Biochimica et Biophysica Acta 816 (1985) 83–92
Elsevier

BBA 72576

Rapid, high-yield purification of cell surface membrane using colloidal magnetite coated with polyvinylamine: Sedimentation versus magnetic isolation

Wayne F. Patton, Jeongsoon Kim and Bruce S. Jacobson

Department of Biochemistry, University of Massachusetts, Amherst, MA 01003 (U.S.A.)

(Received November 2nd, 1984)

Key words: Membrane isolation; Cell surface; Magnetic separation; Polyvinylamine-magnetite; (*D. discoideum*)

A new technique for the magnetic isolation of external plasma membrane from *Dictyostelium discoideum* is described and compared to a previously published procedure employing sedimentation of silica-coated plasma membrane. The magnetic isolation technique involves coating intact cells with a polyvinylamine-magnetite colloid and overcoating with polyacrylate to form a dense pellicle. The magnetite pellicle totally coated the cells and was not internalized. Coated cells were lysed and membrane fragments retrieved from the cell homogenate using a diverging field electromagnet. The membrane obtained in such a manner was analyzed for marker enzyme activity and cell surface label. The plasma membrane was obtained in high yield (42%) with an average purification of 8-fold. The polyvinylamine-magnetite pellicle shielded the external plasma membrane face to proteolysis by papain and pronase. It also acted as a barrier to α-methylmannoside in concanavalin A-carbohydrate competition studies.

Introduction

The plasma membrane as the mediator of interactions between the cell and its environment plays a critical role in such processes as differential adhesion, cellular movement, and endocytosis and exocytosis. Study of these phenomena requires isolation of relatively pure plasma membrane in a form amenable to experimental manipulation. Choice of the purification procedure used for isolating plasma membrane depends to a large extent on the nature of the investigation for which it is intended.

Classical methods for purifying plasma membrane rely on cell homogenization followed by differential and density gradient centrifugation or partitioning into immiscible aqueous solutions of polymers [1–9]. Preparations of this type, while often being relatively pure, are time-consuming and result in low yield of plasma membrane [1]. The centrifugation techniques have been improved by the modification of homogenization conditions to yield membrane fragments of various sizes [2] or orientation [3]. Separation of subcellular organelles has been facilitated by perturbing their densities [4,5] and by utilizing a variety of density gradient materials [6]. Most of the techniques did not provide control of the sidedness of membrane preparations and many resulted in the selection of nonrepresentative plasma membrane subdomains [7]. The ability to selectively probe the internal surface of the membrane was difficult, since it

* Supplementary information to this article is deposited with, and can be obtained from, Elsevier Science Publishers BV (Biomedical Division), BBA Data Deposition, P.O. Box 1345, 1000 BH Amsterdam, The Netherlands. Reference should be made to BBA/DD/309/72576/816 (1985) 83. The supplementary information consists of a technical drawing of the diverging field electromagnet.

Abbreviations: Mes, 4-morpholineethanesulfonic acid; Tris, 2-amino-2-hydroxymethylpropane-1,3-diol.

required the isolation of inside-out vesicles.

Other procedures were developed to isolate the plasma membrane so that it was maintained as open sheets with the outside surface shielded and the inside exposed for direct probing [10–13]. The procedures were based upon the ionic interaction of cells which were negatively charged with large positively charged beads. The cells were shorn away leaving behind sheets of plasma membrane. The procedure required an excess of cells to saturate the beads and the yields of plasma membrane were low. To overcome this difficulty, another technique based upon the ionic interaction of an inert support with the plasma membrane was developed [14]. Cells were coated with positively charged colloidal silica and overcoated with an anionic polymer. Several coats could be applied and the cells were ruptured. The density of the coated membrane was so great that it could be rapidly sedimented in a centrifuge tube. The yield of plasma membrane was high and the membrane was held as an open sheet with the outside surface shielded and the inside exposed for direct probing. Here we describe the synthesis of positively charged colloidal magnetite which is used as the inert pellicle, allowing the plasma membrane to be isolated by an applied magnetic field. The use of colloidal magnetite as a very general nonspecific probe that interacts with the whole cell surface is substantially different from other magnetite-based probes with lectins or immunoglobulin covalently linked to them to produce highly specific interactions with particular membrane receptors for cell separation and scanning electron microscopy tagging [15–22].

Materials and Methods

Materials. Dictyostelium discoideum strain AX-3 was generously donated by Dr. Richard Kessin of Harvard University. Media and supplies for culturing *D. discoideum* were purchased from Difco Laboratories, Detroit, MI and Becton Dickinson and Co., Cockeysville, MD. Polyacrylic acid (M_r 90000) was obtained from Aldrich Chemical Co. and polyvinylamine (M_r 20000) from Polysciences. Carrier-free $Na^{125}I$ was purchased from New England Nuclear. All other chemicals including concanavalin A, the proteases, and the substrates for the marker enzymes were purchased from Sigma Chemical Co., St. Louis, MO.

