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AFFINITY CHROMATOGRAPHY OF CELLS AND CELL MEMBRANES

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1. INTRODUCTION

The principles and practice of affinity chromatography, as applied to macromolecules, have been the subjects of several excellent review articles[1–4] and therefore only a brief resume is presented here. The technique is based on the exceptional ability of biological active macromolecules to bind complementary ligands specifically and reversibly and is realised by covalently attaching the ligand to an insoluble support. In theory, only molecules with appreciable affinity for the immobilized ligand will be retained; others will pass through unretarded. Specifically adsorbed molecules can then be recovered by bioelution with a competitive counter ligand. In principle, affinity chromatography can be applied when any particular immobilized ligand interacts specifically with a biomolecule.

The last decade has witnessed an extensive development of affinity methods in the isolation of enzymes, their inhibitors, antibodies and antigens, nucleic acids, transport and receptor proteins, and a large number of other products. Two monographs[5,6] have been devoted to affinity chromatography dealing exclusively with these systems. The present widespread interest in and extension of this approach to other complex systems, such as cells and cell membranes, have prompted us to classify affinity chromatography into two groups, "molecular" and "cellular", as shown in Table 1.

TABLE 1

TYPES AND APPLICATIONS OF AFFINITY CHROMATOGRAPHY

Type	*Applications*
Molecular affinity chromatography	Enzymes, antibodies, antigens, binding or receptor proteins, complementary proteins, repressor proteins, denatured and chemically modified proteins, nucleic acids and nucleotides, concentration of dilute solutions, storage of otherwise unstable proteins in immobilized form, investigation of kinetic sequences and mechanisms, purification of synthetic macromolecules.
Cellular affinity chromatography	Cells, cell organelles, cell membranes, phages and viruses.

In the literature, affinity techniques which feature cells and cell membranes are variously described as affinity density perturbation, fibre fractionation of cells, immunoaffinity chromatography, or affinity partioning. In all cases, the reference is to affinity chromatographic systems which utilize solid support and a biospecific absorbent. No one title is completely informative. Moreover, these terms could result

in confusion in the long run, mainly from the academic point of view. Therefore, the present discussion is facilitated by using a more general term for these separation procedures, namely cellular affinity chromatography. Under the umbrella of the proposed definition, various terms will be used interchangeably in this review. It should be stressed that we have chosen the running title "cellular affinity chromatography" in the broader sense and will include cells, organelles, membranes, viruses and phages. The potential applications of both forms of affinity chromatography are summarized in Table 1.

The present topic, cellular affinity chromatography, is in its infancy at which stage a comprehensive review is possible without a flood of references. It includes all the published work up to December, 1979. Of work in a closely related approach, such as membrane isolation on cationic beads, citations are limited to a couple of entries into the literature.

2. PRINCIPLE

There is an obvious need in biomedical sciences for methods that utilize chemical differences at the cell surface as a basis for the isolation and fractionation of cell populations. It is well known that the cell membrane has a dynamic and complex structure having many characteristic functional roles. Affinity fractionation and purification of functionally different cells or cell organelles offer unique possibilities for achieving separations which are difficult and even impossible in some cases by using physical differences among cell types[7]. The technique of affinity chromatography exploits the specificity of the binding sites located in the surface of cells. The principle of cellular affinity chromatography is presented in Fig. 1. It allows cells to be selectively retained on an immobilized ligand which interacts specifically with a cell surface component of the adsorbed population. Cells may be recovered subsequently by adding a soluble competitive agent in the buffer. The basic requirements of the technique are the following:

(1) It should be possible to couple covalently a ligand molecule to an insoluble support which, when coupled, should still react biospecifically with the binding site in the surface membrane.

(2) The binding site should be available on the outer surface of the cell membrane.

(3) The system designed should allow recovery of cells by the use of a compound competing for the affinity sites.

(4) The elution should occur under conditions which are compatible with the maintenance of cell integrity and expression of bioactivity.

3. CHOICE OF MATRIX MATERIAL

The required attributes of an insoluble support, or matrix material, for cellular affinity chromatography are that it should: (a) be in a bead form; (b) be chemically and mechanically stable; (c) have good flow characteristics; (d) not physically entrap cells; (e) permit covalent coupling of biologically active molecules in an unaltered form; (f) not absorb cells non-specifically; (g) affect minimally if at all the viability of the chromatographed cells.

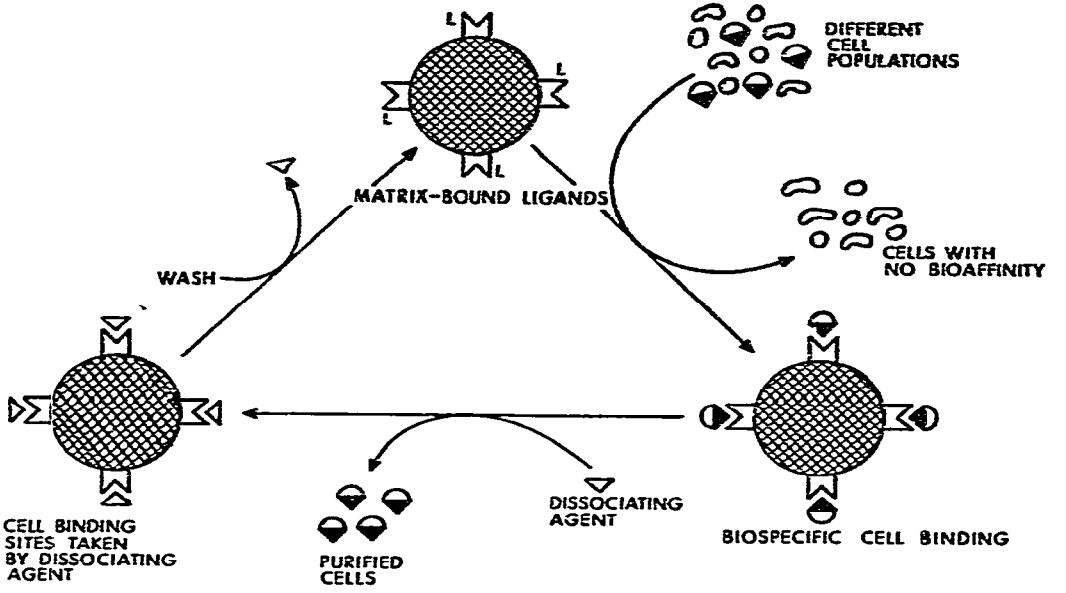


Fig. 1. Diagrammatic representation of cell sequestration by affinity chromatography. The hatched area represents the matrix material and L stands for the immobilized ligand.

Cell sequestration according to their binding affinity for a solid support, coated with a biospecific reagent, has been attempted in a variety of systems. Plastic, glass, polyacrylamide, nylon fibres and agarose have been used as a solid support[8–14] with absorbed or covalently attached substances such as lectins antigens, antibodies or hormones, providing the requisite specificity. The major obstacle in manufacturing solid matrices for receptor-specific cell separations is that these materials should be inert to be characteristic "sticky" properties of cells. Cells are readily adsorbed on to glass and other charged or hydrophobic materials[8,9] and consequently the usefulness of such solid supports is impaired by the non-specific adhesion of a large number of cells. In some studies, however, antigen-coated polyacrylamide[11,12] and agarose[13] have been found to be satisfactory for cell separation. The use of large polyacrylamide beads results in a considerably less non-specific retention of cells, although the problem still exists. On the other hand, activation of agarose with cyanogen bromide[15] fractures the polysaccharide beads and may lead to inefficient cell fractionation. A major disadvantage in employing nylon fibres[14] is the possible perturbing effect on the cell metabolism or function. This particular structure also extends to the method of removing cells from derivatized nylon fibres[14].

Apparently the various materials used have advantages and disadvantages. Although a solid support that fulfills all possible requirements has not yet been found, agarose in the form of a beaded gel seems to be the most generally acceptable. It is at present undoubtedly the most commonly used solid support. The main producers of agarose are Pharmacia (Uppsala, Sweden), under the trade-name Sepharose, and Bio-Rad Labs.* (Richmond, CA, U.S.A.) under the trade-name Bio-Gel A. Cyanogen

* Only firms known to the authors are mentioned. It should in no case be considered as implying the recommendation of any particular firm of product.

bromide-activated Sepharose 6MB i a specially designed product from Pharmacia with properties which make it the matrix of choice for cellular affinity chromatography.

