Anisotropy of anionic sites on plasma membranes has been implicated in contact phenomena at cell surfaces (16, 17). Recent evidence indicates that charge anisotropy may be important among intracellular membranes also, since distribution of anionic sites has been related to structural organization of mitochondrial membranes (8).

The use of cationic ferritin has been introduced by Danon et al. (2) as a marker to demonstrate anionic sites on membrane surfaces by electron microscopy. This visual probe has been applied to study anionic sites on cell surfaces (2, 7) and on mitochondrial membranes (8). In the present study, cationic ferritin has been used to demonstrate anionic sites on the surface of the Golgi complex in relation to the structural organization of this membrane system.

MATERIALS AND METHODS

Preparation of Golgi Membranes

Golgi membranes were isolated from the rat liver according to the method of Sturgess et al. (13). The Golgi membranes were removed from the sucrose gradient and either suspended in 0.1 M sodium phosphate containing 0.25 M sucrose (pH 7.4) to study intact Golgi complex or washed and resuspended in 1 mM sodium bicarbonate (pH 7.4) to study unstacked Golgi complex. Membranes were either unfixed or fixed for 30 min in 1% glutaraldehyde in 0.1 M sodium phosphate buffer or in 1 mM sodium bicarbonate and then washed in the same buffer; these were incubated with different concentrations of cationic ferritin (Miles-Labs. Inc., Kankakee, Ill.) in the range from 40 µg to 1.2 mg/mg Golgi protein. For electron microscopy, samples of the membrane pellet were fixed in glutaraldehyde and then processed and sectioned as described previously (13). Unstained sections were examined at 60 kV with a Philips EM 200 electron microscope.

For galactosyltransferase activity, samples of the membrane pellets were sonicated and assayed as described in detail previously (14), using N-acetyl-D-glucosamine as exogenous acceptor. The components of the reaction were separated by high voltage electrophoresis.

Enzyme activity was expressed as disintegrations per minute [14C]galactose transferred per milligrams Golgi protein in 30 min at 37°C. Protein content was determined according to the method of Lowry et al. (9).

RESULTS

Distribution of Anionic Binding Sites

The distribution of anionic sites was investigated on the surfaces of the Golgi membranes isolated from rat liver, using cationic ferritin at concentrations from 40 µg to 1.2 mg/mg Golgi protein. Selective distribution of anionic sites on surfaces of different structural components of the Golgi complex was studied, first on the intact Golgi complex and second on the unstacked Golgi complex.

INTACT GOLGI COMPLEX: In isolated Golgi-rich fractions, resuspended in sodium phosphate buffer containing 0.25 M sucrose, the Golgi complex remained intact, with stacked cisternae continuous with networks of fine tubules and with secretory vesicles (13). Cationic ferritin was bound to the Golgi complex rapidly, within 15 s incubation, and showed a characteristic distribution of binding sites.

With unfixed membranes and low concentrations of cationic ferritin (40–100 µg/mg Golgi protein), binding occurred only to the surface of the tubular network and to small vesicles. No binding was observed either on the cisternae or secretory vesicles. At 200 µg/mg Golgi protein, more cationic ferritin was bound to the tubular network. Binding to the more peripheral tubules was greater than to those close to the cisternae. In addition, some binding occurred at the periphery of the cisternae, where the tubular network arises, but no ferritin was bound to the central region of the cisternae or to the secretory vesicles. At 400–600 µg/mg Golgi protein, cationic ferritin was bound on the convex faces of the outermost cisternae, and at the interface between adjacent cisternae in the stacked Golgi complex (Fig. 2a). There was little further binding to small vesicles or to the tubular network of the Golgi complex, which indicated that anionic sites were saturated at 400 µg.
FIGURES 1 and 2  Electron micrographs of intact Golgi complex isolated from rat liver and reacted with cationic ferritin.
cationic ferritin. At increasing concentrations of cationic ferritin (>400 μg), agglutination of membranes occurred, the cisternae and vesicles tended to swell and rupture, and, at 1 mg cationic ferritin per milligrams Golgi protein, there was little normal structural integrity.

In glutaraldehyde-fixed membranes, the density of cationic ferritin on membrane surfaces appeared less than on fixed membranes. This distribution of binding sites was similar in both fixed and unfixed membranes (Fig. 2b).

**Unstacked Golgi Complex:** The Golgi-rich fractions, washed and resuspended in sodium bicarbonate buffer, contained unstacked Golgi complex with cisternae dispersed and separated. Some of the continuity between the tubular network and cisternae was lost, but the structural components could be distinguished (Figs. 3–8).