Growth and harvesting of cells. Cellular slime mold, *D. discoideum*, axenic strain AX3 was grown on rotary shakers as previously described [23]. The cells were grown to late log phase of growth ((6–8) $\cdot 10^6$ cells/ml) and were harvested by centrifugation for 2 min at $350 \times g$. Cells were washed in ice-cold 120 mM sorbitol buffered to pH 6.5 with 20 mM Mes. Pelleted cells were always redispersed by gentle manual agitation to avoid cell rupture. The integrity of the plasma membrane must be maintained during the application of the magnetite pellicle in order to ensure minimal contamination from other organelles.

Magnetite synthesis and sizing. Colloidal magnetite was generated by chemical coprecipitation from basic solution. 6 g anhydrous $FeCl_2$ and 16.2 g anhydrous $FeCl_3$ were added to 1.5 l of distilled water at 95°C. While stirring this solution, 150 ml of 10% NaOH was added. The resulting precipitate was pelleted at $150 \times g$ for 10 min. The supernatant was discarded and the pellet was resuspended in 500 ml distilled water. The pelleting procedure was repeated three more times and the final pellet was resuspended in 500 ml distilled water adjusted to pH 2.0 with HCl. This resulted in dispersion of the colloid. The colloidal solution was filtered three times through glass wool, diluted to 1.5 l, and heated to 100°C. Polyvinylamine was added to a final concentration of 1 mg/ml. The solution was probe-sonicated for 10 min at 50 watts to insure colloidal dispersion. This polyvinylamine-magnetite stock solution has a shelf life of approx. 6 months. Solutions kept for longer periods of time lose their magnetic properties due to the slow oxidation of magnetite to hematite in aqueous media.

The magnetite was sized by evaporating the continuous phase to dryness and examining the particles by transmission electron microscopy. Size was determined by comparing the particle images directly to a calibrated diffraction grating (2160 lines/mm). An alternative sizing procedure was used as well, since the sample preparation technique for microscopy could lead to aggregation of the magnetite. The second sizing procedure was performed in solution by Quasi-Elastic Laser Light Scattering using a neon laser ($\lambda = 632.5$ nm) at

room temperature and with incident beam 90° with respect to the detector [24].

Pellicle formation. The following procedure was used to generate a colloidal polyvinylamine-magnetite-polyacrylate pellicle on the cell surface. Sorbitol at 140 mM and buffered to pH 6.5 with 20 mM Mes was used as a wash buffer throughout the procedure. To avoid magnetite sedimentation with the cells the flocculation concentration of 12 mM for monovalent salts like NaCl was not exceeded in the wash buffer. Since colloid flocculation depends on ion valence, divalent and trivalent salts will cause precipitation at much lower concentrations. 5 ml of 2 mg/ml magnetite was diluted to 12 ml with wash buffer in a 50 ml polycarbonate centrifuge tube. About 2 ml of freshly washed, packed cells (approx. $3 \cdot 10^9$ cells) were suspended in enough wash buffer to give a 50% cell suspension. While gently mixing the colloidal polyvinylamine-magnetite on a Vortex Genie (Fischer Scientific, Medford, MA) adjusted to the lowest setting, the cell suspension was added dropwise into the colloidal polyvinylamine-magnetite. The cell-colloid suspension was then diluted to 45 ml with wash buffer and centrifuged for 2 min at $150 \times g$. Unbound colloid was discarded in the supernatant and the pelleted cells resuspended in 5 ml of wash buffer. The cells were then gently vortexed as described above and 5 ml of 2 mg/ml polyacrylate (M_r 90 000) in wash buffer titrated to pH 6.5 with NaOH was added dropwise. The suspension was again diluted to 45 ml and pelleted as above to remove the cells from excess polyacrylate. Generally, the process described above was repeated once more to make a denser pellicle consisting of two coatings of polyvinylamine-magnetite and two coatings of polyacrylate.

Pellicle-coated cells were washed twice in wash buffer, resuspended to 20 ml in a lysis buffer of 10 mM Tris (pH 8.0) and placed in a Parr nitrogen pressure bomb (Parr Instrument Co., Moline, IL) which was packed with ice. The bomb was charged to 800 psi with nitrogen and allowed to equilibrate for 5 min. Nitrogen-cavitation of the samples was induced by slowly releasing the cell suspension from the Parr bomb. The extent of cell lysis was verified by viewing the lysate under a phase-contrast microscope.