4. AFFINITY LIGANDS

4.1. Lectins

Lectins are a group of plant proteins which share the ability to selectively bind to specific carbohydrate receptors. They have proved to be extremely useful as analytical tools in biochemistry and cell biology. Lectins exhibit a high degree of discrimination among complex carbohydrates[16] and have been widely utilized as probes for carbohydrates present on cell surfaces[17]. Because of their specific carbohydrate binding properties, lectins can be conveniently used for isolating and purifying cells or subcellular particles that contain specific carbohydrate groups which are fully exposed. Table 2 lists some of the commercially available lectins. Notably the usefulness of a particular lectin depends, to a large extent, upon its ability to interact specifically with one of a very limited number of monosaccharides (Table 2). It should also be kept in mind that the reaction of lectins with oligosacharides is highly complex depending not only on the type of monosaccharide present, but also on their sequence and the nature of the glycosidic linkages involved[18]. Recently it has been demonstrated[19] that a minimum of two interacting mannose residues are required for binding to concanavalin A (Con A), and the residues linked to these mannoses, such as sialic acid, can either strengthen or weaken binding to the affinity column[18].

4.2. Matrix-bound lectins

Immobilized lectins are attractive candidates for use in cellular affinity chromatography because all cells have carbohydrates on their surface and because the binding of these cells, in theory, can be reversed under gentle conditions by the addition of the specific lectin-binding sugar to the eluting buffer. Cells may be expected to be selectively separated on a matrix-bound lectin due to differences in the content or accessibility of surface sugars or simply because of differing binding affinities for the lectin. Some of the known immobilized lectins are now commercially available and the most commonly used are briefly described below.

4.2.1. Concanavalin A–Sepharose. Agarose-bound Con A is available under the name Con A–Sepharose. Sepharose 4B is linked to Con A by the cyanogen bromide activation method[15,20]. According to Pharmacia, the Con A content is about 8 mg per ml of swollen gel. It is supplied as a suspension in 0.1 *M* acetate buffer of pH 6, containing 1 *M* sodium chloride, 1 m*M* magnesium, calcium and manganese chlorides and 0.02% of merthiolate added as a protecting agent. The carbohydrate-containing moieties in the sample are adsorbed at neutral pH and, after washing out unbound components, are eluted simply and under gentle conditions using the competitive inhibitor, methyl-α-D-mannoside[21,22].

4.2.2. Lentil lectin–Sepharose 4B. It is a group specific adsorbent. Lentil lectin normally is of the same specificity as Con A (Table 2), but it shows a different discriminatory ability to oligosaccharides and may recognize an N-acetylglucosaminyl–mannobiose unit instead of the mannobiosyl–N–acetylglucosamine unit recognized by Con A[21]. It retains its binding characteristics in solution of sodium deoxycholate commonly employed for solubilizing components from cell membranes.

TABLE 2

CHARACTERISTICS OF SOME COMMERCIALLY AVAILABLE LECTINS

	Concanavalin A	*Lentil lectin*	*Wheat germ agglutinin*	*Ricinus communis agglutinin*	
				RCA 60	*RCA 120*
Source	Jack bean (*Canavalia ensiformis*)	Common lentil (*Lens culinaris*)	Wheat germ (*Triticum vulgare*)	Castor bean (*Ricinus communis*)	Castor bean (*Ricinus communis*)
Sugar specificity	α-D-Mannose like residues	α-D-Mannose like residues	N-Acetyl-D-glucosamine	N-Acetyl-L-galactosamine	D-Galactose
Molecular weight	51,000 (dimer)	52,000	36,000	60,000	120,000
Metal ion requirement	Ca^{2+}, Mn^{2+}	Ca^{2+}, Mn^{2+}	None known	None known	None known

4.2.3. Wheat germ lectin–Sepharose 6MB. The designation 6MB indicates that the swollen beads are of large size (macrobeads) and have a narrow range (200–300 μm in diameter) — properties that are essential for minimum entrapment of cells during the chromatographic separation. It consists of purified N-acetylglucosamine-specific lectin from wheat germ, covalently linked to Sepharose 6MB by the cyanogen bromide activation method[20]. It is available in suspension, 10 ml of sedimentated gel in 0.9% sodium chloride solution containing 0.01% merthiolate as protecting agent. In order to avoid the dissociation of lectin into sub-units, the gel should be in a medium of pH higher than 3.5. The manufacturers recommend storing the gel in a refrigerator at 3–8°C.

4.2.4. Lectin–glycoprotein–Sepharose. A new affinity system, consisting of lectins specifically adsorbed to a glycoprotein (hog gastric mucin blood group A+H substance) that is, in turn, covalently linked to Sepharose, has recently been suggested for cell sequestration[23]. In this case crude preparations of lectins may be used, separate coupling of each lectin to Sepharose is not required, and non-biological adsorption does not occur. Moreover, systems of different specificities can be constructed by adsorbing a lectin to a single glycoprotein–Sepharose conjugate. The cells bound to such a system are readily recovered (together with the lectin) with a specific sugar.

4.3. Ligands other than lectins

4.3.1. Scope of applications of macrobeads of Sepharose 6MB. Cellular affinity chromatography on macrobeads of Sepharose may be used to purify cells or cell organelles by two different ways. In the first of these, the affinity adsorbent is employed to selectively recover all cells possessing a particular surface marker. The remaining cells, which do not carry this marker, will pass through the adsorbent unretarded and comprise the fraction of interest. Protein A, a receptor-specific protein from *Staphylococcus aureus*, coupled to Sepharose 6MB is especially vital for this type of purification[24] since it can be applied to cells bearing any surface antigen to which antibodies of the immunoglobulin G type can be raised. Physical entrapping of cells and non-specific adsorption to Protein A–Sepharose 6MB is negligible and the adsorbent can be utilized more than 40 times without showing a significant decrease in capacity[25].

The second approach is to bind selectively cells of interest to a suitably chosen ligand–macrobead derivative. Unbound cells and soluble contaminants can be eliminated by washing, following which the cells are recovered by bioelution with a competitive counter-ligand. This strategy has been adopted to successfully purify acetylcholine receptor-bearing neuron cells from sympathetic ganglia[26]. A 95% pure fraction of viable and electrically active neuron cells was obtained using Sepharose 6MB coupled with α-bungarotoxin[26].

4.3.2. Related media. Obviously Sepharose 6MB shows great promise of being able to yield defined populations of cells, separated on the basis of their surface parameters. Nevertheless, Sephadex G-10, originally developed for other purposes, has been found to be useful for special applications in cell sequestration[27]. An elegant example of a special application of a well-known gel filtration medium is the preparation of platelets by a rapid and non-disruptive procedure involving chromatography on Sepharose 2B (ref. 28). But more elaborate claims for the use of gel filtration medium in cell isolation must be viewed with utmost caution.

4.3.3. Non-lectin ligands. Apart from lectins, cell separation on affinity columns could also exploit hormones, neurotransmitters and related ligands — as long as they exhibit high affinity for surface receptors that are cell specific, and can be effectively coupled to appropriate gel matrices. For example, neurones have neurotransmitter receptors which provide one basis for functional classification and potentially offer targets for affinity probes of high specificity[26]. In a reference to membrane binding to insulin–agarose cited as unpublished data in a review[29], the possibility of utilizing immobilized hormone derivatives to separate cell populations according to specific functions was suggested. Indeed, Venter *et al.*[30] have advocated the potential of drugs and hormones, covalently bound to glass or Sepharose beads, in the isolation of cultured tumor cells. Furthermore, the use of competitive enzyme inhibitors in cellular affinity chromatography remains an attractive possibility. It would, however, be crucial to predetermine that the binding site on the membrane surface be externally exposed under the experimental conditions. In short, details of membrane-bound enzymes provide many future challenges in problems related to cellular affinity chromatography.

