THERMOTROPIC LATERAL TRANSLATIONAL MOTION OF INTRAMEMBRANE PARTICLES IN THE INNER MITOCHONDRIAL MEMBRANE AND ITS INHIBITION BY ARTIFICIAL PERIPHERAL PROTEINS

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ABSTRACT

Freeze fracturing and deep etching have been used to study thermotropic lateral translational motion of intramembrane particles and membrane surface anionic groups in the inner mitochondrial membrane. When the inner membrane is equilibrated at low temperature, the fracture faces of both halves of the membrane reveal a lateral separation between intramembrane particles and particle-free, large smooth patches. Such separation is completely reversed through free lateral translational diffusion by reversing the temperature. The low temperature-induced, particle-free, smooth membrane patches appear to represent regions of protein-excluding, ordered bilayer lipid which form during thermotropic liquid crystalline to gel state phase transitions. When polycationic ferritin is electrostatically bound to anionic groups exposed at the membrane surface at concentrations which inhibit the activities of cytochrome *c* oxidase and succinate permease, the bound ferritin migrates with intramembrane particles during the thermotropic lateral separation between the membrane particles and smooth patches. When bound polycationic ferritin is cross-bridged with native ferritin, an artificial peripheral protein lattice forms in association with the surface anionic groups and diminishes the thermotropic lateral translational motion of intramembrane particles in the membrane. These results reveal that the anionic groups of metabolically active integral proteins which are known to be exposed at the surface of the inner mitochondrial membrane migrate with intramembrane particles in the plane of the membrane under conditions which induce lipid-protein lateral separations. In addition, cross-bridging of the anionic groups through an artificial peripheral protein lattice appears to diminish such induced lipid-protein lateral separations.

Freeze-fracture electron microscopy has revealed that the energy-transducing, inner mitochondrial membrane contains a high density of intramembrane particles when compared to other cell membranes (11-13). This observation is thought to reflect the low lipid to protein ratio in this functionally and structurally complex membrane and is consistent with the general belief that intramembrane particles represent integral proteins which are intercalated in the hydrophobic bilayer lipid continuum of the membrane.

Studies of model membrane systems have

shown that the integral proteins rhodopsin, glycophorin, and microsomal ATPase appear as intramembrane particles when reconstituted in lipid vesicles and, further, undergo low temperature-induced lateral translational motion in the plane of the lipid bilayer (3, 6, 20). In relation to these findings, we have recently presented evidence for thermotropic lateral translational motion of intramembrane particles in both membranes of the isolated, intact mitochondrion (19). The lateral motion of intramembrane particles was expressed as separations between particle-rich membrane regions (presumbly rich in integral proteins) and particle-free membrane regions (presumably rich in bilayer lipid) observed in both fracture faces of the membranes. Such lateral separations were determined to be completely reversible and not destructive to oxidative phosphorylation (19). These temperature-induced lateral separations are believed to reflect, as in the more simplified reconstituted systems, a liquid crystalline to gel state (disorder to order) phase transition in the bilayer lipids of the membranes which results in a two-dimensional lipid-lipid, and, therefore, lipid-protein lateral separation in the plane of the membrane.

We present here a study designed to test further the potential for lateral translational motion by the intramembrane particles in the inner mitochondrial membrane by using methods designed either to induce or inhibit the process of lateral translational motion of membrane integral proteins. We have utilized several of our recent findings in this regard, namely that some metabolically active integral proteins of the inner membrane are completely transmembranous, are known to contain anionic groups which are exposed at the membrane surface, and are functionally inhibited when such groups are bound by polycationic ferritin (14–16). Thus, polycationic ferritin was bound to such anionic groups on the surface of the inner membrane to determine whether these groups migrate with intramembrane particles under conditions which induce thermotropic lipid-protein lateral separations. Conversely, surface anionic groups were cross-bridged through a lattice of bound polycationic and native ferritin to determine whether such an artificial peripheral protein lattice could inhibit lateral translational motion of intramembrane particles under conditions which induce thermotropic lipid-protein lateral separations.

MATERIALS AND METHODS

Liver mitochondria were isolated from male Sprague-Dawley rats in an isolation medium containing 70 mM sucrose, 220 mM mannitol, 2 mM *N*-2-hydroxyethylpiperazine *N'*-2-ethane sulfonic acid (HEPES), 0.5 mg bovine serum albumin (BSA)/ml, and KOH to pH 7.4. This medium is designated H medium. Subsequent removal of the outer membrane and purification of the inner membrane-matrix (mitoplast) fraction was carried out by use of a controlled digitonin incubation (12, 24). The complex topography of the inner membrane-matrix preparation was converted to a simple spherical configuration by washing and resuspending in a 7.5 times diluted (40 mosM) BSA-free H medium as described earlier (12). This medium is designated H_{40} medium.

For ferritin binding at 25°C, 5 ml of H_{40} medium containing 300, 600, or 900 μg of polycationic ferritin was mixed thoroughly at 25°C with 5 ml of H_{40} medium containing inner membranes (10-mg protein) to give a final concentration of 30, 60, or 90 μg of polycationic ferritin/mg membrane protein/ml. After incubation for 5 min, 10 ml of H_{40} medium was added followed by 5 ml of H_{40} medium containing 9 mg of native (anionic) ferritin and incubated for 5 min more. For control samples, the membranes were incubated with only polycationic ferritin, native ferritin, or neither. The membranes were then pelleted at 10°C at 10,000 *g* for 10 min and resuspended in either 300 times diluted BSA-free H medium for deep etching or H_{40} medium containing 30% glycerol for freeze fracturing. Membranes in the 30% glycerol medium were divided into several aliquots, centrifuged, and the pellets subsequently equilibrated at 25°C or −10°C for at least 10 min. The membranes were then transferred to gold-nickel specimen holders which were equilibrated at corresponding temperatures for 5 min and rapidly frozen in Freon 22 (Virginia Chemicals, Inc., Portsmouth, Mass.) precooled by liquid nitrogen.