Plasma membrane recovery from the cell homogenate. A diverging field electromagnet * was designed for the extraction of magnetite-coated plasma membrane from lysed cells. The electromagnet consists of 5000 turns of No. 34 copper wire ($R = 85\Omega$). The body of the magnet was made of hardened steel. One pole of the magnet was designed to accomodate a 50 ml polycarbonate centrifuge tube that has been modified with a release valve at the bottom for removal of cell homogenate not attracted to the magnet. The diameter of this pole piece is 2 cm. The magnet is connected to a power source such as an electrophoresis power supply and operated at 250 mA constant current. Homogenate from the Parr bomb was placed in the diverging magnetic field for 1–2 min. Material not attracted to the magnet was eluted through the bottom of the centrifuge tube and the tube was filled with lysis buffer without removing the centrifuge tube from the pole piece of the magnet. The tube was agitated which released the material attached to the magnet. This insured that contaminants trapped within the magnetite-coated membranes were released into the wash buffer. The coated membranes reattach to the magnet and after 30 s the lysis buffer was eluted from the bottom of the tube. This washing step may be repeated a number of times to insure removal of cytoplasmic contaminants. The magnet was then turned off and the polyvinylamine-magnetite-coated membranes were washed into the centrifuge tube with a stream of lysis buffer. The plasma membrane preparation may be concentrated by pelleting for 10 min at $800 \times g$.

Chemical and enzyme assays. Magnetite and polyvinylamine interfere with protein determination, but by adding sodium dodecyl sulfate to the fractions to make them 2.5% w/v in detergent and heating the samples for 10 min at 70°C the magnetite could be pelleted in a microfuge, leaving the protein in the supernatant. Protein concentration was determined on the supernatant by the method of Lowry et al. [25].

Milder extraction procedures were necessary for the enzyme assays. A variety of detergents at different concentrations were investigated for these assays. Maximal activity of the enzymes used here was obtained when samples were made 0.2% in

* Databank footnote on p. 83.

SDS and heated at 37°C for 20–30 min. 90% of the protein could be extracted from the colloid under these conditions. Plasma membrane preparations were characterized enzymatically by determining the amount of alkaline phosphatase activity [26] and 5′-nucleotidase activity [27]. Contamination by subcellular organelles was assayed by previously published procedures. Lysosomes by β-*N*-acetylglucosaminidase [28], endoplasmic reticulum by glucose-6-phosphatase [29], mitochondria by succinate dehydrogenase [30], and soluble cytosol by lactate dehydrogenase [31]. Iron concentration was determined colorimetrically after converting the iron oxide to $FeCl_3$ using concentrated HCl [32]. Protein was found not to interfere with the iron determination at concentrations normally encountered in the membrane preparations (0.5 mg/ml).

Radioiodination of concanavalin A and plasma membrane. Concanavalin A was isotopically labeled by reaction with carrier-free $Na^{125}I$ as previously described [33]. *D. discoideum* cell surfaces were trace-labeled with the iodinated lectin by incubation of a 2.5% cell suspension for 10 min on ice in a buffer of 50 mM potassium phosphate, 40 mM KCl, 10 mM $MgCl_2$ (pH 6.5) containing 2 μg/ml ^{125}I-concanavalin A. Radioactivity was counted in a Packard Model PGD Prias Automatic Gamma Counter.

Polyvinylamine binding to magnetite. 1 ml of 2 mg/ml magnetite was incubated at 70°C for 30 min with increasing concentrations of polyvinylamine. The resulting polyvinylamine-magnetite colloid was pelleted by centrifuging at $13000 \times g$ for 15 min. The pelleted colloid was resuspended in distilled water and repelleted. Both supernatants were analyzed for free polyvinylamine and compared to standards made in the absence of magnetite. The procedure of Lowry et al. [25] for analysis of proteins was used to quantitate the polyvinylamine.

Scanning electron microscopy. Magnetite-coated cells and uncoated cells were washed in 100 mM potassium phosphate buffer (pH 6.5). Cells were then resuspended in freshly made 1% glutaraldehyde, 1% OsO_4 in H_2O [34]. Samples were allowed to fix for 30–40 min at 4°C with gentle swirling every 10 min. Cells were then washed again in phosphate buffer and resuspended in 2% OsO_4 for an additional hour at 4°C. Samples were applied to gelatin-coated coverslips and allowed to incubate in ice for 30–60 min. The samples were then washed in cold distilled water and dehydrated in a graded ethanol series. Use of acetone at this step seemed to cause solubilization of the polyvinylamine and disruption of the magnetite pellicle. Samples were dried in a Polaron Critical Point Drier using carbon dioxide as the transition fluid. Samples were gold-coated to a thickness of 250 Å in a Polaron Specimen Coater and examined using a Jeol Scanning Electron Microscope.

Results

Colloid sizing

Transmission electron microscopy indicated that the modal diameter of the magnetite particles was 7 ± 5 nm. The aggregates of magnetite averaged 86 ± 30 nm in diameter. Laser light scattering measurements indicated that the aggregates were not an artifact of sample preparation. Estimates of the diameter of the aggregates in solution from this technique were approx. 140 nm. The discrepancy in size estimates between the two techniques may be due to the polyvinylamine which is not electron dense and would not contribute to the estimate of diameter in the electron microscope.

