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APPLICATION OF LATEX MICROSPHERES IN THE ISOLATION OF PLASMA MEMBRANES

AFFINITY DENSITY PERTURBATION OF ERYTHROCYTE MEMBRANES

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SUMMARY

Immunolatex spheres, originally developed as visual markers for scanning electron miscroscopy, were employed as membrane density perturbation reagents. Methacrylate spheres were bound to antibody molecules and used to label antigens on erythrocytes. Ghosts prepared from labeled cells were subjected to isopycnic centrifugation on continuous sucrose and dextran gradients. It was found that the labeled erythrocyte membranes had a substantially higher density than unlabeled membranes. The extent to which the membrane density was shifted on a given gradient depended on the number, size and density of the latex spheres and could be closely predicted by theory. These results suggest that the reagents and techniques described here have potential application for the isolation of plasma membranes from more complex cell types.

INTRODUCTION

One of the major difficulties encountered in purifying plasma membranes from animal cells has been in separating out other cellular membranes having similar physical properties. Differential and isopycnic centrifugation of cell homogenates have been used routinely to fractionate membrane fragments differing in size, shape and density [1]. Plasma membranes isolated by this methodology, however, have often been found to be contaminated with membranes derived from cellular organelles [2].

Recently, Wallach and coworkers [3] introduced a new membrane fractionation method, termed affinity density perturbation. In this technique, the density of membrane vesicles bearing lectin receptors was increased by the binding of concanavalin A-virus complexes.

In this paper we describe a modification of the affinity density perturbation

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method designed to specifically isolate plasma membranes. This technique utilizes immunolatex spheres, previously employed as visual markers for scanning electron microscopy [4, 5], to label cell surface antigens. The labeled cells are then disrupted and the homogenate is subjected to isopycnic centrifugation. Plasma membranes will band at a higher density on a continuous gradient due to the bound latex-antibody conjugates and hence can be separated from membranes with a lighter density.

We have used red blood cells as a model system to test the applicability of latex spheres to serve as membrane density perturbation reagents. The effect of the number, size and density of the latex spheres on the density of labeled erythrocyte membranes is reported.

MATERIALS AND METHODS

Methylmethacrylate, 2-hydroxyethylmethacrylate, methacrylic acid and ethyleneglycol dimethacrylate were obtained from Rohm and Haas and purified by vacuum distillation as previously described [5]. Glutaraldehyde (25 % aqueous solution) was purchased from Polysciences; 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide from the Ott Chemical Co.; CNBr from Matheson, Coleman and Bell and dansyl-ε-lysine from Sigma Chem. Co. Rabbit IgG was obtained from Miles-Pentex and goat anti-rabbit γ-globulin antiserum from Antibodies Inc. Rabbit antiserum against human erythrocytes (rabbit anti-erythrocyte antiserum) having a hemagglutination titer of 2^{11} was obtained from Cappel Laboratories.

Phosphate-buffered saline, pH 7.40, was composed of 8.0 g of NaCl, 0.2 g of KCl, 0.2 g of KH_2PO_4 and 1.15 g Na_2HPO_4 per l of distilled water. 310 mosM buffer according to Dodge et al. [6], pH 7.4, was prepared by dissolving 2.742 g $NaH_2PO_4 \cdot H_2O$ and 12.747 g of Na_2HPO_4 into 1 l of solution. The corresponding 20 mosM buffer was then prepared from the 310 mosM buffer by dilution.

Gradients were made with either ultra-pure sucrose (Schwarz-Mann), dextran of average molecular weight 40 000 (Sigma) or glycerol (Mallinckrodt).