5. TECHNIQUES

5.1. Fibre fractionation of cells

5.1.1. Principle. This method separates cells on the basis of their ability to be bound specifically and reversibly to strung fibres derivatized with molecules such as antibodies, antigens, or lectins[31–33]. The basic principle underlying this approach is depicted in Fig. 2. A petri dish containing a polyethylene frame with strung nylon fibres (the length of the largest fibre being 2.5 cm) makes a very simple, efficient and inexpensive separation device. Sequestration can be accomplished by specific binding to a component on the cell surface, or by differences in the binding affinity, or on the basis of number and distribution of cell surface receptors of the same specificity.

In practice, affinity ligands are coupled in a suitable chemical form with nylon fibres strung on a frame. The cells are then agitated with the fibre in a suitable medium and the non-sorbed cells are washed away. The adsorbed cells then may be transferred

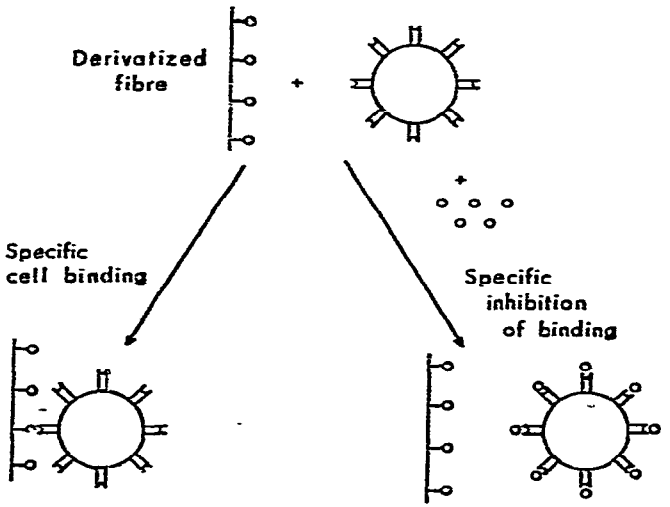


Fig. 2. General scheme of fibre fractionation[33].

into another medium for further characterization or they can be set free into the medium by plucking them from the taut fibre with a nedele. This mechanical step serves to shear the cells from their points of attachment. Affinity ligands can also be coupled with the fibres through special linkers, allowing the release of cells by a specific chemical or enzymatic cleavage.

The fibre fractionation method (Fig. 2) is applicable to a variety of cells. Using the lectin–Con A as the binding agent, Edelman and co-workers[31] were able to fractionate a mixture of thymocytes and erythrocytes, and with antigens as the binding agents, a specific isolation of immune cells was achieved[34]. The use of antigen-derivatized nylon fibres[35] provides a possible approach for the quantitative study of clones of committed cells in immunized and unimmunized animals. A classical example is the isolation of antigen-binding cells from spleens of immune and non-immune mice[34].

5.1.2. Advantages. This technique has a number of merits over other fractionation procedures.

(1) Many cells have a natural tendency to adhere to surfaces, and a serious difficulty encountered with bead column methods is the non-specific binding of cells[8,9,36]. With fibre fractionation, the simple centrifugation of solid support minimizes non-specific binding of cells.

(2) The simple spatial arrangement of the fibres permits direct observation and quantitation of the cells.

(3) The fractionated cells can be manipulated on the fibres under a variety of conditions, and the behavior of single cells can be monitored throughout an experiment.

(4) As with column methods, cells that bind to fibres are firmly attached and cannot in general be removed by incubation with a competitive inhibitor alone. They can, however, be rapidly and quantitatively released by plucking the taut fibre with a needle. The mechanical method of removing cells also has an advantage over bioelution in that it is not linked to cases in which a competitive inhibitor of binding is available.

5.1.3. Problems. In the application of fibre fractionation techniques to cellular systems other than the immune systems there are two major problems to be considered: dissociation of the cells and choice of ligand for the fibre. Apparently an important concern in cell dissociation is that the procedure employed be chosen to avoid the loss of receptors from the cell surface. On the other hand, the choice of ligand depends upon the system and specific purposes of fractionation. Some common ligands include lectins, enzymes, hormones, and antibodies directed against cell surface antigens.

5.2. Cell sequestration by immunological ligands

The complex heterogeneity of cell populations in the central nervous system severely limits the study of many important questions in neurobiology. An innovative approach to resolve complex cell populations would be to utilize immunological techniques, because cell surface has been shown to encode specificities for discrimination among cell types. There are three different approaches that are currently being used in conjunction with immunological ligands: fluorescence activated cell sorting, magnetophoresis, and immunoaffinity chromatography. We will examine each ap-

proach briefly as major emphasis in this section is focused on immunoaffinity approach.

5.2.1. Fluorescence activated cell analysis and sorting. The principles of this technique have been described elsewhere[37]. Here it is sufficient to state that this technique provides both unique data analysis and sorting capabilities on a cell-by-cell basis in relatively large quantities. The maximum sorting rate, without sacrificing specificity is approximately $5–10 \cdot 10^6$ cells/h. Analysis of both cell surface and intracellular moieties of viable cells has been demonstrated using fluorogenic substrates and fluorescent ligands. In order to separate viable cells for functional studies, attention has been primarily on the immune system as well as on the central nervous system[38,39].

5.2.2. Magnetophoresis. In this approach the ligand is bound to magnetic microspheres[40]. The magnetic microspheres bind selectively to the cells of interest, and these cells can be resolved by passage through a divergent magnetic field. In contrast to the fluorescence activated approach, there is no limitation on the number of cells to be separated. Cell-by-cell analysis is not feasible; separation may be affected only on the basis of cell surface moieties. This technique has been successfully employed to separate oligodendrocytes from mouse cerebellum[41].

5.2.3. Immunoaffinity chromatography. A more generally applicable immunoaffinity approach would be of immense value in cell biology, immunology, neurobiology, and virology. The requirement for specificity suggests the use of a method employing solid supports coupled to proteins capable of binding the cell-surface components. Wigzell and Anderson[36] first introduced affinity chromatography for the removal of antigen-binding cells. Subsequently, immunoadsorption of cells to an antibody-coated polyurethane foam was reported by Evans and co-workers[42,43]. In these studies, a high capacity for the binding of cells was demonstrated by using erythrocytes and polyurethane foam coupled with anti-erythrocytic globulin as a model system. The specificity of the foam-bound antibody for cell-specific antigens was shown by using erythrocytes attached to artificial haptens[43]. At about the same time, specific methods for the isolation of antibody-forming cells were reported with various degrees of success[44–49]. In addition, there have been some recent publications on the immunoaffinity chromatography of cells[50–52]. However, the applicability of this approach, in general, is limited by the fact that the structures of the surface components are usually not known, nor are they generally available in soluble form for use as competitive inhibitors of cell binding.

When fractionating a complex tissue such as brain, experiments should logically begin by separating it into its constituent cell classes. Unfortunately, at this time there are practical limits on following this up. Cell sequestration on immunoaffinity columns is only the first necessary step towards the more ambitious goal of neuronal cell separation by affinity methods[53].

Finally, it may be possible to extend the advantages of the immunoaffinity approach to the concurrent use of other cell-specific ligands, by pretreating the cell suspension with a selective ligand and sequestering the ligand-coated cell on gels derivatized with antibody against the ligand itself.

5.3. Cell–column chromatography

Conversely, a technique for immobilizing whole cells on a solid support in order to fractionate cell specific cell-binding components has recently been explored[54].

It is called cell–column chromatography and requires glutaraldehyde-fixed cells immobilized on Sephadex beads. By adopting this strategy, immunoglobulins that specifically bound and agglutinated the same cells as those originally fixed on the column were isolated from non-immune sera of various species[54]. The cell–column method appears to be valuable for the isolation of a variety of antibodies directed against cell surfaces.

5.4. Affinity density perturbation

There is now abundant evidence that biological membranes are fluid in nature with constituent proteins and lipid molecules able to move relative to one another in the plane of the membrane[55–58]. Studies of cellular regulatory processes have been focused in recent years largely on the plasma membrane. Thus, cell activation by some hormones, the induction of differentiation or the activation of lymphocytes is initiated by the interaction of ligands (*i.e.* hormones, mesenchymal factors, or antigens or lectins, respectively) with specialized structures of the outer membrane[59–63]. A variety of membrane changes have been described which occur after the binding of a ligand to the membrane. For studies of these triggering events, it is necessary to isolate these interesting membrane areas that carry the corresponding receptors. In 1973, Wallach *et al.*[64] described a novel affinity approach to isolate such membrane areas[65,66]. It is called affinity density perturbation and its principle is illustrated in Fig. 3.