Optimization of polyvinylamine concentration added to magnetite

It was determined that approx. 0.2 mg of polyvinylamine binds per mg of iron to saturate the magnetite colloid surface. The relative specific activity and yield of magnetically extracted plasma membrane was monitored as a function of polyvinylamine concentration using ^{125}I-concanavalin A as a cell surface marker. Table I shows the dramatic effect addition of polyvinylamine has on yield of plasma membrane. A modest improvement in the relative specific activity of the membrane preparation is also noted at 100% saturation of the magnetite surface with polyvinylamine. The same trend in relative specific activity was observed using alkaline phosphatase as the membrane marker (data not shown).

Preliminary analysis by Mössbauer spec-

TABLE I

YIELD AND RELATIVE SPECIFIC ACTIVITY OF MAGNETICALLY EXTRACTED PLASMA MEMBRANE AS A FUNCTION OF COLLOIDAL MAGNETITE SATURATION WITH POLYVINYLAMINE

Polyvinylamine saturation of the magnetite colloid was determined as described in Materials and Methods. Magnetically fractionated cell homogenates were analyzed for enrichment and yield of the external cell surface marker concanavalin A.

% Saturation of magnetite with polyvinylamine	Yield (%)	Relative specific activity
0	11.3±2.0	3.9±0.4
50	20.6±3.8	3.9±0.4
100	48.8±3.1	7.0±1.1
175	32.5±3.1	4.5±0.5

troscopy on samples of magnetite and magnetite with polyvinylamine added seems to show spectral line broadening upon addition of surfactant which is consistent with chemical adsorption to the magnetite [33].

Verification of magnetite pellicle formation

The formation of the magnetite pellicle was observed using scanning electron microscopy to insure that the whole cell surface was covered by magnetite. As shown in Fig. 1, cells were found to be completely coated with the magnetite colloid. To verify that the coating observed was not simply polyvinylamine, whole cell response to an applied magnetic field was measured. Unlysed polyvinylamine-magnetite-coated cells were suspended in wash buffer and placed in the magnetic field. Aliquots of solution were removed at various time-points and assayed for protein and iron. As shown in Fig. 2, both iron concentration and protein concentration decreased in the solution as the magnetite-coated cells were drawn to the pole of the magnet. The comigration would not have occurred had the cells not been coated with polyvinylamine-magnetite.

Protection of the external face of the cell surface by polyvinylamine-magnetite

Shielding of the external surface of the plasma membrane by the polyvinylamine-magnetite to such probes as proteases is essential if the isolation scheme is to be used in transbilayer mapping of plasma membrane proteins as previously done with silica-coated membranes [14]. Accessibility was determined as follows: the external surface of the cells was labeled with [^{125}I]concanavalin A and either treated with protease or a ligand to compete off the concanavalin A before and after coating with polyvinylamine-magnetite. The amount of ^{125}I released from uncoated cells compared with the coated is a measure of the protection or shielding of the external side of the plasma membrane.

Table II shows the percentage shielding of the magnetite-coated cell surface relative to uncoated cells. Also included in the table are values obtained using the silica technique [14]. The magnetite pellicle afforded significant shielding to the external plasma membrane surface. Application of one or three coats of magnetite to the plasma membrane did not significantly change the amount of surface protection observed.

The possibility that the shielding phenomenon was an artifact caused by release of ^{125}I-concanavalin A from the cell surface followed by nonspecific binding to the magnetite pellicle was also examined. Cells which had been externally labelled with ^{125}I-concanavalin A were either mixed with an equal concentration of unlabelled cells or with an equal concentration of cells coated with two layers of magnetite. If the protection phenomenon were due to the release of the lectin from the cell surface followed by nonspecifc binding of the lectin to the magnetite, it would be expected that lectin-bound cells incubated with magnetite-coated cells might also show an artifactual protection phenomenon. As Fig. 3 indicates, no artifactual protection phenomenon due to nonspecific binding of the concanavalin A to the magnetite pellicle could be detected.

Plasma membrane characterization

Enrichment of plasma membrane in the magnetically extracted fraction of the cell homogenate was monitored using marker enzymes and radioiodinated concanavalin A. Although there is some controversy as to whether or not 5′-nucleotidase and alkaline phosphatase represent separate enzymes in *D. discoideum* [26,36–38], both activities are thought to be localized primarily in the plasma membrane [39,40]. Iodinated concanavalin A was