Synthesis of latex spheres

Acrylic latex spheres consisting of 53 % methylmethacrylate, 30 % 2-hydroxyethylmethacrylate, 10 % methacrylic acid and 7 % ethyleneglycol dimethacrylate were synthesized by aqueous emulsion copolymerization as previously described [5]. Briefly, spheres having an average diameter of 100 nm (1000 Å) were prepared by adding 5.8 g methylmethacrylate, 3.3 g 2-hydroxyethylmethacrylate, 1.1 g methacrylic acid, 0.8 g ethyleneglycol dimethacrylate, 0.1 g of sodium dodecyl sulfate and 0.01 g of $(NH_4)_2S_2O_8$ to 88.9 ml of water. This yielded a total methacrylate monomer concentration of 11 %. The reaction mixture was saturated with argon and polymerization was carried out at 98 °C for 1 h in sealed, tumbling containers. Copolymer spheres having a diameter of 30 nm (300 Å) were polymerized under similar conditions from a solution containing 3 % total monomer. Ionic impurities were removed by passing the latex suspension, adjusted to pH 7.0, through a mixed-bed ion-exchange column consisting of Biorad AG 1-X10 and AG 50-W-X12 resins.

Purification of goat anti-rabbit IgG antibodies

Goat anti-rabbit IgG antibodies were purified from whole serum on an immu-

noadsorbent column consisting of rabbit IgG coupled to Sepharose 4B by the CNBr method [7, 8]. NaSCN (3 M) in 0.01 M phosphate buffer, pH 7.0, was used to elute the goat anti-rabbit IgG from the column. The eluate containing the antibody was dialyzed exhaustively against phosphate-buffered saline over 3–4 days at 4 °C. Anti-rabbit IgG activity was assayed by immunodiffusion.

Preparation of fluorescent immunolatex spheres

Acrylic latex spheres were tagged with fluorochrome, dansyl-ε-lysine, using the CNBr reaction, and subsequently coupled to goat anti-rabbit IgG antibodies by a two-step glutaraldehyde reaction as previously described [5]. Typically, 10 ml of latex spheres (40–50 mg/ml) were activated with 100 mg of CNBr at pH 10.5 for 15 min at 25 °C. The suspension was then cooled to 4 °C and added to an equal volume of 10 mM dansyl-ε-lysine in 0.2 M sodium carbonate buffer at pH 10.0. The reaction was allowed to proceed overnight at 4 °C. Uncoupled dansyl-ε-lysine was removed by dialysis against several changes of 0.1 M NaCl over 4–5 days.

The fluorescent latex spheres were derivatized with diaminoheptane by adding 20 mg of 1-ethyl-3-(3-dimethyl-aminopropyl)carbodiimide to 10 ml of latex spheres (20–30 mg/ml) in 0.01 M diaminoheptane at pH 7.0. The carbodiimide reaction was carried out at 4 °C for 2 h. Free diaminoheptane was removed by exhaustive dialysis against phosphate-buffered saline.

The diaminoheptane-derivatized spheres were activated with 1.25 % glutaraldehyde at 25 °C for 1 h. Excess glutaraldehyde was then removed by dialysis overnight at 4 °C against several changes of phosphate-buffered saline. 5 mg of goat anti-rabbit IgG antibodies were added to 10 ml of the glutaraldehyde-activated spheres and the mixture was stirred at 25 °C for 3–5 h. The reaction was stopped by the addition of 1 ml of 0.1 M glycine, pH 8.0.

Goat anti-rabbit IgG antibody-latex conjugates were separated from unbound antibody by centrifugation on a discontinuous sucrose gradient as previously described [5]. Conjugate solutions (20 mg/ml) were stored at 4 °C in tightly closed vials until used.

Preparation of erythrocyte ghosts and membrane vesicles

Type 0, Rh^+ blood was collected into acid/citrate/dextrose solution. Erythrocytes were washed three times in phosphate-buffered saline by centrifugation ($500 \times g$, 15 min). Erythrocyte ghosts were prepared by the procedure of Dodge et al. [6] and stored frozen at −20 °C in 0.1 M sucrose. Membrane vesicles were prepared from ghosts by sudden decompression in an Artisan pressure homogenizer [9] after equilibration with N_2 for 25 min at 700 lb/inch^2.

Labeling of cells and ghosts

Erythrocytes and ghosts were labeled by an indirect (sandwich) technique. Aliquots, containing $4–5 \cdot 10^8$ erythrocytes, equilibrated to 4 °C, were pelleted ($500 \times g$, 10 min) and resuspended into 25 µl rabbit anti-human erythrocyte antiserum. In the control sample, 25 µl of phosphate-buffered saline was substituted for the erythrocyte-specific antiserum. The cells were incubated for 1 h at 4 °C with occasional shaking to prevent settling and minimize formation of large aggregates. 3–4 ml cold phosphate-buffered saline were added and the cells were pelleted ($400 \times g$, 20 min).