For ferritin binding at −10°C, the procedure was the same as described above with the exception that all steps were carried out at −10°C. To prevent freezing, the H medium contained 25% glycerol. For deep etching, membranes at −10°C in 25% glycerol were fixed at −10°C overnight with 1.5% glutaraldehyde in 25% glycerol. After fixation, the glycerol was washed out at 0°C with 300 times diluted BSA-free H medium. Samples for deep etching were collected and equilibrated as described above for freeze fracturing. Additional details of experimental procedure are included in the figure legends and in the text.

Native and polycationic ferritin were purchased from Miles Laboratories Inc., Miles Research Products, Elkhart, Ind. Polycationic ferritin was the *N,N*-dimethyl-1,3 propanediamine conjugate of ferritin prepared at pH 6.5 and contained approx. 65 cationic charges at physiological pH (5).

Fracturing of specimens, vacuum sublimation, and platinum-carbon replication was carried out at −100°C

at 2 × 10^{-6} torr in a Balzers BA360 freeze-etching apparatus equipped with electron guns (Balzers High Vacuum Corp., Santa Ana, Calif.). Electron micrographs were taken with a Philips 300 electron microscope operated at 80 kV.

RESULTS

Inner Membrane Preparation for Freeze Fracture

The inner mitochondrial membrane has an exceptionally complex surface topography which unfortunately does not offer a sufficiently large surface area for observation by freeze-fracture techniques (1, 12). Figures 1 and 2 show the osmotic unfolding of the inner membrane designed to produce a membrane configuration which offers a large, uncomplicated topography for observation after deep etching or freeze fracturing. Starting with freshly isolated liver mitochondria in the condensed configuration (9, 10), a purified inner membrane-matrix fraction (Fig. 1) is prepared after removal of the outer membrane by controlled digitonin treatment (12). Figure 2 demonstrates the spherical configuration of the purified inner membrane-matrix preparation in the 40 mosM preparatory medium (12). The spherical inner membrane preparation is active in electron transport and oxidative phosphorylation (22) and was used throughout the study reported here. Sphere formation is totally reversible.

Low Temperature-Induced Lateral Translational Motion of Intramembrane Particles

When the inner membrane was rapidly frozen from 25°C, intramembrane particles were invariably observed to be randomly dispersed in a smooth continuum of the membrane in both the convex (Fig. 3) and concave (Fig. 4) fracture faces. Since the membrane conforms to a near perfect sphere, we will, consistent with our previous studies (19) and to maintain simplicity, apply the terms convex fracture face (inner half membrane) and concave fracture face (outer half membrane).

When the inner membrane was cooled slowly to −10°C before rapid freezing, the fracture faces of both halves of the membrane revealed that lateral separations occurred between the intramembrane particles and the smooth continuum of the membrane (Fig. 5). The rather large, particle-free smooth patches which form in the membrane are thought to represent regions rich in ordered lipids which develop during thermotropic liquid crystalline to gel state phase transitions (3, 6, 19, 20, 28). Consistent with our previous observations on intact mitochondria (19), these smooth patches and related lateral translational motion of intramembrane particles first appeared in the inner membrane when the slowly decreasing temperature reached approx. −4°C.

Cross-Bridging of Membrane Surface Anionic Groups through an Artificial Peripheral Protein Lattice

Polycationic ferritin at a high concentration (90 μg/mg protein) was permitted to bind electrostatically to the surface of the inner membrane at 25°C. Although electron microscope examination of thin sections revealed, as in our previous studies (14, 15), almost complete coverage of the membrane surface by the ligand at this concentration, the ligand could not be adequately resolved on the surface of the membrane by deep etching (Fig. 6). However, with further addition of native (anionic) ferritin, the distribution of the membrane-bound polycationic ferritin could be clearly determined (Fig. 7). Since native ferritin alone does not bind to mitochondrial membranes (15) and since we found that native ferritin precipitated polycationic ferritin from solution, it could be reasonably expected that native ferritin would bind to and cross-bridge the polycationic ferritin which was initially bound to the surface of the inner membrane. Such electrostatic cross-bridging would include various metabolic integral proteins which contain anionic groups exposed at the membrane surface and which are known to be bound and inhibited by polycationic ferritin (14). In this manner, it was anticipated that integral proteins could be immobilized through a relatively continuous artificial peripheral protein lattice over the surface of the membrane. The effectiveness of such cross-bridging would, of course, depend on the degree of polycationic ferritin bound initially.

Since the membrane-bound polycationic ferritin alone could not be resolved by deep etching, it was assumed that the multivalent ligand sank or impressed into the membrane surface. Fracture faces of the inner membrane revealed this to be the case. When the membrane was rapidly frozen from 25°C, fracture faces clearly showed a random distribution of impressions made in the membrane by polycationic ferritin bound to the membrane surface (Fig. 8; cf. Fig. 4). This interesting finding

