidylcholine liposomes immobilized in Superdex 200 by freeze–thawing (see the legend to Fig. 3). Bed dimensions: 5 mm (diam.)×100 mm. Temperature: 23°C. Eluent: 150 m*M* NaCl, 10 m*M* sodium phosphate buffer (pH 7.4). Flow-rate: 0.08 ml min^{-1}. Sample volume: 20 μl. Peptide concentration: 0.3 mg ml^{-1} for each peptide. Bar: A_{220} = 0.05. (Reprinted from [23] with kind permission).

liposomes in suspension as well as on supported lipid bilayers, for example, by plasmon surface resonance technique. An issue to be addressed is to what extent various amphiphilic solutes interact with the polar headgroup region of the liposome surfaces, and to what extent they penetrate the bilayers.

4.4. IAM chromatography and related methods

IAM surfaces [31,32] mimic the structure of biomembrane lipid bilayers. These monolayers, consisting of phospholipid analogues and capping alkanoic anhydrides covalently coupled to silica beads, have been used for membrane protein purification [28–32] and immobilization of microsomes as enzymatic reactors [34,35]. Isoenzymes of cytochrome P-450 and cytochrome P-450 oxidoreductase [28], a cholesterol-transfer protein [29], an N-acylphosphatidylethanolamine synthase [30], and lipases [31,32] are among the purified proteins.

Glycolipid monolayers can be immobilized on paramagnetic beads by hydrophobic adsorption, conjugation by use of methods of the Hakomori type [69] or by use of biotin–avidin systems for manifold applications with gentle and rapid batchwise handling, as reviewed by Rye [70].

4.5. Chromatography on plant cells and cell wall fragments and related techniques

The first example of chromatography on native cells may be represented by chromatographic analysis of the cell-wall pore size of cultured *C. rubrum* plant cells. These cells retained the rate of 3-O-methylglucose efflux for 8 h [36]. Furthermore, stable, aggregated *C. album* cell walls (Fig. 8) prepared from suspension-cultured plant cells [38] were packed into columns for chromatographic size-group fractionation of proteins [41,44] and nucleic acids [40]. Aggregated recombinant protein could be separated efficiently from the monomer [44] and blood cells from urea, myoglobin and vitamin B_{12} [39].

The macromolecular charge also affected the separation due to the presence of several carboxylate groups in each cell wall pore. The ion-exclusion effects were particularly useful for separation of highly charged materials such as nucleic acids [40]. By changing from a high-salt to a low-salt buffer, nucleic acids could be entrapped in the microcapsular aggregates for fractionated release [71].

Plant cells show intriguing properties for other applications. The ability of plant cells to bind metal ions 'has fostered considerable interest in the development of alternate methods for heavy metal

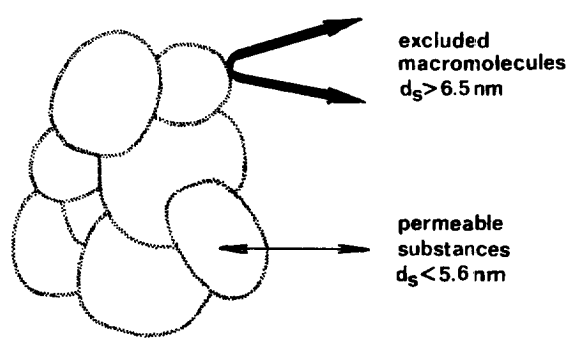

Fig. 8. Schematic illustration of a cluster of porous microcapsules obtained by preparation of plant cell walls with indication of the approximate size selectivity for macromolecules. Stokes' diameters are denoted d_s. (Reprinted from [41], 1992, with kind permission).

removal and recovery from contaminated water' [72]. It is known that Sacred Datura *(D. innoxia)* tolerates heavy metals, and fragments of cultured *D. innoxia* antheral cells show a potential for heavy-metal adsorption for environmental protection purposes [73]. Such material has been immobilized in a polysilicate matrix and packed into columns for frontal affinity chromatographic characterization of metal ion binding properties [73].