Membranes are basically, first physically sheared into minute vesicles. Particles of higher density are added to the membrane fragments carrying the given receptors, to which a specific affinity ligand for the isolated receptor is covalently coupled. The complex is rapidly centrifuged to its isopycnic density, which is higher than that of the perturbant (ligand phage). For convenience of localization and quantification, the membranes and affinity ligands are radioactively labelled with different isotopes. The formation of specific membrane–ligand complexes can be blocked or reversed, if desired, by the addition of reagents with a higher affinity for the receptor, or by an excess of receptor analogue with similar affinity.

A model system is the use of Con A, labelled with ^{125}I, as an affinity ligand[64]. Con A was converted into a density perturbant by glutaraldehyde coupling to purified Coliphage K 29, a stable icosahedron of diameter of 450 Å. The membrane fragments were prepared from hog lymphocytoplasmatic membranes and contained large number of Con A receptors[67]. Interaction of the receptor-bearing membrane fragments with the perturbant reversibly increased the buoyant density in a caesium chloride gradient from about 1.8 for untreated membranes to a broad layer with a marked density at 1.30–1.40. This relatively broad density distribution of the membrane–Con A–K 29 complex shows microheterogeneity in the distribution of receptor sites. Addition of excess of α,α-trehalose which does not possess too great an affinity for Con A ($k = 5.38 \cdot 10^{-3}$ l/mole), was used for dissociation of the complex of Con A with its receptor.

Density-perturbing particles can be made visible under an electron microscope, which enables the receptor topology to be mapped. In principle, therefore, density perturbants may be linked to transmitters, hormones, drugs, specific antigens or specific immunoglobulins, and be employed not only to isolate receptor domains but also to map membrane and cell topology[64–66].

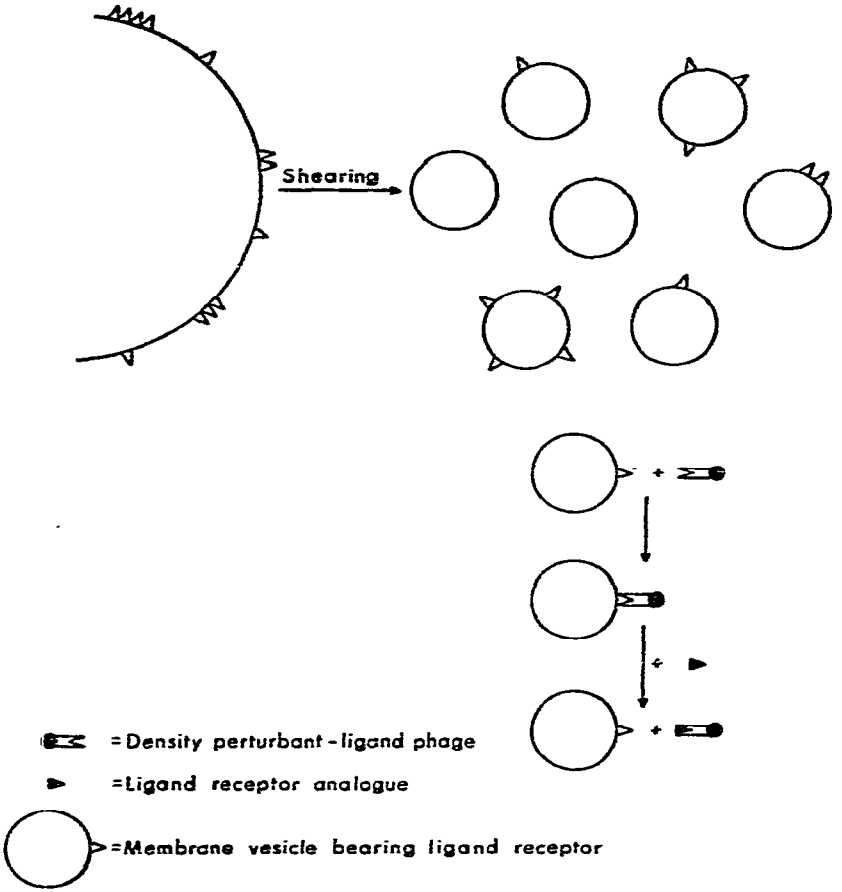


Fig. 3. The principle of affinity density perturbation[64]. A plasma membrane bearing multiple receptors (△) is sheared into membrane fragments carrying different number of receptors in varying distribution. These are reacted with the ligand (Σ) coupled to the density perturbant (●), producing a membrane-receptor–ligand–phage complex. Addition of a low-molecular-weight dissociating agent (▶) returns the membrane and density perturbant to their original densities.

5.5. Affinity-binding buoyant density method

Based on a principle similar to affinity density perturbation[64], Soderman and co-workers[68–70] developed a parallel procedure which takes into account the buoyant density. Operationally it was observed that lymphocytes, which normally float in physiological media, associate with sedimentable insulin–Sepharose beads to produce complexes that either floated or sank depending upon the ratio of the concentrations of cells to beads. A mixture containing viable fat cells and unmodified Sepharose beads rapidly separated into a top layer of cells, followed by a clear infranatant and a sediment of beads. When Sepharose was replaced by insulin–Sepharose, all of the beads floated with the cells, whereas when an excess of insulin–Sepharose was included, cells sedimented with the beads. Apparently the number of cells bound per insulin–Sepharose bead determined the buoyancy of the resultant complex. Interference microscopy was used as a tool to confirm the binding of whole cells to the

beads[69,70]. In addition, treatment of the cells with trypsin, anti-insulin serum or 10^{-5} *M* free insulin solution completely abolished the effect of buoyant cells on the beads. Interestingly, these observations are consistent with the formation of a strong reversible bond between the insulin–Sepharose and specific insulin receptor sites on the cell membrane. Let us now consider other affinity approaches for cell membranes that have been developed during the last five years.

5.6. Affinity partioning

Up to 1975, the purification of cells or cellular membrane fragments containing surface receptors has been attempted using affinity chromatography[8–14,31–33,64–66], but the approach appears to be less successful because of difficulties in eluting bound particulate substances from the solid matrix. Moreover, use of high ionic (caesium chloride) gradient medium could result in aggregation and dissociation of protein from lipid, and therefore severely limits the applicability of earlier methods[31–33,64–66] as general fractionation procedures for biomembranes. To overcome these problems, Flanagan and Barondes[71] described a method termed "affinity partioning". It was originally applied[72] for separating soluble proteins in aqueous polymer two-phase systems by adding a polymer-ligand with a relatively high affinity for a binding site on the protein to be purified, and a solubility preference for one of the phases. Since cells and cell fragments[73] can be partitioned and recovered from aqueous polymer two-phases systems, it seemed possible that the principle of affinity partioning could be used in their fractionation. With the absence of an insoluble matrix, it was anticipated that the problems of recovery would be obviated. As a result, the approach has been successfully applied to partially purified cholinergic receptor enriched membranes derived from electroplax of *Torpedo california*[74]. This approach requires: (1) the membranes to contain high receptor density; (2) a detailed knowledge of the ligand specificity and molecular properties of the binding site; (3) sequential affinity steps, because the purification achieved with a single affinity step was comparable with that achieved by sucrose density gradient fractionation[75].

5.7. Affinity fractionation of membrane vesicles

One of the most amazing properties of living cells is their ability to receive and respond to biochemical signals. A particularly attractive mode of informational transfer is via plasma membranes, which are asymmetrical and glycoproteins are mainly exposed on their external surface[76–78]. When vesiculated they can have carbohydrates stemming either on the inside (inside-out vesicles) or on the outside surface (right side-out vesicles). As mentioned earlier, Con A is known to bind specifically α-D-glucose and α-D-mannose and their derivatives[21,79]. Zachowski and Paraf[80] adopted this strategy for the separation of two plasma membrane populations by a Con A polymer. The membrane vesicles retained by the Con A were shown to be right side out, whereas vesicles not retained by the polymer were considered to be inside out. In a parallel study, Brunner *et al.*[81] published their first results on the fractionation of membranes vesicles using Con A–Sepharose. Detailed reports of studies along these lines appeared later[82–86]. If this protocol is extended to other membrane ligands (hormone, growth factors), it may become an ideal tool in membrane investigation.