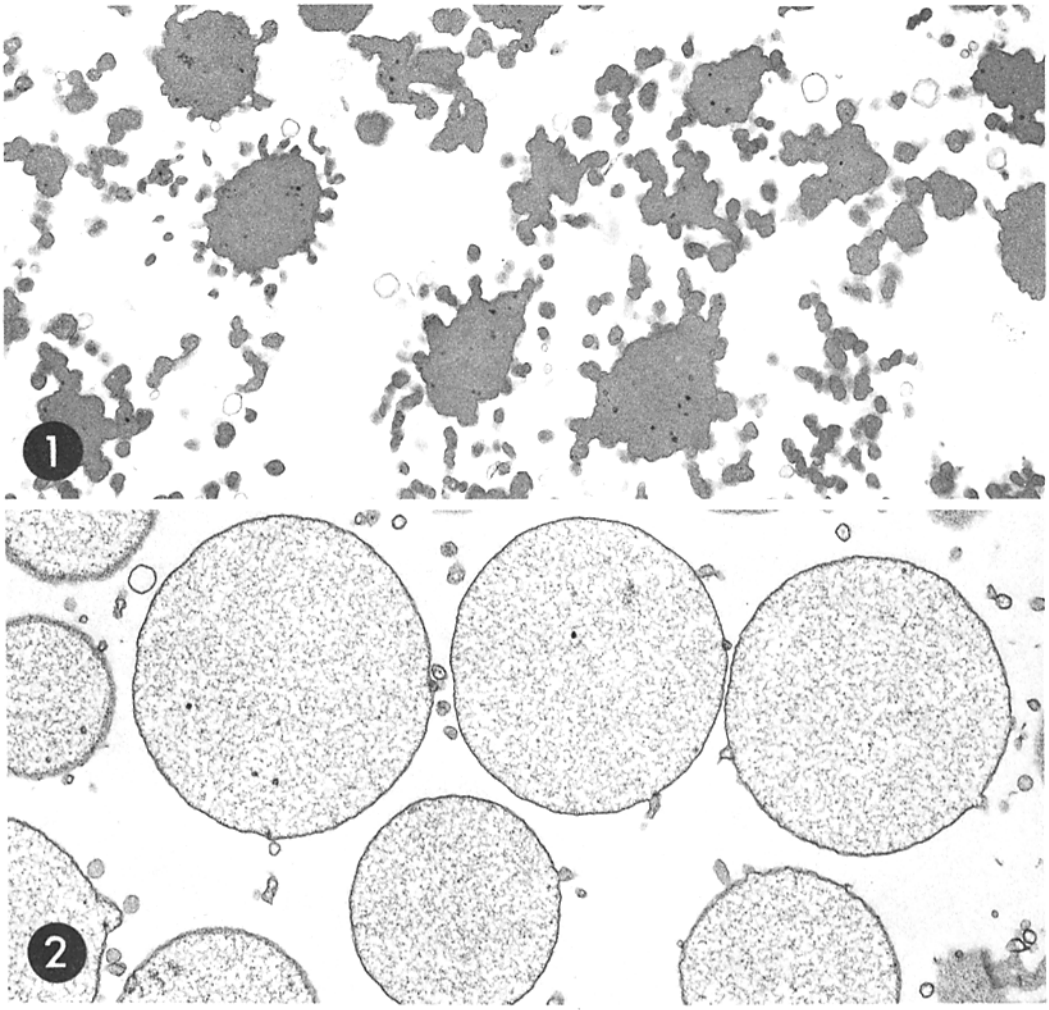


FIGURE 1 Condensed configuration of the outer membrane free, purified inner membrane-matrix preparation. × 20,000.

FIGURE 2 Spherical configuration of the purified inner membrane-matrix preparation. × 20,000.

permitted simultaneous observation of the distribution of the large polycationic ferritin molecules on the membrane surface and the distribution of the smaller intramembrane particles in the concave fracture face of the membrane (Fig. 8).

When the initial binding of polycationic ferritin was carried out at lower concentrations (30 and 60 μg/mg protein) and followed by native ferritin, impressions of the surface-bound ferritin occurred much farther apart since less ferritin binds at these concentrations (14, 15), and the ligand appeared to impress deeper into the membrane surface (Fig. 9). Thus, with the subsequent addition of native ferritin, a more complete cross-bridging of membrane surface anionic groups via a peripheral protein lattice could be expected when an initially large amount of polycationic ferritin was bound (Fig. 8) compared to an initially small amount of polycationic ferritin (Fig. 9).

Comigration of Surface Anionic Groups and Intramembrane Particles, and Immobilization by an Artificial Peripheral Protein Lattice

When the inner mitochondrial membrane containing the larger quantity of bound, latticed ferri-