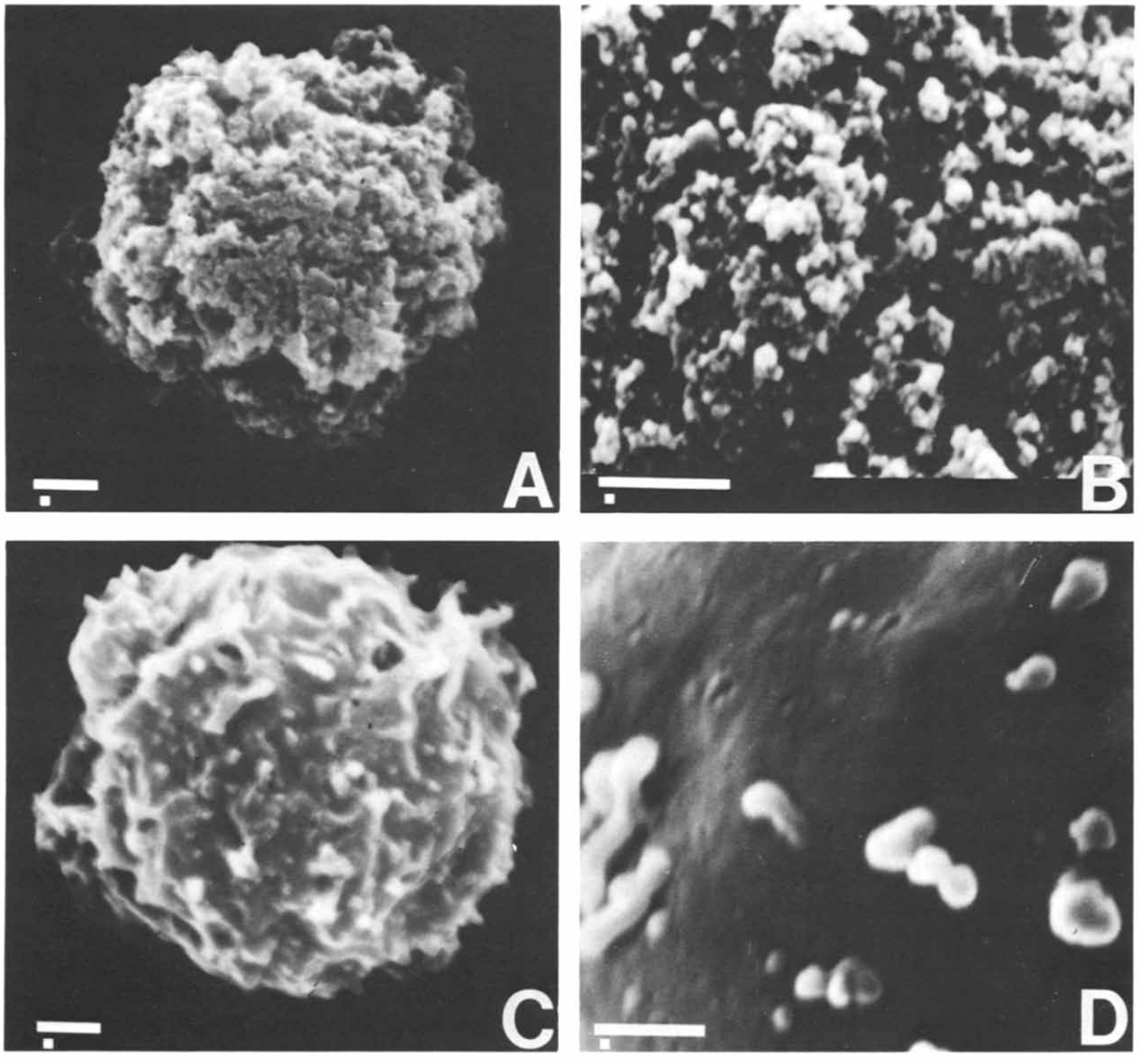

Fig. 1. Binding of polyvinylamine-magnetite to the cell surface of *D. discoideum*. Cells were coated with two layers of polyvinylamine-magnetite. Scanning electron microscopy revealed that a thick pellicle of polyvinylamine-magnetite was deposited on the surface of the cells as shown at low and high magnification (A, B). For comparison, uncoated cells are also shown at low and high magnification (C, D). Scale bars represent 1 μm.

used as a specific marker for external plasma membrane as described in Materials and Methods. Enrichment of magnetite was also monitored since it should increase to approximately the same extent as the ^{125}I-concanavalin A external label in the membrane fraction.

β-*N*-Acetylglucosaminidase was used as a marker enzyme for lysosomes, while glucose-6-phosphatase served as a marker for the endoplasmic reticulum. Succinate dehydrogenase and lactate dehydrogenase were used as markers of the mitochondria and cytoplasm, respectively. Both of these activities were found to be below the sensitivity of our assays due to the presence of magnetite in the samples. Attempts to remove the magnetite prior to activity determination with de-

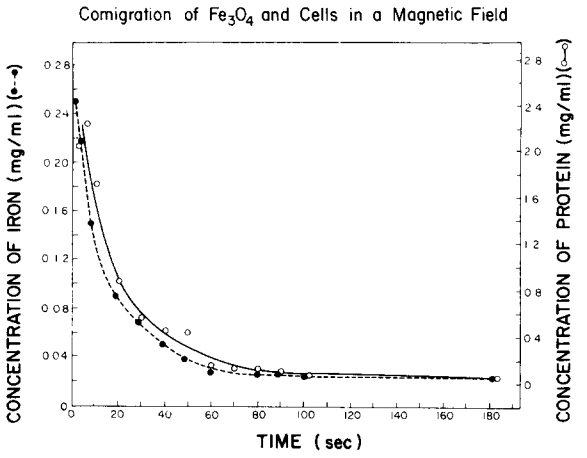

Fig. 2. Time-course of polyvinylamine-magnetite-coated cell attraction to the diverging field electromagnet. Details of this experiment are presented in the text. The comigration of magnetite and protein in the magnetic field demonstrates that the pellicle observed in the scanning electron microscopy is composed of magnetite and not simply polyvinylamine. It should also be noted that the magnet is able to extract the coated cells in less than 2 min.