In an attractive non-chromatographic utilization of cell wall materials, subunits of *Bacillus coagulans* crystalline cell surface layers (S-layers) were recrystallized on positively charged stearylamine–phosphatidylcholine–cholesterol liposomes, cross-linked with glutaraldehyde and used for oriented adsorption and ensuing covalent coupling of macromolecules in a densely packed manner [74] (Fig. 9). This type of material, which offers several potential applications, has recently been used for immobilizing monolayers of glucose oxidase for the development of a fibre-optic micro-integrated glucose biosensor

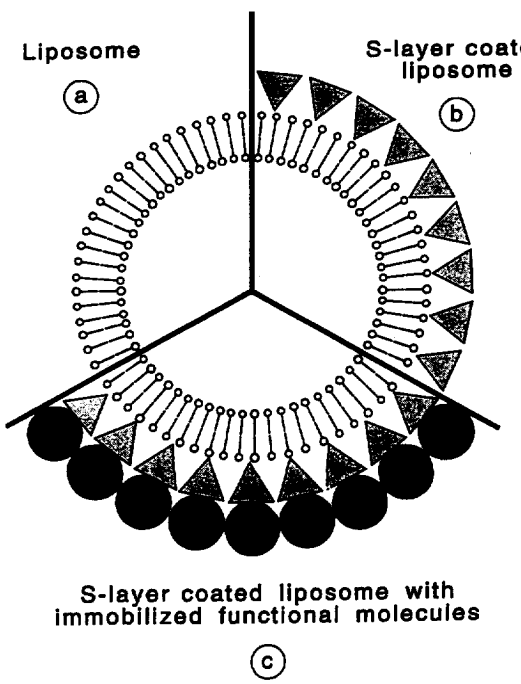

Fig. 9. Schematic illustration of a liposome (a) coated with a crystalline surface layer (b) covalently coupled to functional macromolecules (c). (Reprinted from [74], 1995, with kind permission).

[75], and could possibly also be immobilized in gel particles or beads for chromatographic use.

5. Conclusions and future perspectives

The materials of biological origin that have been used as chromatographic stationary phases for separation and interaction studies show a variety of properties and widely different stabilities. Novel materials, useful matrices and various modes of application are being developed or can be anticipated. Research on the preparation of chromatographic support materials for entrapment or adsorption of cells or biomolecular assemblies and, in many cases, on details of the separation mechanisms, would be helpful to extend the applicability of the methods. The usefulness of analyses and separations of interacting solutes on cells, subcellular particles or proteoliposomes is indisputable. Among several applications, BAC systems rnay, in the future, be used for screening and separation of interactants obtained from natural sources. However, a suitable detection system must be found and a set of columns of different bed sizes must be used to catch interactants of widely varying affinities and with differing numbers of binding sites. Preparative affinity separations on immobilized cells or biomolecular assemblies may become possible [76].

The plant cell-wall separation systems provide the unique property of sharp size and charge selectivity between included and excluded species. Future characterization of the cell-wall pore structure by electron-microscopy techniques or atomic-force microscopy would be valuable for correlation with the chromatographic data now available.

In the applications described in the present paper, most of the raw materials for preparation of the stationary phases are available in fairly large quantities, which has been advantageous for methodological development. The collected experience will facilitate applications of more rare biomaterials.

6. List of abbreviations

BAC	Biomembrane affinity chromatography
Glut1	The human red cell glucose transporter

IAM Immobilized artificial membrane
TRC Transport retention chromatography

7. Note added in proof

Entrapment of liposomes in gel beads (Superdex 200 prep grade, Pharmacia Biotech, Uppsala, Sweden) upon freeze–thawing (see Section 2.2 and Fig. 1) occurred in a thick shell in the outer region of the beads, whereas the bead cores did not contain any lipids (recent scanning laser confocal microscopy data, A. Lundqvist, G. Ocklind and P. Lundahl).

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