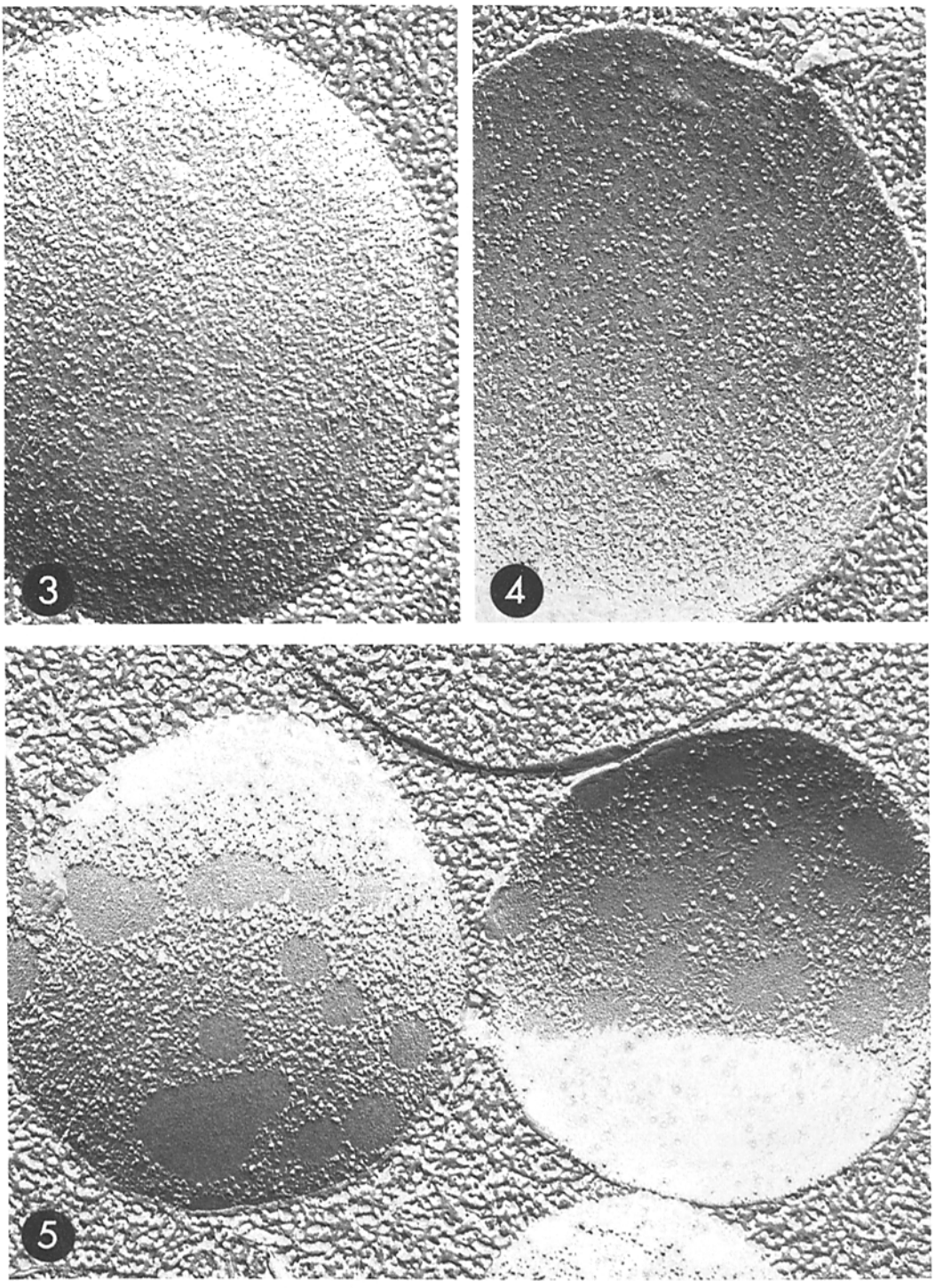


FIGURE 3 Convex fracture face of inner membrane rapidly frozen from 25°C. The fracture face shows a random distribution of intramembrane particles. × 67,000.

FIGURE 4 Concave fracture face of inner membrane rapidly frozen from 25°C. The fracture face shows a random distribution of intramembrane particles. × 67,000.

FIGURE 5 Convex and concave fracture faces of inner membranes equilibrated at −10°C and then rapidly frozen. Both halves of the membranes show fracture faces with a high degree of lateral separation between intramembrane particles and smooth patches. × 67,000.