This washing procedure was repeated three more times. The final pellet was resuspended in 200 μl goat anti-rabbit IgG-latex conjugate (0.5–20 mg/ml), and incubated with occasional shaking for 30 min at 25 °C. Each sample was then diluted with 4–5 ml phosphate-buffered saline and centrifuged at $300 \times g$ for 20 min. Supernatant containing unbound latex conjugates was removed. Cell samples were washed once more with phosphate-buffered saline and resuspended in 0.4 ml 310 mosM buffer according to Dodge et al. [6]. 25-μl aliquots were removed for scanning electron microscope studies. The remaining cells in each sample were lysed by the addition of 6 ml of 20 mosM buffer according to Dodge et al. [6]. The ghosts were pelleted at $6000 \times g$ for 20 min, washed with 10 ml of the 20 mosM buffer, resuspended in 0.3 ml of phosphate-buffered saline and layered on the gradient for isopycnic centrifugation.

Frozen and thawed membranes were labeled similarly except that incubation in antisera was at 25 °C for 30 min and a higher g force ($20\,000 \times g$, 10 min) was used to pellet the membranes. Unbound latex conjugates were not separated from labeled membranes prior to layering onto the gradients.

Determination of isopycnic density

Continuous linear density gradients of sucrose (25–58 %, ρ = 1.11–1.26 $g \cdot cm^{-3}$), dextran (5–40 %, ρ = 1.02–1.17 $g \cdot cm^{-3}$) or glycerol (20–80 %, ρ = 1.05–1.20 $g \cdot cm^{-3}$) were prepared with a Buchler density gradient mixer. All gradient solutions were prepared with phosphate-buffered saline. Samples were layered onto the gradients and centrifuged to equilibrium at 4 °C and $100\,000 \times g$ in a Beckman SW 39L rotor. Typical centrifugation times were 1–1.5 h for sucrose gradients, 4 h for glycerol gradients and 14–15 h for dextran gradients or for small vesicles in sucrose. Prolonged centrifugation (16 h) in sucrose gradient resulted in aggregation of labeled membranes, but the final density was the same as that after 1 h of centrifugation.

Each gradient was fractionated into 150-μl fractions and the refractive index was measured to determine the density. The presence of membrane or latex conjugates was determined from light scattering measurements at 280 nm after dilution with 0.5 ml phosphate-buffered saline.

Determination of degree of labeling

Labeled erythrocytes were washed 3–4 times, fixed with 1.25 % glutaraldehyde in phosphate-buffered saline and centrifuged onto ionene-coated coverslips as previously described [5]. Samples were dehydrated in a series of ethanol solutions (50–100 %), critical-point dried from Freon 13, and coated with gold-palladium. Samples were examined with an ETEC scanning electron microscope operating at 20 kV.

The degree of labeling was determined from scanning electron micrographs by counting the number of latex spheres visible on one surface of labeled erythrocytes (including the rim). 4–8 cells from each sample were counted. The count for each sample was averaged and doubled and used as an estimate of the total number of latex spheres on the whole cell. This procedure tends to underestimate the total number since some latex spheres on the rim of the cell could be obscured.

RESULTS

Properties of erythrocyte membranes and latex-antibody conjugates

Erythrocyte ghosts prepared by lysis of human red blood cells in hypotonic solution [6] were biconcave in shape as viewed by phase contrast microscopy. Ghosts subjected to nitrogen cavitation [9] or sonication were disrupted into small vesicles having a wide distribution of sizes, generally less than 0.5 μm in diameter. Protein composition of ghosts prepared by the procedure of Dodge et al. [6], as determined by gel electrophoresis, was identical to that reported by Fairbanks et al. [10].