With unfixed membranes, cationic ferritin (80–100 μg/mg Golgi protein) was bound at the surfaces of the tubular networks and small vesicles but not to the cisternae or the secretory vesicles (Fig. 3). With 200–400 μg/mg Golgi protein, cationic ferritin was bound increasingly to the tubular network but reached saturation within this range. Some binding occurred towards the periphery of the cisternae (Fig. 4), and occasionally ferritin particles were observed at the convex surface with a patchy distribution (Fig. 5). At 700 μg/mg Golgi protein, cationic ferritin was bound to the convex surface of the cisternae (Fig. 6) and occasionally to the peripheral region of the concave surfaces (Fig. 7). At higher concentrations, 1.2 mg/mg Golgi protein, cationic ferritin was bound to most membrane surfaces, including both convex and concave surfaces of the cisternae (Fig. 8). However, the membranes were ruptured extensively and agglutinated after incubation with the high concentrations of cationic ferritin.

Cationic ferritin was bound to the surfaces of the Golgi membranes as a discrete layer, each particle separated by at least 100 Å from adjacent particles (Fig. 9). There was no tendency for the ferritin particles to aggregate. The number of particles, quantitated on tangential section planes of tubular networks, was approximately 3,000–3,500/μm² membrane at 400 μg cationic ferritin per milligrams Golgi protein. The density did not increase significantly with higher concentrations (>700 μg) of cationic ferritin.

With glutaraldehyde-fixed membranes, distribution and density of binding sites were similar to those described for unfixed membranes (Fig. 10).

**Galactosyltransferase Activity**

Galactosyltransferase activity of the isolated Golgi complex after incubation with cationic ferritin is summarized in Table 1. The activity of galactosyltransferase in control preparations was 4.7 x 10⁵ dpm/mg protein. After incubation with 10 μg cationic ferritin per milligrams Golgi protein, the galactosyltransferase activity increased to 7.0 x 10⁵ dpm/mg protein, and then increased further to a maximum specific activity of 9.5 x 10⁶ dpm/mg protein with 93 μg cationic ferritin per milligrams Golgi protein. At higher concentrations of cationic ferritin (140 μg) the specific activity of galactosyltransferase decreased slightly, from 9.5 to 8.6 x 10⁶ dpm/mg protein.

**DISCUSSION**

The density of anionic sites varies considerably on different membrane surfaces of the Golgi complex, with the greatest density localized to the tubular network. Cisternae show less affinity for cationic ferritin and there is a marked difference in distribution of binding sites on the convex and concave surfaces. This anisotropic distribution of particles may represent differentiation of mem-

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**FIGURE 1** Unfixed membranes with 200-μg cationic ferritin/mg Golgi protein. Ferritin particles are localized on the small vesicles and tubular network (T) of the Golgi complex, but not on the cisternae (C) or secretory vesicles (SV). × 68,000.

**FIGURE 2** (a) Unfixed membranes with 600-μg cationic ferritin/mg Golgi protein. Ferritin particles are localized mainly on the small vesicles and tubular network (T). Labeling of the cisternae (arrows) appears in the intercisternal space and with a patchy distribution on the convex surfaces and more on the peripheral areas of the concave face. Patchy distribution is seen also on the secretory vesicles (SV). × 109,000. (b) Glutaraldehyde-fixed membranes with 800-μg cationic ferritin/mg Golgi protein. Distribution of ferritin particles on cisternae (C) and tubular network (T) is similar to that in unfixed membranes. × 90,000.
Figures 3–8 Electron micrographs of Golgi complex isolated from rat liver, unstacked and reacted with cationic ferritin, without prior fixation.
branes in relation to the functional organization of the Golgi complex.

Polarity of the Golgi complex has been described, in that successive cisternae differ in structure, composition, and function (references 3 and 11). During subcellular fractionation, the Golgi complex remains as an intact organelle with stacked cisternae similar to the arrangement observed in situ (1, 11, 13). With other procedures, this structural integrity is not retained (4-6). In plant cells, the arrangement of cisternae may be due to a layer of parallel fibers in the intercisternal region of the Golgi complex (10, 15), but no structural differentiation has been described in the intercisternal space of Golgi complex in animal cells. The higher density of anionic sites on the surface of one cisterna with a few sites on the adjacent cisternal surface may provide the basis of contact between adjacent cisternae similar to contact phenomena reported for plasma membranes (16, 17) and mitochondrial membranes (8).