5.8. *Membrane isolation using polylysine-coated glass beads*

The method is based on the nonspecific adherence of negatively charged cells to polylysine-coated glass or polyacrylamide beads[87–90]. The portions of the cells not attached to the bead can be sheared away, leaving attached plasma membrane on the bead surface. When intact cells or organelles are bound to the beads, the extracellular surface of the plasma membrane is apposed to the bead. Lysis of the cells and removal of unattached membrane should expose the cytoplasmic surface of the attached membrane[91], as shown in Fig. 4. The cytoplasmic membrane, immobilized and surface exposed outward, may be employed directly in the studies of interactions with the cytoplasmic components, or subjected to enzymatic, chemical or optical analysis. The purified membranes may be eluted from the bead by sonication or use of denaturing agents.

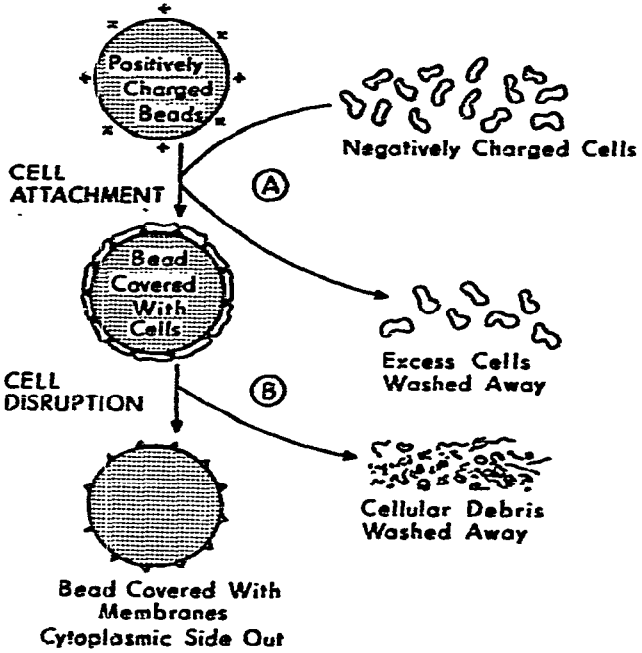


Fig. 4. Schematic illustration of membrane isolation on polylysine-coated glass beads[91].

Isolation on cationic beads minimizes several problems inherent in plasma membrane isolation using other methods[64–66,84]. Typically, the plasma membrane represents a small fraction of the total cell membrane, differing only slightly in density from the other membranes. Bead isolation does not depend on the inherent density differences between cellular membranes, but rather specifically isolates plasma membrane because it is the only membrane which is exposed in the intact cell. Thus, the defining property of the membrane (that it is the external enclosing membrane) is the basis for its specific isolation. Furthermore, the orientation of the isolated membrane is known. Membranes isolated using other techniques[80–84] may vesiculate or break into fragments, leading to a mixture of inside-out and right side-out membrane vesicles. A membrane which has been isolated on cationic beads is attached in one

orientation, with the cytoplasmic surface exposed. Although membrane isolation on beads has several commendable features, certain aspects of the method deserve careful evaluation before it is to be used.

(1) The high surface charge of the bead and its effect upon local pH must be considered when taking into consideration membrane-associated enzymes and pH-dependent membrane properties, because it is known that fixed surface charges and soluble polylysine at low concentrations can greatly alter cell morphology and membrane structure and have profound effects upon membrane function[92–95].

(2) The conditions[89] for optimal membrane isolation on beads (pH 5.0 in sucrose–acetate buffer) may adversely affect sensitive membrane functions. In addition, other cells may require quite different conditions for optimal attachment.

(3) Although the polypeptide composition of membranes eluted from beads is similar to that of membranes on beads, subtle changes in protein conformation or distribution induced by harsh conditions required for the removal of membranes, may limit the usefulness of eluted membranes for functional studies.

In summary, although the above method of membrane isolation on beads may not replace the available isolation methods, it may be valuable for certain applications.

6. ORGANELLES, VIRUSES AND PHAGES

6.1. Organelles

Methods for the separation of subcellular components, *e.g.* sedimentation and density-gradient centrifugation, take advantage of difference in physical properties, such as size, form, mass and densities of the moieties to be resolved[96]. Specific methods based on receptor–ligand interaction have rarely been utilized to isolate organelles. By using Con A–Sepharose beads, separation of mitochondria from crude mitochondrial fractions from rat brain has been demonstrated[97]. On the other hand, histochemical data indicate that Con A binds to some synaptosomes and synapses in sections from a nervous system[98]. Therefore, it seems possible in theory to isolate synaptosomes interacting with Con A from those not interacting. This indeed has been found recently in two different laboratories[99,100]. Furthermore, functional ribosomes from crude bacterial extracts have been purified by affinity chromatography on immobilized streptomycin or gentamicin[101]. Other examples of the application of cellular affinity chromatography to polysomes and ribosomes are indicated in Table 3 (refs. 102–107).

6.2. Viruses

In Table 3, several examples of the isolations of viruses are included[108–115]. One of the most frequently employed ligands is bound antibodies. Kenyon *et al.*[108] first reported isolation of Aleutian mink disease virus on a Sepharose–antibody column. The Sepharose–antibody column was charged with tissue extracts from mink infected with Aleutian disease. Dissociation of the adsorbent–virus complex with 0.75 *M* sodium chloride and a gradient of glycine–HCl released infective particles resembling picornaviruses. In a related study, Sepharose with coupled IgG immunoglobulin was used for the purification of Aleutian disease virus from chronically infected mink[109]. Likewise, Wood *et al.*[110] have obtained a purified preparation of Semliki Forest virus on an immune adsorbent prepared from rabbit or chicken antisera.

TABLE 3

EXAMPLES OF THE USE OF CELLULAR AFFINITY CHROMATOGRAPHY

Substances isolated	*Affinity ligands*	*Solid support or immobilized ligands*	*Reference*
Cells, cell membranes and organelles			
Adipose cells	Insulin	Sepharose 4B	69
Antigen-binding cells from spleens of mice	Antigens	Nylon fibres	34, 35
Antigen-reactive cells from rabbit bone marrow	Antigens	Antigen-coated glass beads	10
Anti-hapten plaque-forming cells	δ-N-2,4-dinitrophenyl-L-ornithine, HCl	Sepharose 2B	119
Anti-hapten specific lymphocytes	Azophenyl-β-lactoside hapten groups	Bio-Gel P-6 with histamine	120
Avian antigen-binding cells	Antigen-coated beads	Polyacrylamide	121, 122
Blood platelets	Sepharose 2B	Sepharose 2B	28
Cells carrying IgG and blood group antigens	Antigen-coated beads	Glass of plastic beads	46
Cells (erythrocytes, thymocytes)	Concanavalin A	Nylon fibres	32
Spleen	Dinitrophenylated serum albumin	Gelatin fibres	32
Populations of specific antibody-producing and specific memory cells	Human or bovine serum albumin or hen ovalbumin	Glass beads or Degalan, V 26	120
Producing antihapten antibodies	Azophenyl-β-glycosides	Bio-Gel P-6 with histamine and hydrazine	48
Cholinergic receptor enriched membranes	α,ω-bis-4-trimethyl-ammonium polyethylene oxide	Dextran	71, 74
Cultured tumor cells	Isoproteronol, corticotropin (ACTH), triiodothyronine	Glass or Sepharose	30
Cultured leukemic cells (L-1210)	Concanavalin A	Nylon	123
Erythrocytes	Anti-erythrocyte antibodies	Reticulated polyester polyurethane foam	42, 43
Erythrocytes	Concanavalin A	Sepharose 6B	31
Fat cells	Insulin	Sepharose 4B	69
Flagellae	Anti-Hb globulins	Sepharose 4B	124
Human lymphocytes	*Lens culinaris* lectin	Sepharose 2B	125
Human T-lymphocytes	Wheat germ agglutinin	Sepharose 4B	118
Human reticulocytes	Transferrin	Sepharose 4B	126
Immunoglobulin-positive cells	Anti-fluorescein antibody	Anti-fluorescein antibody	127
Immune cells	Antigen (serum albumin or ovalbumin)	Glass and plastic beads coated with antigenic protein molecules	36
Immunogenic tumor cells	Concanavalin A	Nylon fibres	128
Immunoglobulin-bearing lymphocytes	Anti-immunoglobulin antibodies	Plastic beads coated with anti-immunoglobulin antibodies	129