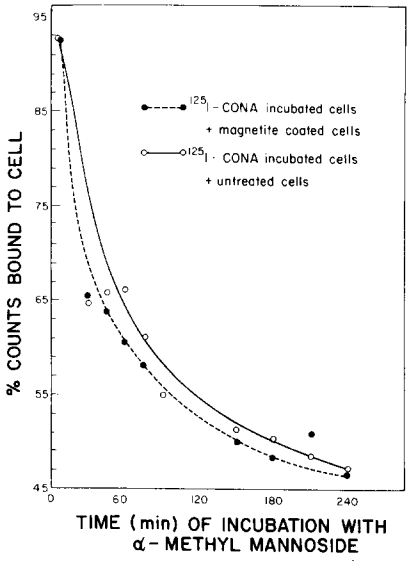

Fig. 3. Analysis of the shielding data for possible artifactual contributions from nonspecific binding of [^{125}I]concanavalin A to the magnetite pellicle. Details of the experiment are presented in the text. Even after 4 h, no nonspecific binding of the lectin to the magnetite pellicle could be detected. CONA, concanavalin A.

tergents failed due to inactivation of the enzymes. By comparing the activity of the homogenate to the smallest amount of activity that could be detected in the membrane fraction in the presence of magnetite, the maximum relative specific activity for lactate dehydrogenase and succinate dehydrogenase was estimated to be less than 0.75.

TABLE II

SHIELDING OF ^{125}I-CONCANAVALIN A ON THE CELL SURFACE BY POLYVINYLAMINE-MAGNETITE OR CATIONIC SILICA TO PROTEOLYSIS OR RELEASE BY α-METHYLMANNOSIDE

Cells were labeled with ^{125}I-concanavalin A and either coated twice with polyvinylamine-magnetite according to the procedures in Materials and Methods or with cationic silica [14]. Coated and uncoated cells were treated with either pronase, papain or α-methylmannoside and after the cells were removed, the amount of ^{125}I remaining bound to the cells was determined. Percent shielding was the amount released from coated cells compared with uncoated.

Treatment	Percent shielding	
	silica coated	magnetite coated
α-Methylmannoside competition	43	53
Pronase digestion	61	54
Papain digestion	79	45

Table III summarizes the results obtained using the various markers. ^{125}I-Concanavalin A and enzyme marker yields were determined for the cell homogenate, the plasma membrane fraction and the discarded supernatant. The combined yield of the plasma membrane and discarded supernatant always represented 95–110% of the total activity in the cell homogenate, indicating minimal enzyme deactivation during the purification procedure. Enrichment of plasma membrane markers and depurification of markers for other organelles is expressed in Table III in terms of relative specific activity; the specific activity of the plasma membrane fraction divided by the specific activity of the cell homogenate. The table also compares the magnetite to the silica technique routinely used in our laboratory [14]. The membranes isolated by the silica technique were pelleted at 800 × *g* for 5 min in a clinical centrifuge. It should be noted that previous reports indicated that gradient centrifu-

TABLE III

COMPOSITIONAL ANALYSIS OF MAGNETICALLY EXTRACTED PLASMA MEMBRANE

The specificity of the markers with respect to subcellular organelles is indicated. Protein yield for magnetically extracted membranes was 5.0%. The numbers in parentheses correspond to relative specific activities obtained in parallel experiments using the silica technique to isolate the plasma membrane [14].

Marker	Source	yield (%)	relative specific activity
[^{125}I]Concanavalin A	external plasma membrane	42	7.8 (6.0)
Magnetite	external plasma membrane	78	10
Alkaline phosphatase	plasma membrane	15	3.3 (2.4)
5'-Nucleotidase	plasma membrane	15	2.8 (1.8)
β-*N*-Acetylglucosaminidase	lysosome	1.2	0.04 (0.40)
Glucose-6-phosphatase	endoplasmic reticulum	4.4	0.30 (0.22)
Succinate dehydrogenase [a]	mitochondria	–	
Lactate dehydrogenase [a]	cytoplasm	–	

[a] The limit of detection by the enzyme was low and the upper limit of relative specific activity was 0.75.

gation of the silica-coated membranes gave degrees of purification almost 2-fold greater than centrifugation in a clinical centrifuge [14].

