PARACRYSTALLINE ARRAYS OF MEMBRANE-TO-MEMBRANE CROSS BRIDGES ASSOCIATED WITH THE INNER SURFACE OF PLASMA MEMBRANE

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ABSTRACT

In cultured cells of the rat kangaroo PtK2 line, veils of the cell surface were observed which consisted of only plasma membrane and paracrystalline arrays of membrane-associated particles sandwiched in between. These membrane-to-membrane cross-bridging 9- to 11-nm wide particles were somewhat columnar-shaped and were arranged on a hexagonal lattice with an interparticle distance of 16 nm. At higher magnification, they revealed an unstained core, thus suggesting a ringlike substructure. Similar arrays of paracrystal-containing veils, which were rather variable in size and frequency, were also observed in other cultured cells. It is hypothesized that these paracrystals represent protein macromolecular complexes associated with the inner plasma membrane surface which crystallize when plasma membranes come into close intracellular contact and other components of the subsurface network are removed.

Paracrystalline arrays of particulate units associated with the plasma membrane have been described on the outer surface in a variety of cells. Prominent examples include the membrane-inserted hexagons of subunits in thickened plaques in luminal surface membranes of the urothelia of mammalian bladder (for references see 9 and 15), regular arrays of intercellular material in various types of clefts and junctions (e.g. references 7 and 13), and the particles coating the surface of the apical endocytic complex of ileal epithelium of suckling rats (for references see 15). A series of granular, filamentous, or densely aggregated indistinct components have been identified in association with the inner side of surface membranes, and certain proteins have been shown to be major constituents of such associations with the inner surface of plasma membranes. Periodically arranged structural components located at the inner side of the plasma membrane are prominent in the apical infoldings of some insect epithelia (for references see 1). They have also been sporadically described in, for example, erythrocyte ghosts (for references see 17) and in the form of the lateral cross bridges with microtubules (reviewed in reference 14), microfilaments (11, 16, 18), and with adjacent intracellular membranes such as components of the endoplasmic reticulum (3, 5, 8) and secretory vesicles (2, 4–6). In the present study, we describe a paracrystalline array of particles associated with the inner surface of the plasma membrane which cross-links adjacent regions of cell surface membrane. Such arrays are particularly frequent and prominent in cells of the
Cells of the established rat kangaroo (Potorous tridactyla) kidney cell line PtK₂, originally obtained from the American Type Culture Collection (via Flow Laboratories GmbH, Bonn, Germany) were grown as monolayer cultures on the surface of plastic dishes (BioQuest, BBL & Falcon Products, Becton, Dickinson & Co., Cockeysville, Md.) or on glass cover slips in modified Eagle's minimal essential medium (Flow Laboratories) supplemented with nonessential amino acids and 10% fetal calf serum, at 0.85 g sodium bicarbonate per liter. Medium was replaced after 3 days of culture, and cultures were subdivided after 4-6 days using 0.25% trypsin (Trypsin 1:250, Difco Laboratories, Detroit, Mich.) and 0.02% EDTA in phosphate-buffered saline (PBS) for detachment, and were replated at a ratio of 1:10. Some cultures were treated with cytochalasin B (10⁻⁵ M or 2 x 10⁻⁶ M, in medium containing 0.5% dimethylsulfoxide) for 1, 2, or 4 h before fixation.

Electron microscopy

Cells grown on plastic dishes were fixed in 2.5% glutaraldehyde (0.05 M sodium cacodylate buffer, pH 7.2, 2.5 mM MgCl₂, 50 mM KCl) at 37°C for 3-5 min and then for 20 min in a refrigerator kept at 4°C. They were postfixed for 2 h in 2% OsO₄, then washed with distilled water and soaked overnight in aqueous 0.5% uranyl acetate. After washing with distilled water, the cells were collected from the plastic dishes by scraping with a spatula or a rubber policeman, dehydrated in graded ethanol solutions, and embedded in Epon 812 by polymerization, the cover slips were removed with the aid of liquid nitrogen, and the cells remaining in the Epon were sectioned as described above. Micrographs were taken with a Siemens 101 electron microscope (Siemens AG, West Berlin).

RESULTS

Cells of the established rat kangaroo kidney epithelial cell lines PtK₁ and PtK₂ are known to have a flattened morphology which is often maintained even during mitosis (for references see 12). The cortex of these cells is rich in skeletal structures such as bundles of microfilaments, microtubules, and individual and aggregated intermediate-sized filaments (Fig. 1 and reference 12). In sections grazing to the surface and the cortex of flat-embedded PtK₂ cells, we frequently noted veils of the plasma membrane, in both tangential and oblique cross sections (Figs. 1 and 2a and b), which showed ordered arrays of intensely stained subunits (Fig. 2). The frequency of such areas was variable from cell to cell, perhaps due to differences in the plane of the specific sections, and they were observed more frequently in cells treated with cytochalasin B. The largest areas of such paracrystals encountered in flat sections were up to ~0.2 μm². Occasionally, large surface areas dominated by the appearance of many of these veils were noted (Fig. 1). In these ordered arrays, monolayers of surface membrane-associated particles (mean diameter of 9.8 ± 0.9 nm) were arranged in a hexagonal array as was also demonstrated by optical diffraction (Fig. 2c and d). The mean center-to-center distance in these hexagonal arrays as measured in the electron micrographs was 165 nm. The corresponding lattice constant as calculated from diffraction reflections was in the range from 15.8 to 17.5 nm. At higher magnification and in specific planes of sections, some of the plasma membrane-associated particles seemed not to be homogeneously stained but rather appeared as annular structures with inner diameters in the range from 3 to 5 nm. Occasionally, we gained the impression that individual subunits are interconnected to one another by densely stained horizontal threadlike connections (e.g. in the inset of Fig. 2a and in the optical reconstructions as in Fig. 2d).

