A RAPID METHOD FOR DETERMINING THE TOPOLOGICAL DISTRIBUTION OF ANIONIC SITES ON MEMBRANE SURFACES*

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SUMMARY

A method is described for the topological localization of cell surface anionic sites using positively charged colloidal iron hydroxide (Gasic et al., Lab. Invest. 18, 63 [1968]). Purified glutaraldehyde-fixed plasma cell membranes or glutaraldehyde-fixed lysed cells are mounted on thin support films and directly stained with colloidal iron at pH 1.8, washed, dried, and then examined by transmission electron microscopy. Using this method the surface anionic residues on rabbit erythrocytes and murine MOPC-70A myeloma cells are presently in randomly spaced clusters. The distribution of anionic residues on rabbit spermatozoa is discontinuous: the sperm tails almost exclusively bind the colloidal iron in a dense random distribution, while the heads show little or no labeling.

The distributions of cell surface anionic residues are thought to play an important role in the interactions of cells with their environment and with other cells (1). The purpose of this communication is to introduce a reliable method for determining topological distributions of anionic surface charges without resorting to conventional plastic embedding techniques and statistical reconstruction of the data obtained from ultrathin sections (2-4). The method is based on the surface binding of positively charged colloidal iron hydroxide (CIH) developed by Gasic, Berwick and Sorrention (5).

METHODS

Cells

Rabbit erythrocytes were obtained from blood drawn by heart puncture into a 20 ml syringe containing 800 units of heparin. After washing several times

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in cold sodium phosphate buffered (pH 7.2) saline (PBS), the cells were divided and half were treated with Vibrio cholerae neuraminidase (10 units/10^6 cells; Calbiochem) for 30 min at 37°C. Both untreated and neuraminidase-treated erythrocytes were washed again in PBS and lysed according to the method of Dodge et al., (6). After lysis and washing in low ionic strength PBS (20 mosmoles) and then in PBS, the cell membrane ghosts were fixed in 1.5% buffered glutaraldehyde for 20 min at room temperature. The ghosts were finally washed twice in PBS and stored at 0°C.

Murine MOPC-70A myeloma cells were obtained from Dr. R. Hyman (original line from Dr. M. Potter) and were grown in BALB/c mice. Plasma membranes of MOPC-70A were isolated according to the method of Dods, Essner and Barclay (7). The washed plasma ghosts were fixed in 1.5% buffered glutaraldehyde and washed twice in PBS and stored at 0°C.

Rabbit spermatozoa (cauda epididymus) were washed several times in PBS and fixed in 1.5% buffered glutaraldehyde as described above.

Colloidal Iron Hydroxide Labeling

Colloidal iron hydroxide was made by a variation of the technique of Gasic et al. (5). 5 ml of 0.5 M $FeCl_3$ was added rapidly to 60 ml of boiling distilled water. After cooling, 10 ml of glacial acetic acid was added to the CIH sol and the pH was adjusted to 1.8.

Intact cells or plasma membrane ghosts were spread flat at an air-water interface and mounted on collodion-coated (carbon-strengthened) electron microscope grids as previously described (8, 9). The mounted membranes were treated for 3 min with a 5% solution of bovine serum albumin (in distilled water). The excess albumin was removed by touching the grids to a surface of distilled water. Without drying, a large drop of the freshly prepared CIH sol was added to each of the grids at room temperature, and the staining was terminated after 2-3 min. by quickly floating the CIH-treated grids on several large drops (5-6) of 12% acetic acid followed by distilled water (2). The total wash time never exceeded 1 min, including the two distilled water

rinses. After removal of excess water with a filter paper the grids were air dried prior to examination by transmission electron microscopy.

RESULTS

Mounted glutaraldehyde-fixed plasma membranes can be specifically stained with CIH at pH 1.8 to obtain the topological distribution of surface anionic residues. Rabbit erythrocyte membranes bind the CIH in discrete clusters that are more or less randomly spaced on the membrane surface (Fig. 1). Neuraminidase-treated rabbit erythrocyte membranes show very sparse CIH labeling and no clusters of CIH (Fig. 2). Murine MOPC-70A myeloma plasma membranes also bind CIH in discrete clusters (Fig. 3), but these tend to be more variable in size than the clusters found on rabbit erythrocyte membranes.

Cell membranes may have variable distributions of CIH sites; this variability is probably dependent on local concentrations of anionic residues. For example, rabbit spermatozoa are complex haploid cells with localized concentrations of antigens (10) and saccharides (11). Spermatozoon cells isolated from the cauda epididymas have high densities of bound CIH on their tail surfaces (Fig. 4), but little or no bound CIH on the head regions (now shown).