The isopycnic densities of erythrocyte ghosts and vesicles in several gradients are summarized in Table I. The density of the ghosts depends on their permeability to the gradient material and on the effect of osmotic pressure [1, 11, 12]. Since membranes are permeable to glycerol [2], the observed density of ghosts in a glycerol gradient ($\rho = 1.187$ g $\cdot$ cm^{-3}) is essentially the hydrated density of the erythrocyte membrane. The densities in sucrose and in dextran gradients are lower than in glycerol and presumably correspond to the buoyant density of the ghosts subjected to the osmotic pressure induced by the non-permeating gradient material. This interpretation is in accord with the observations of Bodemann and Passow [13]. Their type II (sealed) ghosts and our ghosts have similar sucrose gradient densities.

TABLE I

ISOPYCNIC DENSITY OF ERYTHROCYTE GHOSTS AND LATEX CONJUGATES

Erythrocyte ghosts were prepared by the procedure of Dodge et al. [6]. Densities were then determined by equilibrium centrifugation at 4 °C.

Sample	Gradient material	Density (g $\cdot$ cm^{-3})
Ghost	Glycerol	1.187
Ghost	Sucrose	1.169
Ghost	Dextran	1.088
Latex-antibody conjugates (100 nm diameter)	Sucrose	1.232
Latex-antibody conjugates (300 nm diameter)	Sucrose	1.232

The reduction in volume of the sealed ghosts (or vesicles) which arises from the osmotic effects of a non-permeant gradient can be calculated from the observed buoyant density (ρ_v) using the relation

$$\rho_v = \frac{\rho_m V_m + \rho_s V_s}{V_m + V_s} \tag{1}$$

where ρ_m is the hydrated membrane density (the isopycnic density in a permeating gradient, e.g. glycerol), V_m is the membrane volume, V_s is the volume of the fluid inside the vesicle at equilibrium and ρ_s is the density of the fluid inside the vesicle.

For erythrocyte ghosts in a sucrose gradient with $\rho_m = 1.187$ g $\cdot$ cm^{-3}, $\rho_v = 1.169$ g $\cdot$ cm^{-3}, $\rho_s = 1.0$ g $\cdot$ cm^{-3}, membrane surface area $A_m = 145$ μm^2 [14],

membrane thickness h_m 7 nm and V_m $A_m \cdot h_m$ $1.01 \cdot 10^{-12}$ cm^3. V_v was calculated to be $1.08 \cdot 10^{-13}$ cm^3. Since the mean corpuscular volume is $8.7 \cdot 10^{-11}$ cm^3 [15], V_v is only 0.1 % of the original ghost volume.

A similar calculation in dextran (ρ 1.088 $g \cdot cm^{-3}$) leads to a V_v of $1.14 \cdot 10^{-12}$ cm^3, approx. 1.3 % of the original ghost volume.

The density of latex-antibody conjugates is also given in Table I. Differences in conjugate density with different size latex spheres were undetectable.