Sections perpendicular to these layers of surface membrane-associated particles were especially frequently found in cells sectioned perpendicular to the plane of the substratum (Fig. 2e-f). Such sections revealed that the regularly arranged, densely stained particles were contained in surface membrane veils and connected homologous inner surfaces of closely apposed regions of surface membrane of the same cell. Such surface membrane veils or folds containing these particle paracrystals were traced up to a total length of ~2 μm (e.g. Fig. 2e and f). Frequently, such symmetrical layers composed of two plasma membrane sheets and a sandwiched layer of regularly arranged particles seemed to represent bridges between otherwise normally structured cytoplasmic regions. In other regions, they appeared as plasma
FIGURE 1 Survey electron micrograph of a section obliquely grazing to the surfaces of cultured rat kangaroo PtK₂ cells. Note the predominance of bundles of microfilaments (some are denoted mf), intermediate-sized (7-10 nm) filaments (if), microtubules (mt), ribosomes, endoplasmic reticulum (ER), and small vesicles in the cell cortex. Numerous veils and folds of the cell surface membrane containing paracrystalline arrays of densely stained particles are identified (arrows). Bar, 0.5 μm. × 76,000.
membrane folds constituting special cell surface cavity formations. In such cross sections, the intermembranous particles appeared slightly columnar (Fig. 2f and g) with an outer diameter of 9–11 nm and a height of 11–16 nm. The center-to-center distance in such sections was between 14 and 18 nm. The micrographs suggest that structures normally associated with the inner plasma membrane surface, such as microfilaments and cortical web structures, are totally excluded from the paracrystal-containing regions.

While paracrystalline intermembranous cross-bridge structures in such surface membrane veils were especially frequent in PtK₂ cells, they were also observed in other cultured cell lines that are maintained in our laboratory, such as a murine sarcoma 180 cell grown in monolayer. Thus, the paracrystalline arrays of particles serving as cross-links between different plasma membrane regions are not limited to the rat kangaroo cell line described.

DISCUSSION
The described type of paracrystalline monolayer of particles associated with the inner surface of plasma membranes is, to our knowledge, the first demonstration of a highly ordered cytoplasmic component which cross-links two plasma membrane regions of the same cell. It represents a type of membrane-to-membrane cross-bridge element (5) which closely resembles the regularly spaced cross-bridge elements between the inner aspect of the surface membrane and various forms of secretory vesicles (2, 4, 6). This structure seems to be of widespread occurrence in cultured cells, although it is particularly rich in the PtK₂ cell line. The reason for this variation in frequency is not clear, nor do we know of any function which might be associated with these structures. Preliminary attempts to isolate such paracrystal-containing plaques of plasma membrane sandwich have shown that they are rather rigid and tend to break into plates, thus suggesting that these components confer a certain degree of mechanical stability to cell surface regions.

Regarding the origin of the inner plasma membrane surface-associated particles contained in these paracrystal sheets, two alternatives can be envisaged: (a) the paracrystals represent a component, probably of proteinaceous nature, which is specific and unique to these membrane-associated structures; (b) the paracrystals represent components which are normally associated with the inner plasma membrane surface and are contained in the submembranous feltwork of granular and fibrillar material. It is obvious that cytochemical studies as well as biochemical analysis of the isolated paracrystal material are necessary for the elucidation of the composition and function of these structures.

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**Figure 2** Details of the surface membrane-associated paracrystalline arrays of densely stained particles as revealed in tangential (a–d) and transverse (e–g) sections. At higher magnifications (inset in a and b), the particles exhibit relatively electron-transparent cores, suggestive of an annular structure, and threadlike interconnections (e.g., between the particles denoted by arrowheads in the inset in a). An optical diffraction pattern of such an array is presented in Fig. 2c. The reflections can be indexed on a hexagonal lattice which is somewhat distorted, presumably due to sectioning. Angles between lattice vectors (arrows) range from 55° to 65° with lattice constants from 15.5 to 17.5 nm. The pattern shows the 10 and 11 reflections (six each). A filtered image (cf. reference 10) of such areas, using reflections out to a resolution of a/2 (~8 nm), is presented in Fig. 2d. The ringlike structures of the original are resolved into dots located roughly on the threefold axes of the hexagonal lattice, thus producing a honeycomblike appearance. The distance between the dots is ~10 nm.) Cross sections through plates consisting of two layers of surface membrane with the paracrystal sandwiched (e–g) are oriented in the original plane of cell substratum attachment. Note that such paracrystals bridge two apparently normal cytoplasmic regions (A and B). Details of the intermembranous particles are recognized in Fig. 2f and reveal their columnellalike shape. Bars, (a) 0.2 nm, inset 33 nm; (b) 33 nm; (e and f) 0.5 μm; (g) 0.1 μm. (a) × 110,000, inset × 360,000; (b) × 300,000; (e) × 110,000; (f) × 100,000; (g) × 340,000.
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