Discussion

The colloidal polyvinylamine-magnetite and colloidal silica plasma membrane isolation techniques illustrate a general principle of density perturbation for subcellular fractionation. The polyvinylamine-magnetite technique also permits the plasma membrane to respond to magnetic fields. This is of particular value when the plasma membrane fraction is to be extensively washed to remove subcellular contaminants. Typically, the membrane fraction obtained with the polyvinylamine-magnetite may be washed 20 times in 20 min. To wash the silica pellet to the same extent requires more than 2 h. Magnetic isolation of plasma membrane also significantly reduces lysosomal contamination when compared to the centrifugal process employed in the silica technique. If the polyvinylamine-magnetite-treated cell homogenate is pelleted using a clinical centrifuge, lysosomal contamination was found to be approximately the same as for the silica procedure. It should be stressed at this point that in our hands both techniques routinely generate plasma membrane of approximately the same quality in terms of yield and plasma membrane marker purification.

Addition of saturating amounts of polyvinylamine to the colloidal magnetite is critical to extraction of plasma membrane of high yield and purity. The polyvinylamine probably serves two important roles in this membrane purification procedure. It acts as a dispersing agent in the colloidal magnetite solution and in an analogous manner as certain detergents added to colloid preparations it provides a molecular film around the magnetite particles. This serves as an elastic cushion preventing particle agglomeration. The polyvinylamine also confers upon the particles a strong net positive charge essential for binding the negatively-charged cells. Magnetite at room temperature has an isoelectric point of about 6.7, at which point it precipitates out of solution. Applying polyvinylamine allowed purification of membrane using magnetite while maintaining physiological buffer conditions, i.e., pH 7.0. It is believed that addition of polyvinylamine in amounts greater than necessary for magnetite saturation is only slightly detrimental to membrane purification. Since polyvinylamine reacts to the protein assay of Lowry et al. [25] and since it is concentrated in the membrane pellet, relative specific activities ob-

tained at greater than saturating concentrations of the polymer tend to be underestimates of the purity of the preparation.

Another important aspect of density perturbation with a noninternalized inorganic support is that only externally oriented plasma membrane is isolated. Internalized plasma membrane is not isolated as a component of the dense pellicle. This is readily apparent when one compares the purification of ^{125}I-concanavalin A with either alkaline phosphatase or 5′-nucleotidase. By comparing activities of 5′-nucleotidase and alkaline phosphatase in whole cells with those treated with Triton X-100 and subjected to rigorous lysis in the Parr bomb, it was determined that only 50% of the enzyme activity was associated with the external plasma membrane. This is consistent with other published values for these marker enzyems [40]. Since ^{125}I-concanavalin A is a marker of external plasma membrane it is recovered at much higher specific activity and yield. Magnetite enrichment exceeds that of concanavalin A due to the copurification of noncolloidal magnetite agglomerates in the plasma membrane preparation. These agglomerates do not appear to be cell-associated when viewed by phase-contrast microscopy. Thus, concanavalin A provides the best estimate of plasma membrane purification by polyvinylamine-magnetite.

The polyvinylamine-magnetite plasma membrane isolation technique should serve as a useful adjunct to other existing techniques. It is especially suitable for membrane topological studies. Since the inert pellicle formed on the outer surface of the plasma membrane is less permeable to labeling reagents and blocks access to the extracellular membrane surface, membranes isolated by the technique may be selectively labelled from both sides of the membrane for transbilayer mapping studies (e.g., Ref. 14). The technique may also have application in the study of internalized membranes such as endosomes and lysosomes as these organelles should be easily separable from the plasma membrane. Classical centrifugal techniques cannot separate pinocytic vesicles from plasma membrane due to their similar densities [41].

The polyvinylamine-magnetite technique for plasma membrane isolation is rapid and gives plasma membrane in high yield. Like the silica technique, plasma membrane may be obtained in approx. 2 h. The external plasma membrane is readily identified as the fraction attracted to a diverging electromagnet. Centrifugal techniques employed in the past result in more than three plasma membrane fractions [37,44,45]. Das and Henderson [45] found their two most dense plasma membrane fractions to have identical polypeptide composition although the components were present in different stoichiometries. The third fraction lacked approx. 8% of the polypeptides identified in the other fractions by two-dimensional gel electrophoretic analysis. It is thus likely that plasma membrane isolated by centrifugal techniques represents a mixture of internal plasma membrane, external plasma membrane, cytoskeletal components, and some intracellular organelles resulting in multiple membrane fractions of differing composition and marker specific activity. The centrifugation techniques also tend to be long and arduous, often requiring 20 or more hours for completion.

Acknowledgements

We wish to thank Dr. Chris Woodcock and Wendy Marston for their assistance with the electron microscopy. We also thank Dr. Ken Langley for the use of his laser light scattering facility and Dr. Judy Shiozawa for her valuable advice and insight. This work was supported in part from grants from the N.I.H. GM25461 and GM29127. B.S.J. is an Established Investigator for the American Heart Association.