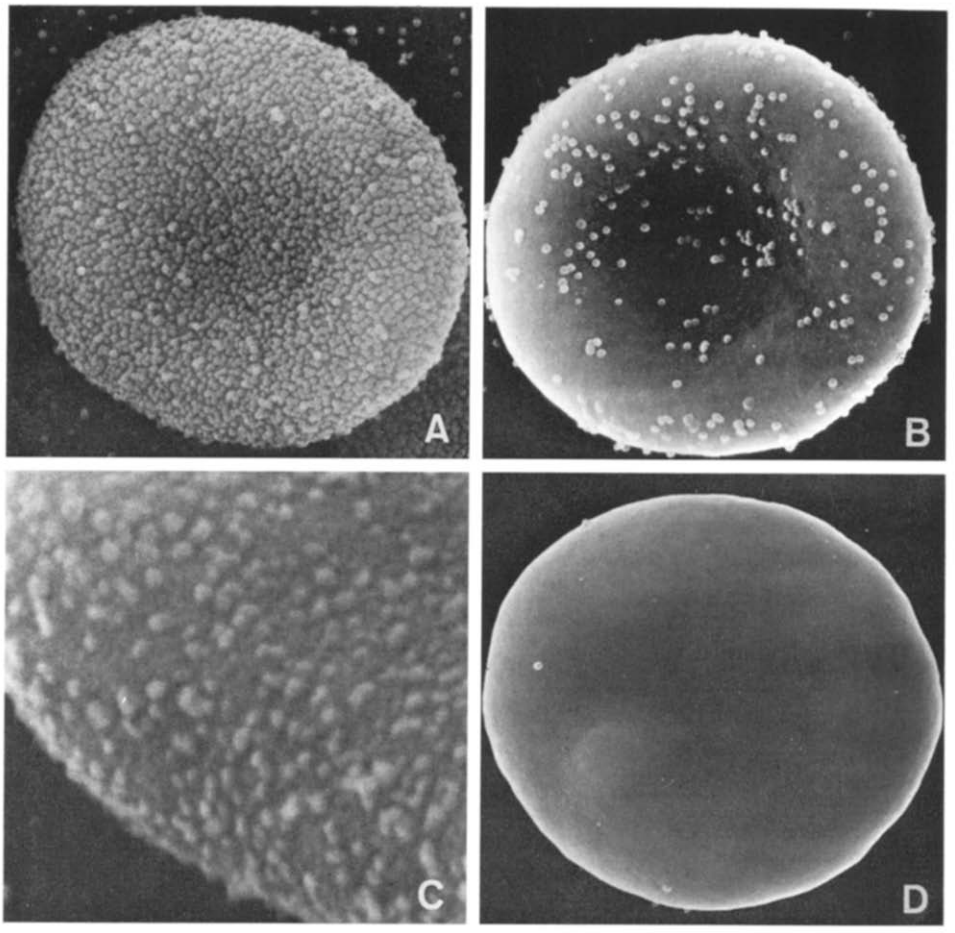

Fig. 1. Scanning electron micrographs of human erythrocytes treated with immunolatex conjugates. (A) Erythrocytes sensitized with rabbit anti-erythrocyte antibodies and subsequently labeled with goat anti-rabbit IgG antibody-latex conjugates at a concentration of 20 mg/ml. Spheres have an average diameter of 100 nm, but appear slightly larger in the micrographs due to the gold-palladium coating (9200). (B) Erythrocyte treated as in A, but with a concentration of 0.5 mg/ml of immunolatex conjugate (9200). (C) Erythrocyte at high magnification treated as in A, but labeled with immunolatex spheres (8 mg/ml) having a diameter of approx. 30 nm (50 000). These spheres, however, are more heterogeneous in size compared to 100-nm spheres (see ref. 5). (D) Control for A. Unsensitized erythrocyte incubated with 20 mg/ml of 100-nm diameter goat anti-rabbit IgG antibody-latex conjugate (9200).

Erythrocytes labeled with immunolatex spheres

Erythrocytes sensitized with rabbit anti-erythrocyte antibodies can be labeled with goat anti-rabbit IgG antibody-latex conjugates for visualization by scanning electron microscopy [4]. Fig. 1a shows an erythrocyte densely labeled with immunolatex spheres having an average diameter of 100 nm (1000 Å). The number of spheres bound per cell depends on the concentration of the reagent used in the labeling procedure [4]: at low concentrations of immunolatex spheres, a sparse, random distribution of spheres is observed (Fig. 1b). Immunolatex spheres having a diameter of approx. 30 nm can also serve as markers (Fig. 1c). Non-specific binding of immunolatex spheres to unsensitized cells was extremely low (Fig. 1d).

When the labeled erythrocytes were hemolysed and washed by centrifugation, the dansyl-lysine tagged immunolatex spheres remained bound to the erythrocyte membranes as confirmed by fluorescent and scanning electron microscopy.

Density perturbation of erythrocyte membranes

As shown in the sucrose density gradients in Fig. 2, ghosts prepared from erythrocytes labeled with fluorescent immunolatex spheres band at a position between the unlabeled ghosts and the immunolatex conjugates. These membrane bands were intensely fluorescent under ultraviolet light, thus confirming the presence of bound