TABLE 3 *(continued)*

Substances isolated	*Affinity ligands*	*Solid support or immobilized ligands*	*Reference*
Immunological memory cells	Antigen-coated glass or plastic beads	Glass or plastic beads	44
Immunospecific precursor cells from unimmunized mice	Azophenyl-β-lactoside	Bio-Gel P-6 with hydrazine and histamine	11
Inside-out membrane vesicles from pig lymphocytes	Concanavalin A Anti-lymphocytic serum	Sepharose 4B	84
Inside-out plasma membranes from erythrocytes	Polylysine-coated glass beads	Polylysine-coated glass beads	87, 88
Inside-out vesicles from rat liver mitochondria	Cytochrome *c*	Sepharose 4B	85
Lymphocytes	β-Lactoside haptens	Acrylamide	49
Anti-hapten specific	Phytomitogens	Sepharose 4B	136
Specific immunocompetent T	Anti-idiotypic antibodies	Degalan beads coated with antibodies	130
(T and B cells) from spleens of mouse	Antigens (hapten–bovine serum albumin conjugates, *Limulus* haemocyanine or concanavalin A)	Nylon fibres	131
	Human immunoglobulin (HGG) after treatment with anti-HGG antisera	Surface of tissue culture grade plastic ware	132
(T and B cells) from rat thoracic duct lymph	Anti-rat F $(ab')_2$ antibody	Sephadex G-200	133
Lymphocyte membrane vesicles	Concanavalin A	Coliphage K 29	65, 66
Lymphocyte plasma membrane	Concanavalin A	Sepharose 4B	134, 135
Lymphoid cells	Bovine serum albumin or its derivatives	Nylon fibres	14
	Antigen-coated beads	Glass or plastic	45
Lymphocytes from rat spleen and thymus and mouse spleen	Aggregated rat immunoglobulin	Sepharose 4B	137
Lymph node cells from guinea-pigs	Dinitrophenyl	Polyacrylamide beads	138
Membranes from eucariotic cells	Polylysine-coated beads	Polyacrylamide	88
Mouse bone marrow cells	Wheat germ agglutinin	Sepharose 6MB	139
Murine cytotoxic T-Lymphocytes	Lectin	Sepharose	140
Mouse spleen cells (IgG bearing)	Protein A of *Staphylococcus aureus* (sp A)	Sepharose 6MB	24
Neural cell separation	Normal rabbit globulin	Sepharose 6MB	53
Ovalbumin-synthesizing polysomes in complex with anti-albumin antibody	Ovalbumin	Ovalbumin cross-linked with glutaraldehyde	107
Peripheral blood T lymphocytes	*Helix pomatia* lectin	Sepharose	153

(Continued on page 488)

TABLE 3 *(continued)*

Substances isolated	*Affinity ligands*	*Solid support or immobilized ligands*	*Reference*
Plasma membrane from erythrocytes	Polylysine-coated beads	Glass	90, 91
Plasma membranes from HeLa cells	Polylysine-coated glass beads	Polylysine-coated polyacrylamide beads	89
Plasma membranes from pig lymph node cells	Concanavalin A	K 29 Coliphage	64
Plasma membrane vesicles from eukariotic cells	Concanavalin A	Sepharose 4B	80
Plasma membrane vesicles from thymocytes	Concanavalin A	Sepharose 4B	81–83
Polyribosomes from mouse plasmacytoma producing IgG 1 immunoglobulin type X	Complex of mouse immunoglobulin with rabbit antibodies	Amino–cellulose	141
Polysomes (albumin-synthesizing)	Anugen–antibody complex	Amino–cellulose	142, 143
Polysomes (galactosidase-specific)	*p*-Aminophenyl-D-thio-galactopyranoside	Sepharose with 3-aminosuccinyl-1,6-diaminohexane	144
Polysomes	Antibody to specific protein	Agarose	102
	Pyridoxamine phosphate	Sepharose 4B with ethylenediamine and succinic anhydride	103
Proliferated cells	Concanavalin A	Sepharose 4B	145
Rat basophilic leukemia cells	Lentin–lectin	Sepharose 4B	146
Reticulocyte ribosomes	Polyuridylic acid	Sepharose 4B	104
Ribosomes from *E. coli*	Streptomycin or gentamicin	Indubiose 4A	101
Ribosomes synthesizing tyrosine aminotransferase from hepatoma tissue culture cells	Pyridoxamine phosphate	Sepharose 4B	105
Splenocytes	*Helix pomatia* A hemagglutinin	Sepharose	154
Spleen lymphocytes	Insulin, Con A	Sepharose 4B	148
Sympathetic ganglion neurones	α-Bungarotoxin	Sepharose 6MB	26
Synaptic plasma membrane fraction from guinea pig brain	Concanavalin A	Sepharose 4B	149
Synaptosomes	Concanavalin A	Sepharose 4B	99,100
Synaptic vesicles	Sepharose 6MB	Sepharose 6MB	151
Tissue culture cells (HeLa, SV 3T3)	*Lens culinaris* lectin	Sepharose 2B	150
Thymocytes	Anti-thymocyte globulin	Sepharose 4B	147
Thymocytes	Anti-peanut agglutinin	Sepharose	152
Translating ribosomes	Periodate-oxidized polyuridylic acid	CM-Cellulose with dihyrazide of dithioglycolic acid	106
Phages and viruses			
Bactericphage SKV 1	*Shigella sonnei* liposaccharide	CNBr-activated Sepharose 4B	117

TABLE 3 *(continued)*

Substances isolated	*Affinity ligands*	*Solid support or immobilized ligands*	*Reference*
T-4 Phage	Poly DL-lysine	Sepharose 2B	116
Aleutian mink disease virus	Antibody–Sepharose	Antibody–Sepharose 4B	108
	Immunoglobulin from chronically infected mink	Immunoglobulin from chronically infected mink	109
Foot-and-mouth disease virus (FMDV)	FNDV antibodies	Sepharose 4B	111
Influenza virus	γ-Globulin of rabbits immunized with influenza virus	Disulphide-linked γ-globulin with N-acetyl-homocysteine thiolactone	112
Murine type-C virus p 30 precurose protein	Single-stranded DNA	Cellulose	113
Plant virus	Antibodies	Antibodies cross-linked by glutaraldehyde	114
Semliki forest virus	Antibody–Sepharose	Antibody–Sepharose	110
Tobacco mosaic virus	Anti-tobacco mosaic virus antibodies	*p*-Aminobenzyl cellulose	115

6.3. Phages

Initially, Sundberg and Hoglund[116] developed a procedure for the purification and concentration of T4 phages from lysates of T4-infected *E. coli*. Later it was shown[117] that coupling of lipopolysaccharide to Sepharose matrix yields a receptor which can adsorb bacteriophage specifically. It was also demonstrated[117] that the binding of the phage to the receptor can be reversed and a significant portion (up to 60%) of the active phage particles could be recovered. To our knowledge, these are the only two examples of affinity purification of phages.

7. EXAMPLES OF THE USE OF CELLULAR AFFINITY CHROMATOGRAPHY

The availability of specific ligands utilizing surface properties of cells to form specific and reversible complexes has facilitated enormously the isolation of cells, cell membranes, organelles and viruses, as reviewed in Table 3. In addition to isolated substances, Table 3 also gives the affinity ligand used, the solid support and the appropriate reference(s). Additional examples of cellular affinity chromatography are included in the Table[118–154]. Table 3 includes, in addition to isolations carried out by typical bioaffinity chromatography, also those examples in which surface charge is made use of[87–91]. This enumeration of the uses of cellular affinity chromatography does not exhaust its possibilities, which are many and varied.