The significance of the high density of anionic sites on the surface of one cisterna with a few sites on the adjacent cisternal surface may reflect an increased proportion of sialic acid residues associated with the membrane in glycoprotein biosynthesis. This implies, however, that carbohydrate residues of glycoproteins are exposed at the cytoplasmic surface as well as at the intracisternal surface of the Golgi complex as described previously (12). This finding is supported by recent evidence that the tubular network of the Golgi complex binds lectins such as concanavalin A.

However, some binding, particularly at higher concentrations of cationic ferritin, may result from membrane damage with subsequent leakage and adsorption of glycosylated macromolecules to membrane surfaces. After glutaraldehyde fixation, lesser binding of cationic ferritin presumably reflects stabilization of the membranes with less membrane damage. The adsorption of glycoproteins does not explain the anisotropic distribution of binding sites which occurs rapidly, and under different incubation conditions. Similar binding of cationic ferritin is observed in both fixed and unfixed membranes, providing evidence that the distribution of anionic sites does not result from interaction of the multivalent agent, cationic ferritin with the membranes.

With binding of cationic ferritin to the tubular network, there is an increase in total galactosyltransferase activity of the Golgi complex. The maximum stimulation occurs between 45-90 µg cationic ferritin per milligrams Golgi protein, when binding only to the tubular network is observed. The mechanism by which transferase activity is stimulated is the subject of further investigation. The stimulation may be due to a direct effect on the enzyme or it may be mediated through membrane changes.

SUMMARY

The distribution of anionic binding sites has been investigated in the isolated Golgi complex using cationic ferritin. The greatest density of anionic sites occurs on the tubular network and small vesicles, and this binding is accompanied by increased levels of galactosyltransferase activity. The density of anionic sites on the cisternae is less than on the tubules and shows anisotropic distri-

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**FIGURE 3** 100-µg cationic ferritin/mg Golgi protein. Ferritin is localized mainly to small vesicles associated with the tubular network (T, arrows). × 116,000.

**FIGURE 4** 400-µg cationic ferritin. Ferritin is localized to the small vesicles and tubules (T) with few particles in patches at the periphery of the cisternae (arrows). × 90,000.

**FIGURE 5** 400-600-µg cationic ferritin/mg Golgi protein. Ferritin is localized in patches on the convex surface of the Golgi cisternae (C), with none on the concave surface. × 91,000.

**FIGURE 6** 600-µg cationic ferritin/mg Golgi protein. Ferritin is distributed along the convex surface of the Golgi cisternae (C) with none on the concave surface. × 87,000.

**FIGURE 7** 700-µg cationic ferritin. Ferritin binds to convex surface of the cisternae (C) and at more peripheral regions of the concave surface (arrows). × 116,000.

**FIGURE 8** 1-mg cationic ferritin. Ferritin binds to all surfaces of the membrane including both cisternal surfaces (C), small vesicles, and tubular network (T). × 107,000.
FIGURE 9 Electron micrographs of Golgi complex isolated from rat liver, unstacked and reacted with 1-mg cationic ferritin/mg Golgi protein to show arrangement of ferritin particles. The ferritin particles are closely apposed to the membrane surface (single arrows) and are separate (double arrows). In tangential section planes (double arrows) up to 3,500 ferritin particles are observed per square micrometer membrane, × 156,400.

FIGURE 10 Electron micrographs of Golgi complex from rat liver, unstacked and reacted with 200-μg cationic ferritin after glutaraldehyde fixation. Ferritin binds to the small vesicles and tubular network (T) and shows anisotropic distribution (arrows) to cisternae (C) similar to that observed in unfixed membranes. × 90,000.

The data is based on results of three experiments, each carried out separately on different Golgi fractions. The membranes, washed in 1 mM sodium bicarbonate, were incubated with different concentrations of cationic ferritin for 30 min, sonicated, and assayed. The assay contained Golgi membranes (0.03 mg protein), 0.05 μmol UDP-[14C]galactose (sp act 1.0 μCi/μmol), 0.25 mg N-acetyl-d-glucosamine, 5 μmol of 2-(N-morpholino)ethane sulfonate buffer pH 5.7, 5 μl of 5% Triton X-100 and 3 μmol of MnCl₂ in a total volume of 50 μl. After incubation for 30 min at 37°C the reaction was stopped with 5 μl of 2% sodium tetraborate containing 0.25 M EDTA.

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