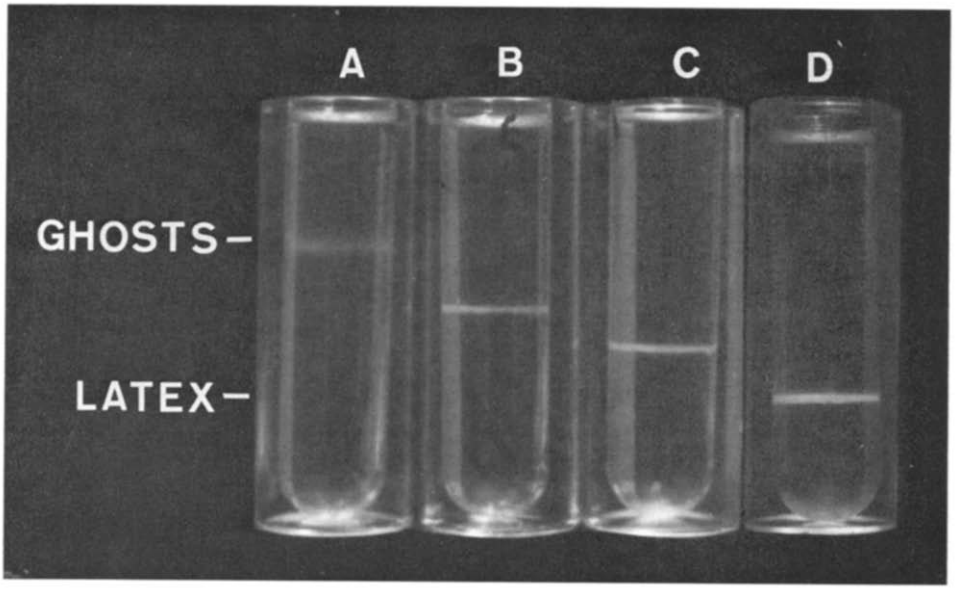

Fig. 2. The isopycnic banding of erythrocyte ghosts and latex spheres on linear sucrose gradients at 4 °C. Gradient A displays ghosts prepared from unsensitized erythrocytes treated with goat anti-rabbit IgG antibody-latex conjugate (100 nm in diameter). The measured density of 1.169 g · cm^{-3} is the same as that of erythrocyte ghosts not exposed to the conjugate. Gradient B shows ghosts derived from erythrocytes sensitized with rabbit anti-erythrocyte antibodies and labeled with immunolatex conjugate. A density of 1.204 g · cm^{-3} was determined and corresponded to 1795 spheres per cell. Gradient C shows ghosts derived from erythrocytes sensitized as in B, but treated with a higher concentration of conjugate. A density of 1.212 g · cm^{-3} was determined and corresponded to 3430 spheres/cell. Gradient D shows the sharp band obtained for latex spheres. A density of 1.232 g · cm^{-3} was measured.

latex reagents. The isopycnic density of the labeled ghosts depended on the concentration of immunolatex used to label the cells and, consequently, on the degree of labeling of the cells. The density of ghosts obtained from erythrocytes either labeled with rabbit anti-erythrocyte antibodies alone or else treated only with the immunolatex conjugates (control) was the same as that measured for ghosts of untreated cells. A similar dependence of density on conjugate concentration was observed when erythrocyte ghosts rather than intact cells were labeled.

The relationship between the number of spheres (100 nm diameter) bound per cell as counted in scanning electron micrographs (for example see Figs 1a and 1b) and the observed density of the labeled ghosts in sucrose gradients is depicted in Fig. 3. When 4500 spheres, having an average diameter of 100 nm, were bound per erythrocyte, a density of 1.215 $g \cdot cm^{-3}$ was achieved. When as few as 300 spheres were bound, a density change of 0.01 relative to unlabeled membrane (control) was found.

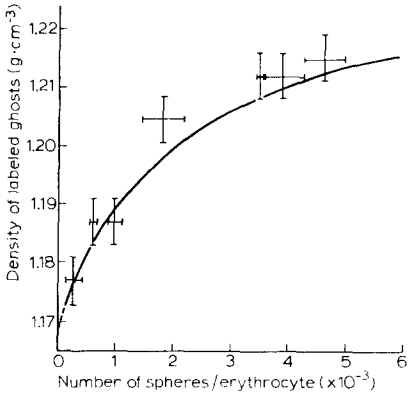

Fig. 3. The relationship between the number of immunolatex spheres (100 nm diameter) bound per erythrocyte and the isopycnic density of the corresponding labeled ghosts. Erythrocytes sensitized with rabbit anti-erythrocyte antibodies were labeled with varying concentrations of immunolatex spheres. The number of spheres was counted from scanning electron micrographs. The densities of the ghosts prepared from the labeled cells were determined by equilibrium centrifugation on linear sucrose gradients at 4 °C. Data are given with standard deviations. Theoretical curve was calculated from Eqn 2.