8. COMPLICATING FACTORS AND SUGGESTIONS

In order to operate any affinity chromatographic separation succesfully, care should be taken to the correct selection of a number of variables. In addition, cellular

affinity chromatography introduces several new aspects not encountered in molecular affinity chromatography, as will be evident later in this discussion. It is clear that a number of parameters are critical in obtaining good results, and these may fluctuate considerably in different situations. Therefore several points can be made to underscore the importance of complicating factors, most of which are related to the use of lectins in cellular affinity chromatography.

8.1. Column versus batchwise procedures

The first point to be addressed is the use of column against batchwise methods. Column procedures are not entirely satisfactory, as non-specific steric retention of particulate matters such as cells occurs in a system of packed gel beads which have been used as matrix for this purpose[14,36,42,43,48]. In densely packed, sedimented gels (diameter of Sepharose 4B beads of 40–190 nm), organelles of sizes ranging from several hundred Ångstroms to some microns will be sterically hindered in movement, flow and release. Batchwise technique under conditions in which gel particles are freely floating and separated from each other are therefore preferable to column methods.

8.2. Lectin amount and bead volume

An important factor governing the rate of cell binding as well as of sugar-specific release is the amount of lectin bound per bead volume. It is difficult to determine how much lectin is actually involved in a particular batch of beads. Furthermore, the geometry of bead activation as well as ligand coupling are still the subjects of controversial discussion[155–159]. Studies[150] with *Lens culinaris* lectin (LCL) indicate that not only is the affinity of LCL to a receptor or hapten important, but probably also important is the firmness of lectin–cell linkage. This has also been demonstrated previously for the binding of cells to immobilize Con A[14]. The formation of stable (but not irreversible) cell–bead complexes seems to take place within a critical range of lectin concentrations on beads. Kinzel *et al.*[150] have suggested that the lectin density at the surface is not solely responsible for the strength of cell–bead linkage.

8.3. Physical factors

In addition to the amount of lectin per bead, its surface structure itself seems to play a certain role, especially in determining the efficiency of cell recovery[150]. Factors responsible for the specific architecture of beads are the agarose concentration, conditions of manufacture and effects of the chemical activation process. Another interesting aspect is the physical behavior of beads and cells in suspension. The motion of the cells together with the beads is necessary to give a maximum number of cell–bead interactions leading to the immobilization of the cell by the lectin. Lectin is obviously necessary to overcome the mechanical forces during incubation, since the cells do not bind to uncovered beads[9,89]. Suspended beads permit the access of cells to their entire surface, thus utilizing the maximum lectin binding capacity. Also in this way the possibility of trapping cells between beads is minimized. Suspended beads allow, in addition, the unretarded liberation of released cells back into the solution. In those circumstances where this factor is crucial, Sepharose 2B beads are preferable because they are suspended almost like cells in the agitated system. This is not the case for 6B-beads which are heavier and tend to stay at the bottom of the vessel.

Furthermore, secondary interactions[14] between cell and bead may take place, which to a certain degree are probably determined by the surface structure of the beads.

8.4. Binding affinities of lectins

Edelman *et al.*[14] have made a significant observation while working with an immobilized lectin, namely Con A–Sepharose. They noticed that the release of cells from Con A–Sepharose upon the addition of specific sugars, without mechnical aid, was difficult, if not impossible. Similar problems which may be due to the same property of Con A have been recorded elsewhere[19,158]. According to these studies, the yield of specifically elutable material was rather low. Immobilized LCL, in contrast, has been shown to release much more elutable material upon elution with specific sugars[150]. One interpretation which is now apparent from the above is that LCL binds the same sugars as Con A (Table 2) but with a binding contrast 50 times lower[159]. It is therefore advisable to take into consideration the binding affinities of different ligands bearing similar specificities.

8.5. Size, form and mobility of particles

Subcellular membraneous particles of unfractionated homogenates are generally very heterogenous in size, form and flexibility and in the number, distribution, density and accessibility of their receptors. This is illustrated in Fig. 5. The degree of binding on the gel surface will largely depend upon the number of actually interacting ligand–receptor pairs, which is unlikely to be identical with the total number of receptors. On the other hand, the ease of detachment depends mainly on the size, form and mobility of the particles, and on the number of actual attachment sites. The susceptibility of particles to shearing forces obviously increases with their size. The likelihood of a particle being released in this way is tentatively rated[97], in diminishing order, by the numbers 1 to 7 in Fig. 5. Small particles with a high density of surface receptors (No. 7) are presumably the most difficult to detach. In an effort to solve these foregoing problems, a technique has been described elsewhere[97].

Fig. 5. Scheme for the binding of subcellular particles to Con A–Sepharose[97].

8.6. Role of shearing forces

An interesting feature of the affinity chromatography of membrane vesicles[82,83] is the dependence of dissociation of the membrane versicles on shearing forces. Neither a gradient nor high concentrations of α-methylmannoside were able to elute significant amounts of membrane material, as depicted in Fig. 6a. Disturbing the

settled gel, in this particular experiment, was found to be sufficient for the dissociation of the membrane vesicles. Moreover, the presence of the inhibitor α-methyl-mannoside was not essential. The binding of membrane vesicles to Con A–Sepharose was prevented if the mixture was stirred throughout the whole separation procedure (Fig. 6b) as well as by the simultaneous addition of α-methylmannoside and membrane vesicles[82]. These difficulties are understandable if the binding of membranes to Con A–Sepharose depends largely on gross non-biological interactions since these bear no relationship to biospecificity. The confusion of non-biological with biospecific effect in the binding process is of more than academic interest and may have considerable practical consequences. Such confusion could result in incorrect generalizations which may adversely affect the design of new systems. It is clearly necessary to exercise great care in distinguishing true bioaffinity from non-specific binding effects, as has previously been stressed in the case of molecular affinity chromatography[160–163].

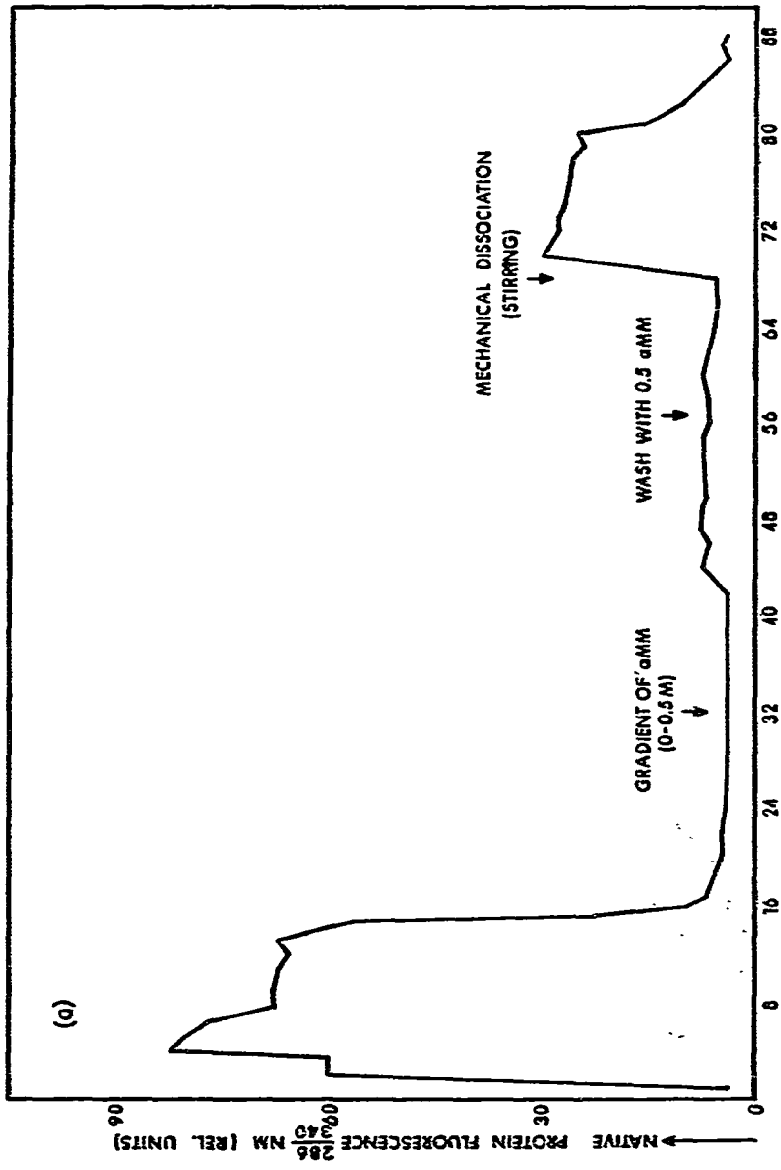
8.7. Concept of mobile multicomponent system

As indicated elsewhere (section 4.3.1.) in this article, a significant enrichment of different cell types can be achieved by affinity chromatography on Sepharose 6MB linked with a lectin. It is very important, however, to investigate in some detail the conditions required for obtaining reproducible cell binding and elution paterns based on specificity of the immobilized lectin. The sugar-specific release of cells from affinity ligand is much more critically dependent on incubation times, incubation temperatures and elution flow-rates[159] than is the case for molecular affinity chromatography. The major reasons for this are related to the much larger size and content of lectin receptors or cells compared to macromolecules. The implication is that the cells can form many stabilizing interactions with the lectin–beads (multipoint attachments) which can render elution difficult. This property of multipoint attachment of cells appears to be an important element in almost all forms of cellular affinity chromatography.