DISCUSSION

Weiss and his colleagues (1-4) have concluded from CIH labeling of glutaraldehyde-fixed cells and mathematical topographic reconstruction of the CIH distributions obtained from ultrathin sections that anionic residues are distributed in clusters on certain cell surfaces. Direct evidence for their hypothesis is provided here. From the distributions of the CIH clusters on erthrocyte membranes it appears that the anionic clusters are randomly dispersed on the membrane structure. Anionic clusters are also present on glutaraldehyde-fixed MOPC-70A myeloma plasma membranes, but these appear to be less uniform in size. The possibility that glutaraldehyde fixation causes the clustered distribution will be discussed in another publication; however, the surface topological distributions of anionic clusters on human erythrocyte

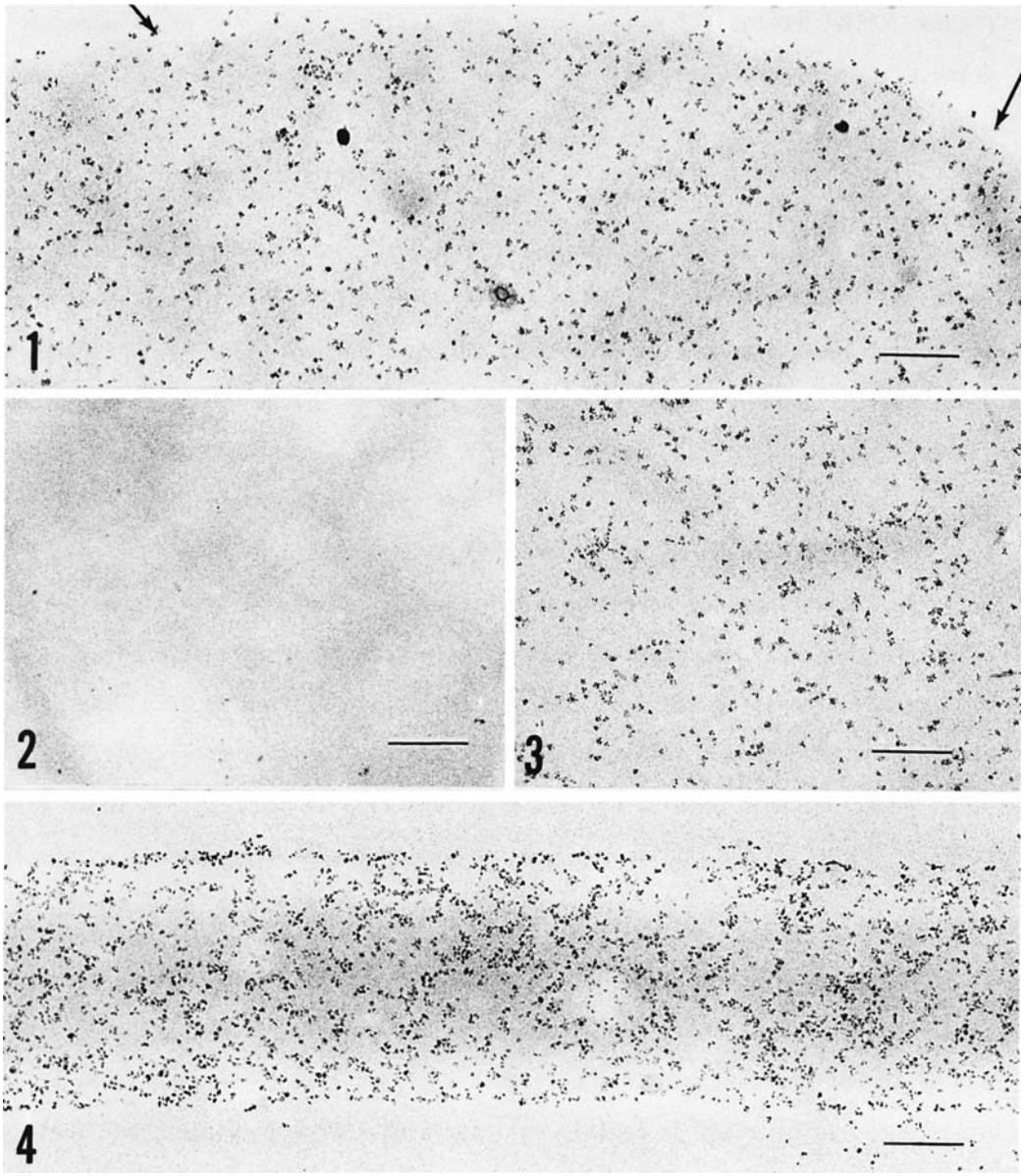

Fig. 1. Topological distribution of colloidal iron hydroxide on a glutaraldehyde-fixed rabbit erythrocyte membrane. The cell membrane edge is indicated by arrows. Marker bars equal 0.1 μm for Figs. 1-4.

Fig. 2. The legend is the same as in Fig. 1 except that rabbit erythrocytes were treated with V. cholerae neuraminidase before lysis and fixation.

Fig. 3. Distribution of colloidal iron hydroxide on a murine MOPC-70A myeloma plasma membrane.

Fig. 4. Colloidal iron hydroxide bound to the tail of a rabbit (cauda epididymal) spermatozoon.

membranes (prior to fixation) have been shown to be sensitive to proteinases, phospholipases and pH treatment (G. L. Nicolson, in preparation) in a similar manner to the distributions of ferritin-conjugated plant agglutinin sites on unfixed membranes (12).

Human erythrocytes have a surface glycoprotein which contains a large fraction of the total membrane sialic acid (13). The sailic anions are postulated to be present on the glycoprotein as terminal residues on several oligosaccharide chains connected to a peptide region that extends out from the membrane surface (14). Similar membrane components containing sialic acid or other acidic residues connected to glycopeptide chains could easily explain the binding of CIH in discrete clusters on the surfaces of rabbit erythrocyte or MOPC-70A myeloma cell membranes.

This CIH staining technique for surface anionic groups has been tested on several other membrane systems (bacterial, microsomal, mitochondrial, and viral membranes) and has proven to be a simple and reliable method.

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