Theoretically, the effect of the degree of labeling on the density of the latex particle-membrane vesicle complex (ρ_c) can be determined from the following equation:

$$\rho_c = \frac{\rho_m V_m + n\rho_b V_b + V_s \rho_s}{V_m + nV_b + V_s} \qquad (2)$$

where n is the number of latex spheres bound to the vesicle and ρ_b, V_b are latex sphere density and volume, respectively. To a first approximation, V_s can be assumed to remain constant (i.e. identical to V_s of original unlabeled vesicles), then ρ_c can be plotted as a function of n. As shown in Fig. 3, there is a good correlation between the experimentally determined points and the calculated curve. Hence, any further

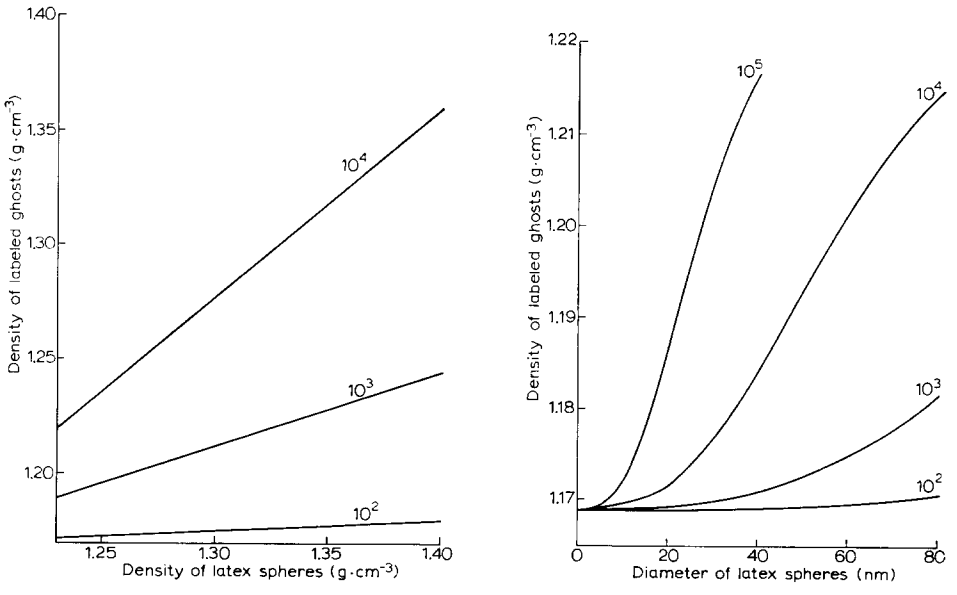

Fig. 4. Dependence of the density of labeled erythrocyte ghosts on the density and size of latex spheres. (A) Effect of increasing density of latex spheres on ghosts labeled with 10^2–10^4 spheres of 100 nm diameter. (B) Effect of different sizes of latex spheres on ghosts labeled with 10^2–10^5 spheres of density 1.232 g · cm^{-3}. Curves were calculated from Eqn 2, using a membrane density = 1.187 g · cm^{-3}, membrane thickness = 7 nm, and membrane area = 145 μm^2.

decrease in V_s must be small. This is to be expected since unlabeled erythrocyte ghosts are already essentially collapsed at isopycnic equilibrium in sucrose.

Eqn 2 can also be used to predict the effect of changing various parameters on the isopycnic density of the membrane-latex complex. As shown in Fig. 4a, there is a linear increase in the isopycnic density of the complex (ρ_c) with an increase in the density of the latex spheres (ρ_b). The effect is especially pronounced with heavy labeling of the cell surface.

For a constant number of bound latex spheres, the isopycnic density of a labeled vesicle (ρ_c) will be higher for larger latex spheres (Fig. 4b). However, a significant density shift can still be obtained with small (approx. 30 nm diameter) spheres provided that heavy labeling occurs.