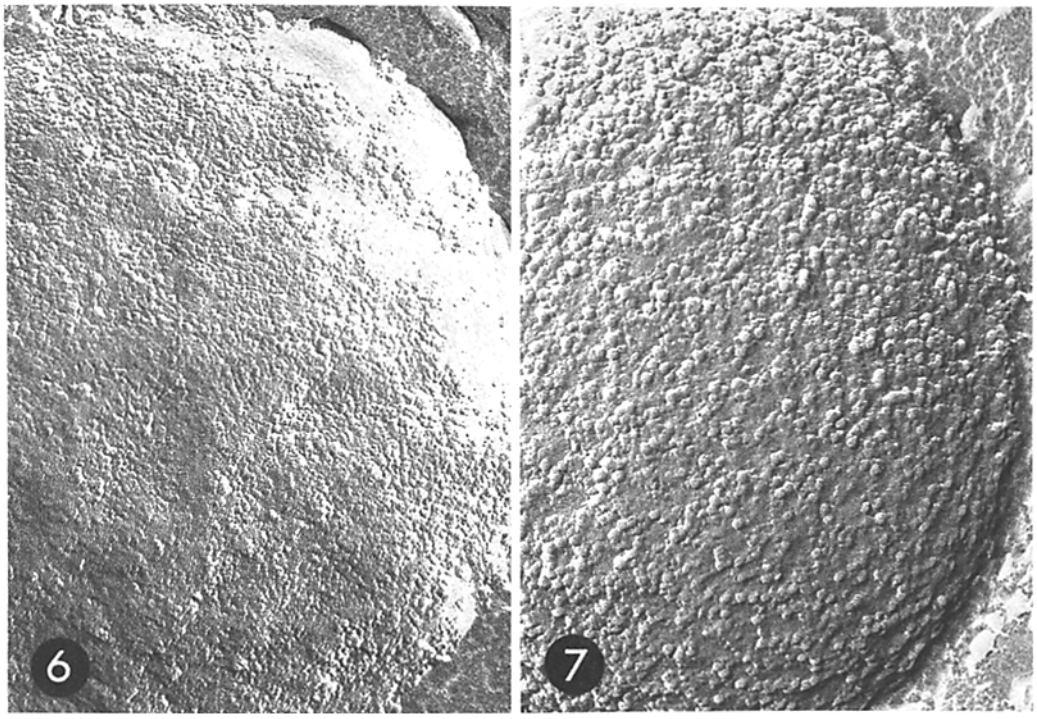


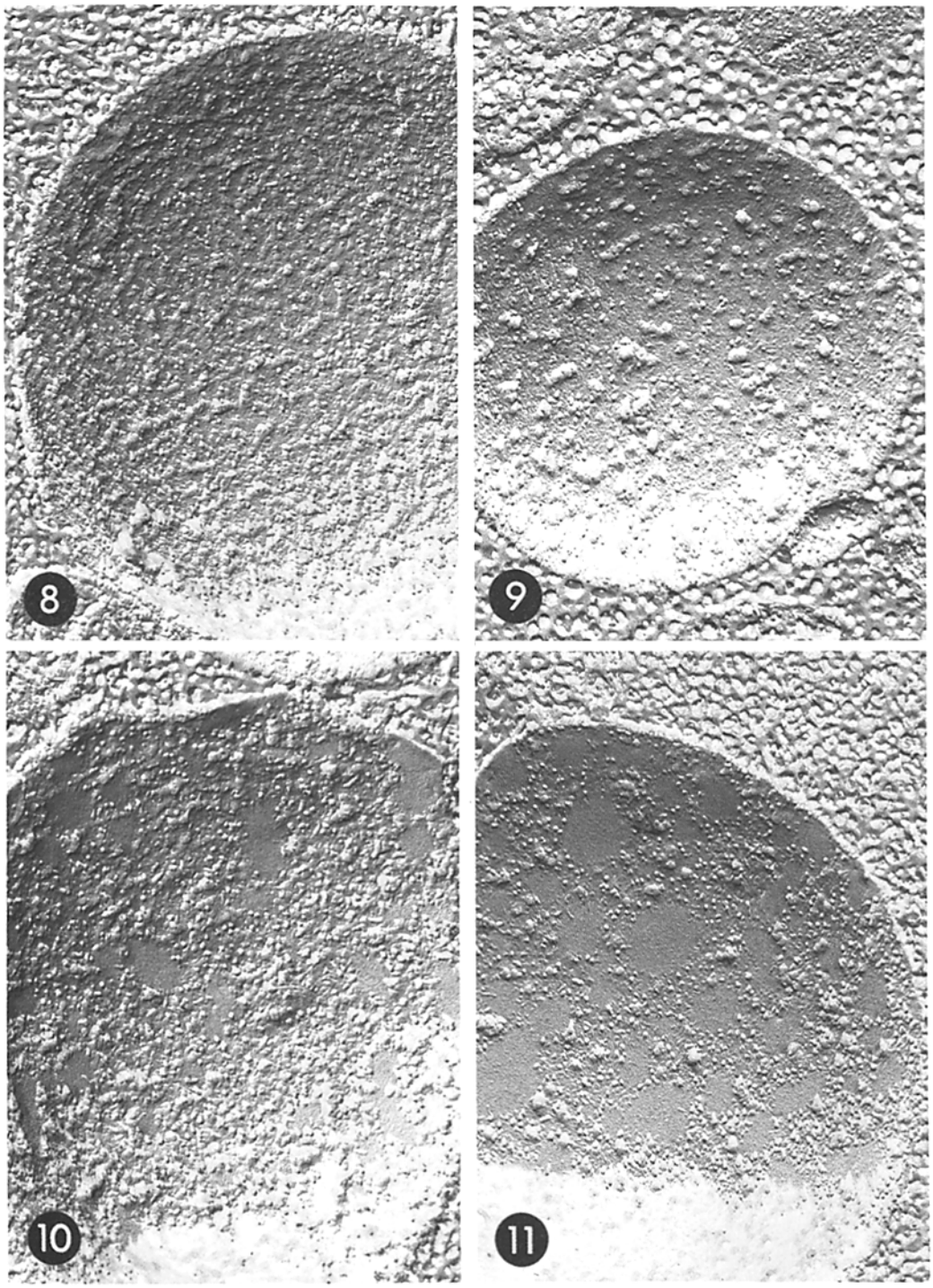
FIGURE 6 Deep-etched surface of inner membrane. Polycationic ferritin (90 μg/mg protein) was permitted to bind to the membranes at 25°C. The membranes were then rapidly frozen. The bound polycationic ferritin cannot be resolved adequately on the membrane surface. × 115,000.

FIGURE 7 Deep-etched surface of inner membrane. Polycationic ferritin (90 μg/mg protein) was permitted to bind to the membranes followed by native ferritin (900 μg/mg protein) at 25°C. The membranes were then rapidly frozen. The membrane surface shows a random distribution of the bound ferritin. × 115,000.

tin was slowly cooled to −10°C followed by rapid freezing, the low temperature-induced lateral separations between the intramembrane particles and smooth patches of the membrane were significantly reduced in both the concave (Fig. 10) and convex (Fig. 12) fracture faces in comparison to the separations which occurred in the membrane not bound by latticed ferritin (Fig. 5). The smooth patches which appeared were generally of limited size and irregular shape. Noted also was that the impressions of the bound ferritin were located only over the particle-rich regions of the membrane (Figs. 10 and 12).

Inner membrane containing the smaller quantity of bound ferritin, however, showed the usual high degree of low temperature-induced lateral separations between the intramembrane particles and smooth patches of the membrane (Figs. 11 and 13). In this case, with a limited degree of ferritin binding, cross-bridging through a ferritin lattice was most unlikely, and therefore no obvious inhibition of the lateral separations occurred. In addition, it was particularly clear in this experiment that ferritin impressions did not occur over the low temperature-induced smooth patches of the membrane and that the surface-bound ferritin migrated in the lateral plane of the membrane with the intramembrane particles.

It was determined that the low temperature-induced lateral separations between the intramembrane particles and smooth patches of the inner membrane were reversible. After inducing lateral separation and aggregation of intramembrane particles at −10°C (Fig. 5), the temperature was raised either to 0°C for 15 min or to 25°C for a few seconds before rapid freezing. No lateral separations between intramembrane particles and smooth membrane patches could be found on either the convex or concave fracture face of the membrane (Fig. 14). The low temperature-in-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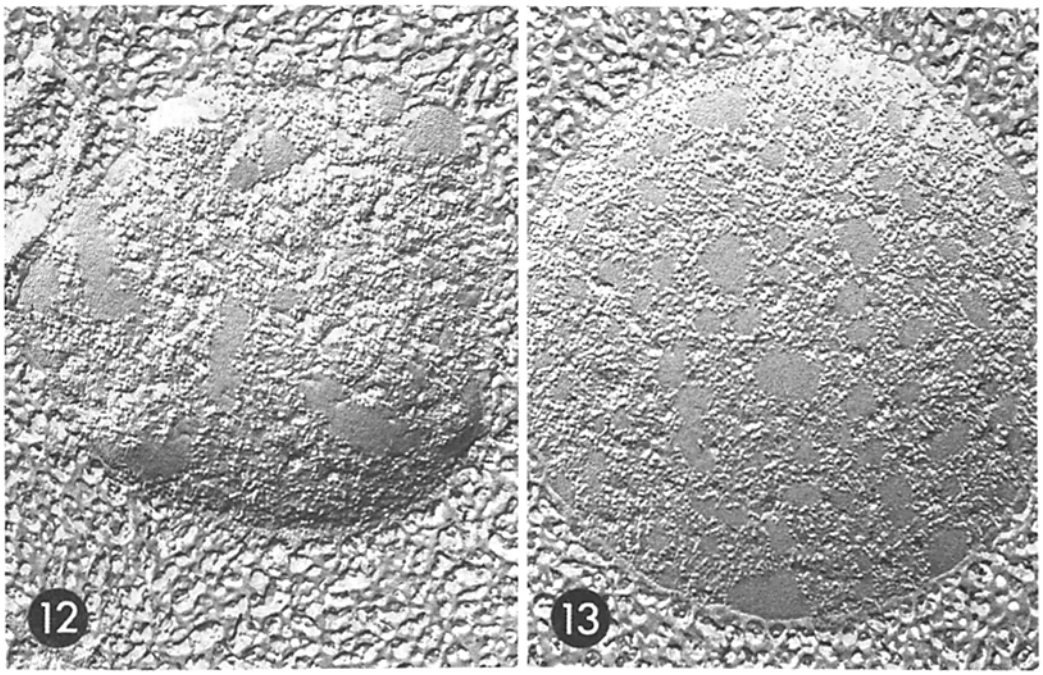


