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GLUTARALDEHYDE INDUCED ALTERATIONS OF MEMBRANE ANIONIC SITES

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Summary

Prefixation of the plasma membranes of cultured baby hamster kidney cells and human fibroblasts with glutaraldehyde can induce an anomalous distribution of surface anionic sites. However, such glutaraldehyde induced changes do not appear to occur in isolated inner mitochondrial membranes.

In recent years many laboratories have undertaken investigations of the distribution and mobility of different molecular components and receptor sites of the plasma membrane and the membranes of various cellular organelles. The distribution of such components has often been studied by light and electron microscopic techniques after fixation with glutaraldehyde. We have utilized the multivalent ligand polycationic ferritin to study the distribution and mobility of anionic sites on the surfaces of cultured baby hamster kidney (BHK) cells both in suspension [1] and attached to a substratum [2], of cultured human skin fibroblasts attached to a substratum [3], and of isolated inner and outer mitochondrial membranes [4,5]. During our studies we observed that under certain conditions, fixation with glutaraldehyde resulted in an anomalous distribution of membrane anionic sites.

We have reported that the native distribution of anionic sites is random and essentially continuous over the surface of BHK cells as determined by incubating the cells with polycationic ferritin (0.32 mg/ml) for 10 s at room temperature. Longer incubations in the presence of polycationic ferritin resulted in a marked redistribution of anionic sites, movement of the sites off of cell microextensions and into clusters on the cell body, and ultimately (after 20 min) substantial clearing of the ligand-bound sites from the cell surface by pinocytosis. In all of our experiments, the reactions were stopped by addition of buffer to dilute out the polycationic ferritin, followed by

fixation with glutaraldehyde. When cells with cleared anionic sites were washed and resuspended in fresh medium containing polycationic ferritin, there was no longer any binding of the ligand to the cell surface. However, if such cleared cells were prefixed with 0.2% glutaraldehyde for 10 min at room temperature and subsequently exposed to polycationic ferritin, the ligand bound uniformly and continuously over the entire cell surface (Fig. 1A and 1A'). Binding of native ferritin did not occur under these conditions. Moreover, binding of polycationic ferritin was not prevented by treatment of the glutaraldehyde fixed cells with a 10-fold molar excess of NH_4Cl, L-lysine, or tris prior to the addition of the ligand. We anticipated that these reagents would quench free aldehyde groups associated with the cell surface as a result of some glutaraldehyde molecules binding to the cells at only one end of the molecule.