References

1 Murray, B.A. (1982) in The Development of *Dictyostelium discoideum* (Loomis, W.F., ed.), pp. 71–116, Academic Press, New York
2 Warren, L., Glick, M.C. and Nass, M.K. (1966) J. Cell Physiol. 68, 269–287
3 Steck, T.L., Strauss, J.H., Weinstein, R.R. and Wallach, D.H. (1970) Science 168, 255–256
4 Klenk, H.D. (1973) in Biomembranes (Chapman, D. and Wallach, D.P., eds.), Vol 2, pp. 145–183, Academic Press, London
5 Leskes, A., Siekevitz, P. and Palade, G.E. (1971) J. Cell. Biol. 49, 264–287
6 Murray, R.K., Suess, R. and Pitot, H.C. (1967) Methods Cancer Res. 2, 239–256
7 Hubbard, A.L., Wall, D.A. and Ma, A. (1983) J. Cell. Biol. 96, 217–229

8 Albertsson, P.A. (1972) Partition of Cell Particles and Macromolecules, 2nd Edn., Wiley-Interscience, New York
9 Walter, H., Krob, D.J. and Brooks, D.E. (1976) Biochemistry 15, 2959–2964
10 Jacobson, B.S. and Branton, D. (1977) Science 195, 302–304
11 Cohen, C.M., Kalish, D.E., Jacobson, B.S. and Branton, D. (1977) J. Cell. Biol. 75, 119–134
12 Jacobson, B.S. (1980) Biochim. Biophys. Acta 600, 769–780
13 Gotlib, L.J. and Searls, D.B. (1980) Biochim. Biophys. Acta 602, 207–212
14 Chaney, L.K. and Jacobson, B.S. (1983) J. Biol. Chem. 258, 10062–10072
15 Rembaum, R.K., Yen, D.H., Kempner, D.H. and Ugelstad, J. (1982) J. Immunol. Methods 52, 341–351
16 Merivuori, H., De la Chapelle, A. and Schroder, J. (1980) Exp. Cell Res. 130, 464–467
17 Margel, S., Zisblatt, S. and Rembaum, A. (1979) J. Immunol. Methods 28, 341–353
18 Kronick, P.L. and Joseph, K. (1978) Science 200, 1074–1076
19 Antoine, J.C., Ternynck, T., Rodrigot, M. and Avrameas, S. (1978) Immunochemistry 15, 443–452
20 Mosbach, K. and Andersson, L. (1977) Nature 270, 259–261
21 Molday, R.S. and Yen, S.P. (1977) Nature 268, 437–438
22 Molday, R.S., Dreyer, W.J., Rembaum, A. and Yen, S.P. (1975) J. Cell Biol. 64, 75–88
23 Franke, J. and Kessin, R. (1977) Proc. Natl. Acad. Sci. USA 74, 2157–2161
24 Bloomfeld, U.A. and Lim, T.K. (1978) Methods Enzymol. 48, 415–495
25 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275
26 Lee, A., Chance, K., Weeks, C. and Weeks, G. (1975) Arch. Biochem. Biophys. 171, 406–417
27 Moore, E.P. (March 6, 1973) U.S. Patent 3, 719, 607
28 Findlay, J., Levy, G.A. and Marsh, C.A. (1958) Biochem. J. 69, 467–476
29 Hubscher, G. and West, G.R. (1965) Nature (Lond.) 205, 799–800
30 Massey, V. (1959) Biochim. Biophys. Acta 34, 255–258
31 Neilands, J. (1955) Methods Enzymol. 1, 449–454
32 Snell, F.D. and Snell, C.T. (1949) in Colorimetric Methods of Analysis, Vol. 2, Van Nostrand, New York
33 Cuatrecasas, P. (1973) Biochemistry 12, 751–758
34 Hayat, M.A. (1981) Fixation for Electron Microscopy, pp. 201–205, Academic Press, New York
35 Du, Y.W., Lu, H.X., Wang, Y.Q. and Wang, T.X. (1983) J. Magn. Magn. Mater. 31–34, 896–898
36 Armant, D.R. and Rutherford, C.L. (1981) J. Biol. Chem. 256, 12710–12718
37 Gilkes, N.R. and Weeks, G. (1977) Biochim. Biophys. Acta 464, 142–156
38 Crean, E.V. and Rossomando, E.F. (1977) Biochem. Biophys. Res. Commun. 75, 488–495
39 Green, A.A. and Newell, P.C. (1974) Biochem. J. 140, 313–322
40 Siu, C.H., Lerner, R.A., Ma, G., Firtel, R.A. and Loomis, W.F. (1976) J. Mol. Biol. 100, 157–178
41 Sugimoto, T. and Matijevic, E. (1980) J. Colloid Interface Sci. 74, 227–242
42 Rossomando, E.F. and Cutler, L.S. (1975) Exp. Cell. Res. 95, 67–78
43 Mellman, I.S., Steinman, R.M., Unkeless, J. and Cohn, Z.A. (1980) J. Cell Biol. 86, 712–722
44 McMahan, D., Miller, M. and Long, S. (1977) Biochim. Biophys. Acta 465, 224–241
45 Das, O. and Henderson, E. (1983) Biochim. Biophys. Acta 464, 45–56