FIGURE 12 Convex fracture face of inner membrane. Same conditions and results as in the legend for Fig. 10. × 67,000.

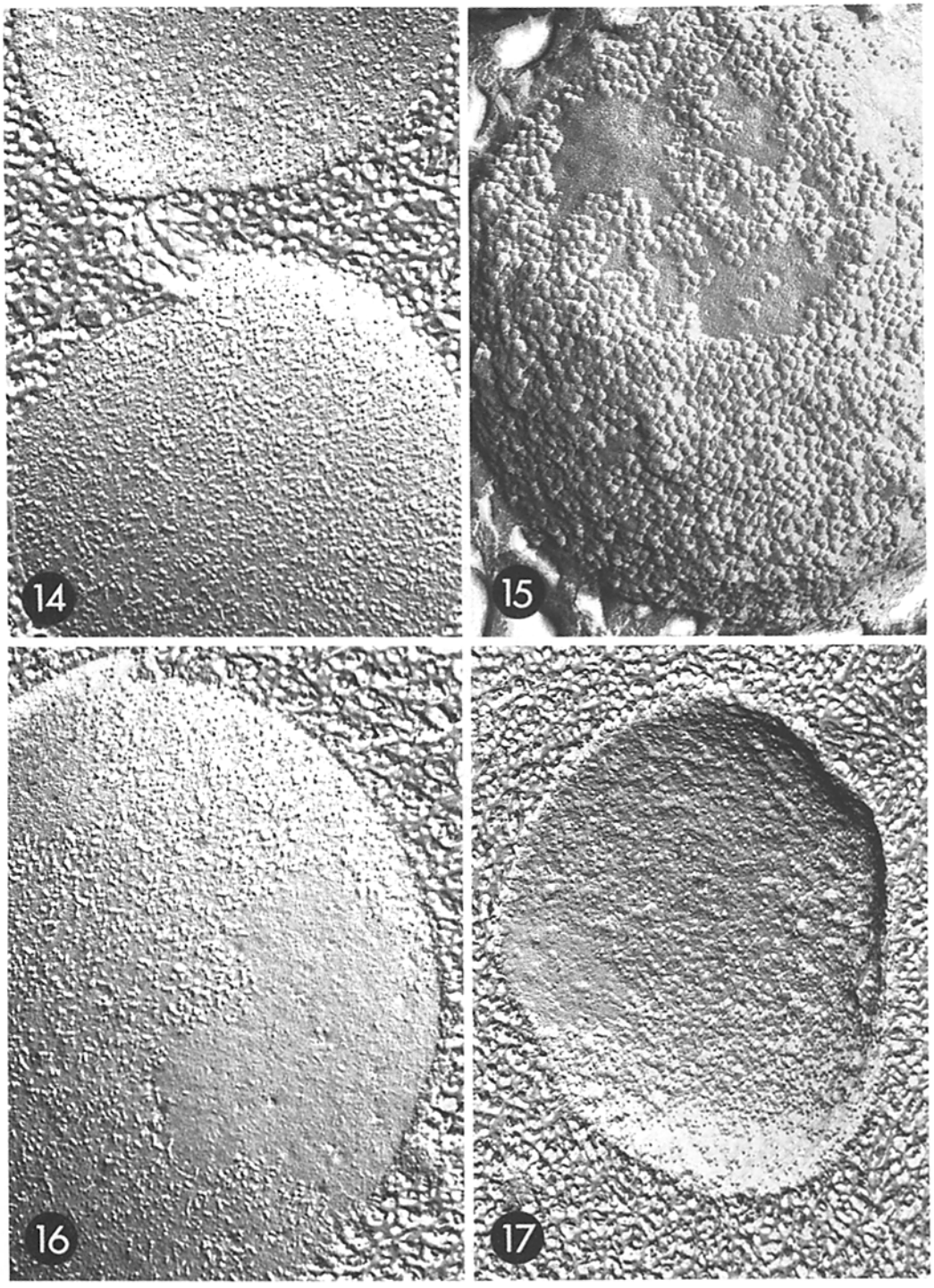
FIGURE 13 Convex fracture face of inner membrane. Same conditions and results as in the legend for Fig. 11. × 67,000.

FIGURE 8 Concave fracture face of inner membrane. Polycationic ferritin (90 μg/mg protein) was permitted to bind to the membranes followed by native ferritin (900 μg/mg protein) at 25°C. The membranes were then rapidly frozen. The fracture face shows a random distribution of intramembrane particles and a high density of shallow impressions of surface-bound ferritin. × 67,000.

FIGURE 9 Concave fracture face of inner membrane. Polycationic ferritin (30 μg/mg protein) was permitted to bind to the membranes followed by native ferritin (900 μg/mg protein) at 25°C. The membranes were then rapidly frozen. The fracture face shows a random distribution of intramembrane particles and a low density of deep impressions of surface-bound ferritin. × 67,000.