The necessity of mechanical forces (stirring) for the dissociation (Fig. 6a) is in itself a remarkable fact. This has also been taken to indicate secondary interactions between membrane structures and the Sepharose matrix occurring after the binding step. The occurrence of short range lateral movement (micropatching) of the receptor molecules in the membrane vesicles, a mobile multicomponent system, is supported by the fact that continuous stirring prevents the binding of the membranes (Fig. 6b). If one may generalize from the Con A model, a special feature of affinity chromatography of membranes vesicles, as a mobile multicomponent system, is that elution with the competitive inhibitor alone is not possible[81–83,164,165]. This has also been shown for lymphocyte plasma membranes[84]. Furthermore, shearing forces allow dissociation of the bound membrane material even in the absence of the competitive inhibitor. This is consistent with results on membranes of plasmacytoma cells[80] as well as for postsynaptic membranes[86]. All these findings reinforce the hypothesis of a mobile multicomponent system in cellular affinity chromatography and further suggest that different interactions seem possible in such a mobile multicomponent system.

8.8. Flow-rates and shearing forces

Recently it has been demonstrated that high elution flow-rates (5 ml/min or greater) are required to release a significant number of mouse bone marrow cells using



(Continued on p. 494)

Fig. 6a.

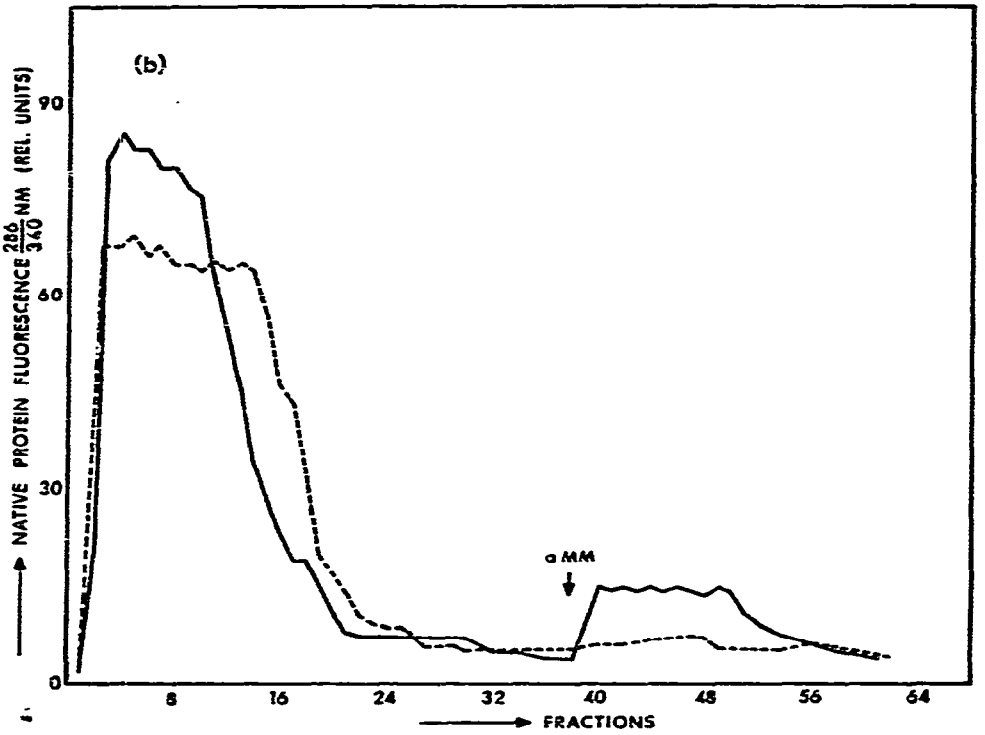


Fig. 6. Affinity chromatography of membrane vesicles[83]. (a) Dissociation of membrane vesicles bound to Con A–Sepharose. A 5-mg amount of microsomal protein was added to 60 ml of Con A–Sepharose (50%, w/w, suspension). After elution of the non-bound membrane fraction, the Con A–Sepharose beads were rinsed with a linear gradient of α-methylmannoside (aMM), ranging from 0 to 0.5 *M* "without" stirring. After that the beads were stirred. (b) Effect of stirring on the binding of membrane vesicles to Con A–Sepharose at 24°C. —, Normal dissociation procedure; – – – –, stirring during all procedures.

wheat germ agglutinin (WGA)–Sepharose 6MB[139]. Although shear forces created by high flow-rates play little role in the initiation of cell release these shear forces are probably required to reduce the number of cell–lectin interactions that have to be broken by the sugar before cell release can occur. High flow-rates would also prevent rebinding of released cells to the column.

The controlled use of shear forces by increased buffer flow-rates should be distinguished from the shear forces generated by mechanical agitation of the beads. High flow-rates are known[139] not to release cells in the absence of competing sugar. In contrast, mechanical agitation of beads released certain percentage of bound cells non-specifically. Thus, the binding of cells to WGA–Sepharose 6MB was reduced by mechanical agitation; 75% of the bone marrow cells were bound when the cells and beads were "gently" mixed, compared with 90% binding when the cells were incubated on a stationary column. It has also been reported that mechanical agitation is important to obtain good contact between cells and beads[150] but, contrary to this, Nicola *et al.*[139] claim that mechanical forces can prevent the binding of some cells. The reason for this discrepancy remains unknown.

8.9. Concluding remarks

Despite the potential usefulness of cellular affinity chromatography as a fractionation and purification technique, there have been few detailed studies of the various parameters that affect these purifications and fractionations. Moreover, many of the reported fractionations have been performed on highly simplified populations of cells[118,150] or with highly specific antigens or antibodies[42,43,48], so that these approaches cannot be readily extrapolated to other cell populations. Also, in some reported methods[14,34,42,43], elution of cells was achieved by mechanical, non-specific methods, thus limiting their applicability to systems where only the cell type of interest binds to the column. Therefore, it is suggested that special attention should be given to defining conditions for specific binding and elution of cells, viability of cells, and for optimizing the cell yields and fractionation achieved.

Finally, the above analysis of the present state of the complicating factor associated with cellular affinity chromatography may appear to be less than optimistic. However, it seems to be a realistic measure of the progress that has been accomplished and may be of significant help in focusing our attention on the precise questions that need to be answered so that these techniques can be applied more effectively.

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10. SUMMARY

This article attempts to introduce the concept of cellular affinity chromatography as it departs from molecular affinity chromatography. Special emphasis is placed on the selection of a solid support as well as on the role of lectins as affinity ligands. Our major goal was to bring to light the basic principles involved, multiple options of ligands and matrices, and different techniques, which may be applied to separate the complex cell population as well as cell membranes. It is hoped that further developments in the field, especially in the selection of proper experimental conditions, ligands and matrix material may provide better results. We have tried to identify some of the potential problems which should be considered before these approaches can be used on a routine basis. Although the review deals primarily with the affinity chromatography of cells and cell membranes, examples are presented for diverse systems such as cell organelles, viruses and phages. A table summarizing the use of cellular affinity chromatography is included. It lists more than 80 examples covering the literature up to December, 1979.

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