Table II summarizes the experimental results obtained when some of these parameters were varied. When 30-nm diameter spheres, instead of 100-nm spheres, were used to label cell surface antigens, a smaller density shift (difference between labeled and unlabeled ghosts) was observed. A greater density shift was found for labeled ghosts in dextran gradients. This difference is primarily due to the lower buoyant density of ghosts in dextran compared to that in sucrose gradients (Table I). The observed isopycnic density in dextran is in agreement with the value calculated from Eqn 2.

A preliminary study indicates that small membrane vesicles, both labeled and unlabeled, have a lower density than the corresponding intact erythrocyte ghosts. The membrane bands of small vesicles were also quite diffuse. The cause of this is

TABLE II

DEPENDENCE OF ERYTHROCYTE MEMBRANE DENSITY SHIFT ON VARIOUS PARAMETERS

Erythrocytes were labeled with immunolatex spheres of the indicated size and then lysed according to a modification of the procedure of Dodge et al. [6]. Vesicles were prepared from the erythrocyte ghosts with an Artisan pressure homogenizer. The densities of the membrane-latex complex were then determined as described in Table I. The number of spheres bound per cell was determined from scanning electron micrographs.

Experiment	Membrane sample	Gradient	Latex size diameter (nm)	No. cells counted	Average No. latex spheres/cell	Density shift ($g \cdot cm^{-3}$)
I	Ghost	Sucrose	100	5	3 476	0.041
	Ghost	Dextran	100	5	3 476	0.067
II	Ghost	Sucrose	100	4	3 322	0.035
	Vesicle	Sucrose	100	4	3 322	0.055*
III	Ghost	Sucrose	30	4	12 074**	0.023
Control	Ghost		100	6	10	

* Vesicles showed up as a fairly diffuse band in the gradient. Density shift value corresponds to the density difference between the center of labeled and unlabeled vesicle bands ($\rho = 1.133\ g \cdot cm^{-3}$).

** The number of spheres in 2.5 μm^2 of the cell surface was counted and the total number of spheres on the cell was estimated. This count is unreliable due to low resolution of the micrographs and the dense labeling.

uncertain, but may be related to an uneven distribution of latex spheres among the vesicles when ghosts were homogenized, osmotic effect on vesicles of different sizes, loss of protein or loss of some latex spheres from the membrane during the homogenization procedure. The last explanation is supported by the presence of a faint latex-conjugate band in the gradient containing labeled small vesicles but not in any other gradients. Nevertheless, as shown in Table II, the density shift is still quite significant and the homogenization procedure potentially can be improved to yield better results.

DISCUSSION

Methacrylate latex microspheres chemically bonded to antibody molecules have been used to label cell surface antigens by the indirect immunochemical method. In addition to serving as visual markers for scanning electron microscopy, these reagents increase the density of plasma membranes. The studies reported here using erythrocyte ghosts as a model system indicate that the extent to which the density of these membranes are shifted on a given gradient depends on the number, size and density of the latex spheres as predicted by theory.

These results suggest that plasma membranes from more complex cell types can be isolated using these latex spheres as density perturbation reagents. With a judicious choice of size and density of the spheres, as well as gradient material, labeled plasma membranes can be separated from other cellular membranes and hence, in principle, can be obtained in a highly purified state for biochemical charac-

terization. In cases where the number of cell surface binding sites for a particular latex-ligand conjugate is low, a substantial increase in membrane density can be achieved with relatively large (approx. 100 nm diameter) methacrylate spheres, as have been used in the present study. However, when a large number of sites are displayed on the cell surface, it is desirable to have small spheres (10–30 nm diameter) having a higher density ($\rho > 1.23$ g · cm^{-3}) for combined studies involving high resolution mapping of sites by electron microscopy and for isolation of plasma membranes by affinity density perturbation. Such spheres have been synthesized (Yen, S. P. S., unpublished) and will be described elsewhere.

Latex spheres chemically bonded to ligands which interact specifically and reversibly with cell surface components (e.g. lectins and hormones) are particularly well suited for the purification of plasma membranes by affinity density perturbation. Currently, we are investigating this approach and also exploring the applicability of these microspheres in the purification of specific cell surface receptors.

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