FIGURE 10 Concave fracture face of inner membrane. Polycationic ferritin (90 μg/mg protein) was permitted to bind to the membrane surface followed by native ferritin (900 μg/mg protein) at 25°C. The membranes were equilibrated at −10°C and then rapidly frozen. The fracture face shows a limited degree of lateral separation between intramembrane particles and smooth patches. Impressions of surface-bound ferritin are confined to the particle-rich areas. × 67,000.

FIGURE 11 Concave fracture face of inner membrane. Polycationic ferritin (30 μg/mg protein) was permitted to bind to the membrane surface followed by native ferritin (900 μg/mg protein) at 25°C. The membranes were equilibrated at −10°C and then rapidly frozen. The fracture face shows a high degree of lateral separation between intramembrane particles and smooth patches. Impressions of surface-bound ferritin are confined to the particle-rich areas. × 67,000.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duced aggregated intramembrane particles therefore disaggregated and reequilibrated completely in the plane of the membrane, presumably through free lateral translational diffusion, when the temperature was raised.

A major finding was that such free lateral diffusion of intramembrane particles could be greatly inhibited by cross-bridging membrane surface anionic groups through a peripheral ferritin lattice. In this experiment, lateral separations between intramembrane particles and smooth patches were induced at −10°C as before (Fig. 5). Membrane surface anionic groups were then cross-bridged through a lattice of polycationic and native ferritin without raising the temperature. Membranes deep etched at this point showed a very closely packed, latticed ferritin incompletely covering the membrane surface and often not binding to patched areas (Fig. 15). The distribution of binding was therefore suggestive of the distribution of aggregated intramembrane particles at −10°C. The temperature was then raised to either 0°C or 25°C before rapid freezing. Membrane fracture faces clearly showed that disaggregation and reequilibration of intramembrane particles through free lateral diffusion did not occur when the temperature was raised to 0°C or above (Figs. 16 and 17).

Thus, it was determined that cross-bridging of membrane surface anionic groups through an artificial peripheral protein lattice after the intramembrane particles were aggregated by low temperature resulted in a significant inhibition of disaggregation of intramembrane particles through free lateral translational diffusion when the temperature was subsequently raised. It is to be noted, however, that there was a very small population of intramembrane particles of less than average diameter which did diffuse back into the smooth patches of the membrane after raising the temperature (Figs. 16 and 17; cf. Fig. 5).

DISCUSSION

The study reported here reveals that relatively long-range lateral translational motion of intramembrane particles occurs in the inner mitochondrial membrane subjected to low temperature. Such thermotropic lateral motion leads to aggregation of intramembrane particles and is most likely the result of the growth of protein-excluding regions of liquid crystalline to gel state phase transitions in the bilayer lipid of the membrane which are observed as smooth, particle-free patches in the freeze-fracture faces of the membrane. Thus, as in a few other natural membrane systems, as well as in several reconstituted membrane systems, exposure to low temperature causes particle-rich regions (integral proteins) to separate laterally from particle-poor regions (gel state lipid) of the membrane (3, 6, 20, 21, 25, 27-29). Rapid disaggregation and complete randomization of intramembrane particles, which occur in the inner mitochondrial membrane after removal of the

FIGURE 14 Concave and convex fracture faces of inner membranes. The membranes were equilibrated at −10°C. The temperature was then raised to 25°C for 5 s followed by rapid freezing. Both halves of the membranes show fracture faces with a random distribution of intramembrane particles. No lateral separations can be detected. × 67,000.

FIGURE 15 Deep-etched surface of inner membranes. Membranes were first equilibrated at −10°C. Polycationic ferritin (90 μg/mg protein) was then permitted to bind to the membranes followed by native ferritin (900 μg/mg protein) at −10°C. The membranes were then fixed with 1.5% glutaraldehyde at −10°C, the glycerol was removed at 0°C, and the membranes were rapidly frozen from 0°C. The membrane surface shows tightly packed bound ferritin and large patches which remain unbound by ferritin. × 115,000.

FIGURE 16 Convex fracture face of inner membrane. Membranes were equilibrated at −10°C. Polycationic ferritin (90 μg/mg protein) was then allowed to bind to the membranes followed by native ferritin (900 μg/mg protein) at −10°C. The temperature was then raised to 0°C for 15 min followed by rapid freezing. The fracture face shows a high degree of lateral separation between intramembrane particles and smooth patches. × 67,000.

FIGURE 17 Concave fracture face of inner membrane. Same conditions and results as in the legend for Fig. 16. × 67,000.

low-temperature perturbation, are consistent with the melting properties of membrane lipids and with the idea that the integral proteins can diffuse in the fluid bilayer lipid of the inner membrane (19).

The first sign of a low temperature-induced lateral separation between intramembrane particles and smooth patches of the inner membrane appears when the slowly decreasing temperature reaches approx. −4°C and continues progressively until the temperature reaches a low of between −12°C and −15°C. Complete lateral randomization occurs if the temperature is then raised to 0°C or above. X-ray diffraction and differential scanning calorimetry reveal that the total lipid component of the complex, two-membrane mitochondrial system has a broad endothermic gel to liquid crystalline phase transition (2, 8, 18). Combining freeze fracture with differential scanning calorimetry, we have determined that the purified spherical inner membrane shows an onset temperature in the transition exotherm of −4°C and an onset temperature in the transition endotherm of −15°C (18). Thus, a clear correlation exists between the appearance of thermotropic lateral separations of intramembrane particles and smooth patches in the inner mitochondrial membrane observed by freeze fracture and the occurrence of thermotropic liquid crystalline-gel state phase transitions determined by differential scanning calorimetry.

It is of interest that the temperature range at which lateral translational motion of intramembrane particles occurs in the specially prepared spherical inner membrane matches the temperature range for particle motion in the inner membrane of the intact mitochondrion (19). Therefore, thermotropic lateral translational motion and reversible aggregation of intramembrane particles occur in the inner membrane over nearly identical temperature ranges, irrespective of the osmotic environment, ionic strength, membrane configuration, or membrane association with the proteins of the matrix of the intact mitochondrion. These findings suggest that no change in lipid transition characteristics or change in protein-protein associations or dissociations occurs during preparation of the spherical inner membrane which can account for the thermotropic lateral translational motion of the intramembrane particles. It should be added here that sphere formation of the inner membrane is completely reversible. Sphere formation at 40 mosM results in an unfolding of the inner membrane without osmotic lysis.