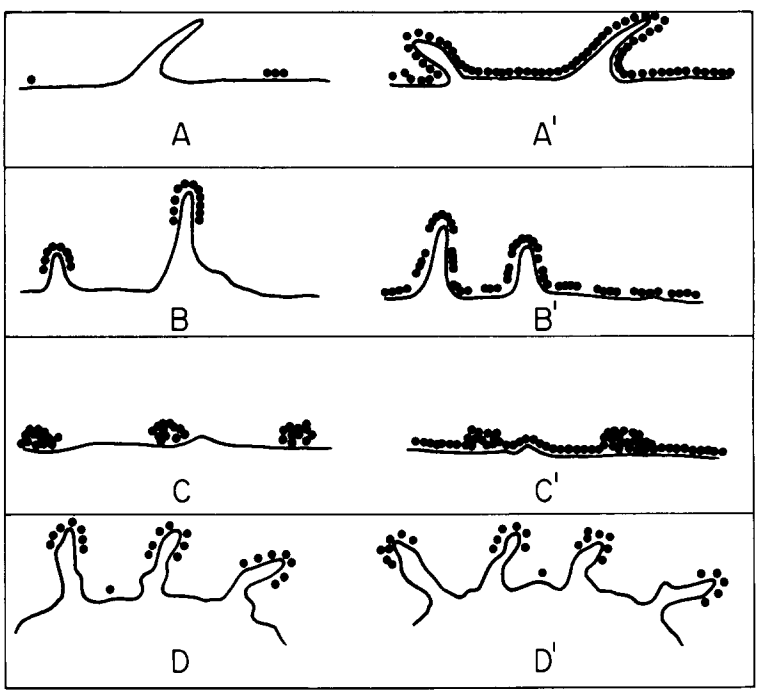

Fig.1. Schematic representation of the distribution of polycationic ferritin on the surfaces of various membranes. A and A': binding of polycationic ferritin to "cleared" BHK cells before and after fixation with glutaraldehyde. B and B': binding of low concentrations of polycationic ferritin to BHK cells before and after fixation with glutaraldehyde. C and C': binding of polycationic ferritin to human skin fibroblasts before and after fixation with glutaraldehyde. D and D': binding of polycationic ferritin to the outer surface of inner mitochondrial membranes before and after fixation with glutaraldehyde. See text for further explanation.

In experiments where BHK cells were incubated for 10 s with a low concentration of polycationic ferritin (0.08 mg/ml) we observed binding of the ligand only to high affinity sites which were located on the surfaces of cell microextensions but not on the cell body. If the cells were prefixed with glutaraldehyde (as above) prior to the addition of polycationic ferritin, the distribution of the probe was observed in continuous, random clusters over

the entire cell surface (Fig. 1B and 1B′). In these experiments the glutaraldehyde fixed cells were quenched with a 10-fold molar excess of NH_4 Cl.

When human skin fibroblasts attached to a substratum were treated with polycationic ferritin (0.32 mg/ml) for 10 s the probe was observed in small piled-up clusters distributed over the entire cell surface. Unlike the BHK cells, further incubation of these cells in the presence of the ligand did not result in any significant redistribution of the bound anionic sites. However, as with BHK cells if these cells were prefixed with glutaraldehyde prior to treatment with polycationic ferritin, the distribution of the ligand-bound anionic sites was observed to be random and covering the entire cell surface (Fig. 1C and 1C .

Studies on the outer surface of the isolated inner mitochondrial membrane revealed membrane surface domains of high affinity and low affinity anionic sites. The distribution of anionic sites did not change when the mitochondrial inner membranes were incubated with polycationic ferritin for short or long time periods. Nor did pre-fixation of the inner membrane with glutaraldehyde (as above) alter the distribution of ligand-bound anionic sites (Fig. 1D and 1D′). In these experiments glutaraldehyde was quenched with a 10-fold molar excess of NH_4 Cl.

These results indicate that prefixation of the plasma membranes of cultured BHK cells and human skin fibroblasts with glutaraldehyde can induce a change in the distribution of surface anionic sites. However, such glutaraldehyde-induced changes do not appear to occur in isolated inner mitochondrial membranes. Two possible explanations are immediately apparent to account for the glutaraldehyde effect: (1) a direct binding and Schiff base formation may occur between the added polycationic ferritin and exposed aldehyde groups of glutaraldehyde bound to the cell surfaces; or (2) a glutaraldehyde-induced reorganization of the membrane resulting in the appearance of "new" anionic sites.

Direct binding between polycationic ferritin and exposed aldehydes of cell surface-bound glutaraldehyde would be the simplest explanation, but is not consonant with the observation that native ferritin, albeit containing fewer free amino groups, did not bind to prefixed cells. Also, treating glutaraldehyde prefixed cells with excess NH_4 Cl, L-lysine, or tris to block free aldehyde groups did not prevent glutaraldehyde induced changes in the distribution of anionic sites. Moreover, no glutaraldehyde-induced alteration was observed in the inner mitochondrial membrane which suggests that the fixation effect relates to the particular nature of the membrane being studied. Based on these considerations — although we cannot conclusively rule out the direct-binding possibility — our data is more consistent with the possibility that glutaraldehyde induces a reorganization of some membrane anionic sites. It could be due to exposure of "masked" anionic groups of membrane components, or possibly (although less likely) the result of a glutaraldehyde-induced lateral redistribution of pre-existing sites. Whatever the ultimate explanation for this finding, our observations emphasize the need for caution when glutaraldehyde is used as a prefixative in studies utilizing surface probes for the purpose of determining the distribution of membrane components.

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