The large number of different metabolically active proteins which are located in the inner mitochondrial membrane has so far precluded the identification of intramembrane particles with specific inner membrane proteins. However, since most, if not all, integral proteins of the inner membrane can be expected to be amphipathic, they will contain a variety of anionic groups exposed at one or both membrane surfaces. Most commonly, these are the free carboxyl groups of glutamic and aspartic acids. We have utilized these anionic groups exposed at the surface of the inner membrane in an effort to further examine lateral translational motion of intramembrane particles as it may relate to lateral translational motion of integral proteins. Polycationic ferritin, a visually detectable multivalent ligand, avidly binds electrostatically to the anionic groups on the surface of the inner membrane (14, 15). The ligand binds specifically, but perhaps not exclusively, to integral proteins such as succinate permease and cytochrome *c* oxidase and inhibits the activity of these proteins (14). Recently, we determined that cytochrome *c* oxidase is completely transmembranous in its orientation in the inner membrane (16). As could be expected, cytochrome *c* oxidase can clearly be observed as an intramembrane particle in cytochrome *c* oxidase-enriched membranes (17) and in reconstituted membranes using the purified oxidase (26). Our present results show that when polycationic ferritin is bound to anionic groups on the surface of the inner membrane at concentrations which inhibit cytochrome *c* oxidase as well as succinate permease, the ligand migrates laterally with the intramembrane particles in the plane of the membrane when the temperature is lowered to induce the liquid crystalline to gel state lipid transition and thus lipid-protein separation.

These results strongly suggest that the intramembrane particles are the various metabolically active integral proteins of the inner mitochondrial membrane. We cannot rule out completely that polycationic ferritin does not bind, at least partially, to anionic phospholipids. We would point out, however, that the bound ligand does not migrate into the particle-free smooth patches of the membrane but rather partitions into the particle-rich regions of the membrane during the low temperature-perturbation. We would point out further that phosphatidyl choline and phosphatidyl ethanolamine account for approx. 80%, while cardiolipin accounts for approx. 20%, of the total phospholipids of the inner membrane (4, 23). Of

the three, cardiolipin, the only anionic phospholipid, does not occur in the outer half of the lipid bilayer of the inner membrane (7).

Of notable interest in the present study was the unexpected but useful finding that polycationic ferritin not only binds to anionic groups on the surface of the inner membrane but impresses deeply into the membrane surface so that its binding distribution can be easily observed by examining the freeze-fracture faces of the membrane. This approach to observing the distribution of the surface-bound ferritin was a distinct advantage over deep etching, since many of our experiments were carried out at −10°C and therefore required the use of glycerol. In a few −10°C experiments in which deep etching was used, it was required that the specimens first be fixed with glutaraldehyde at −10°C and then washed at 0°C to remove the glycerol before rapid freezing (Fig. 15 is a sample of this more difficult procedure). A second, more significant advantage of the polycationic ferritin impression phenomenon is that the distribution of the impressions can clearly be observed simultaneously with the distribution of intramembrane particles on a single concave or convex fracture face of the membrane. The impressing of polycationic ferritin into the surface of the inner mitochondrial membrane appears to depend on the specific physicochemical characteristics of the membrane, since we observed that such impressing does not occur in the outer mitochondrial membrane or in the erythrocyte ghost membrane even though a strong electrostatic binding occurs between the ligand and these membranes. The lack of cholesterol and high content of unsaturated phospholipids is consistent with the low viscosity and high degree of plasticity and fluidity in the bilayer lipid of the inner membrane and may account for the impressing of ferritin into the surface of this membrane (19).

In this study, we have employed polycationic ferritin as an artificial peripheral protein by permitting it to bind to anionic groups of metabolically active integral proteins which are exposed at the membrane surface. Indeed, when polycationic ferritin binds to and inhibits the integral protein cytochrome *c* oxidase, it completely displaces cytochrome *c*, a naturally occurring cationic peripheral protein, from its binding site on the oxidase (16). Once polycationic ferritin binds electrostatically to membrane surface anionic groups, the subsequent addition of native (anionic) ferritin appears to cross-bridge the bound polycationic ferritin electrostatically and therefore cross-bridges anionic groups of membrane integral proteins through a relatively continuous artificial peripheral protein lattice. Since the results reveal that this method clearly diminishes low temperature-induced lateral translational motion and aggregation of intramembrane particles, a logical conclusion is that such peripheral protein latticing results in the immobilization of integral proteins. A second, perhaps more dramatic, observation which emphasizes the effectiveness of an artificial peripheral protein lattice in inhibiting lateral translational motion of intramembrane particles was the irreversibility of particle aggregation. It was observed that intramembrane particles, induced to aggregate at −10°C, disaggregate and equilibrate rapidly to a random distribution when the membrane temperature is raised to 25°C. Such disaggregation and equilibration most likely occurs by free lateral translational diffusion. Our results show that an inhibition of such diffusion occurs when the peripheral ferritin lattice is permitted to bind to the membrane surface anionic groups just before raising the temperature. A very limited population of small intramembrane particles which do manage to diffuse back into the smooth patches of the membrane may be small integral proteins which do not contribute anionic groups to the outer surface of the inner membrane and therefore are not stabilized electrostatically by the peripheral ferritin lattice. In any case, free diffusion of these small intramembrane particles back into the smooth patches of the membrane indicates that the smooth patches, presumed to be rich in gel state bilayer lipid at −10°C, become fluid and penetrable to the small intramembrane particles when the temperature is raised. Related to this observation and of considerable interest is that, when the temperature is raised, it appears that the fluid lipid of such patches cannot diffuse back into the particle-rich regions of the membrane, most likely because the large intramembrane particles are held together by the peripheral ferritin lattice.

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