PERMEABILITY MODULATING MEMBRANE COATS

I. Fine Structure of Fluid Segregation Organelles of Peritrich Contractile Vacuoles

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Many protozoans and some other organisms possess contractile vacuoles (CVs), membranous organelles which help maintain cellular water/electrolyte balance by extracting a dilute solution from the cytoplasmic colloid and expelling it from the cell (35, 37). Although the similarity of CV function in the various organisms has long been recognized, the expected similarity of morphological elements responsible for generating CV fluid has eluded detection (21).

The initial step in this process, termed "fluid segregation" by Pappas and Brandt (34), is carried out by systems of vesicles or tubules which communicate with the main vacuole. The fluid segregation organelles exhibit characteristic differences in shape and relationship to the vacuole in various organisms; however, recent comparative examination of CVs in ciliates, flagellates, amebae, and freshwater sponges has revealed that the fluid segregation organelles are all composed of membrane with a similar specialization or "coat" (26). As described below for the fluid segregation tubules of peritrich protozoans, the coat consists of electron-dense, peg-shaped elements attached to the cytoplasmic dense lamina of the trilaminar membrane. The coat is of considerable comparative interest because morphologically similar specialized membranes occur in several other cells where water and electrolyte transport is emphasized, including insect digestive and excretory cells, and fish chloride cells. For reasons presented below, these similar coats are considered to be involved in membrane permeability modulation (PM).

Epistylis plicatilis, a colonial peritrich protozoan, was processed for electron microscopy by standard methods as described elsewhere (27).

RESULTS AND DISCUSSION

The fluid segregation organelles in *E. plicatilis* are relatively straight tubules (Figs. 1 and 2) of uniform diameter (45–50 nm) similar to those described for other peritrichs by Carasso et al. (12). The tubules are about 1 μm long, and peripherally they terminate blindly in the cytoplasm. Previous reports of continuity between fluid segregation organelles and rough endoplasmic reticulum (RER) have not been confirmed for any of the organisms in our comparative study (26). The fluid segregation tubules are confluent centrally with a 0.5-μm thick network of anastomotic tubules (20–60 nm diameter) which open to the main vacuole at numerous points. Of these membranous structures, only the fluid segregation tubules are composed of PM coated membrane.

In an extremely thin section as shown in Fig. 2, the coat is resolved as 6-nm diameter, peg-shaped elements projecting 12–15 nm into the cytoplasm from their origin at the cytoplasmic dense lamina of the 80-nm thick trilaminar membrane. The pegs show secondary association into pairs separated by a 6-nm gap, and the pairs exhibit tertiary organization into linear arrays which wind helically around the tubules with a pitch of ~100 nm. There are two linear arrays per tubule.

Examination of fluid segregation organelles in phylogenetically diverse organisms suggests that they all are composed of coated membrane. The higher order organization of the coat varies, but all fluid segregation membranes examined to date possess the basic peg-shaped elements projecting from the cytoplasmic dense lamina. In other ciliates (*Paramecium*, *Tetrahymena*, *Dileptus*), the coat on the fluid segregation tubules is in helical linear array as in peritrichs (26). Elongate fluid segregation tubules in chrysomonad phytoflagellates (1, 40), and simple tubules and vesicles in the small soil ameba, *Acanthamoeba castellanii* (9), are covered with PM coat elements. Alkaline phosphatase activity has been demonstrated in the fluid segregation organelles of *Acanthamoeba* (10). In most ameboid (26) and flagellated protozoans (23, 24) and fresh water sponges (11, 26), the fluid segregation organelles are vesicular rather than tubular; however, the luminal diameter of the vesicles is similar to that of the tubules, and the vesicles are composed of PM coated membrane.

Comparative Evidence

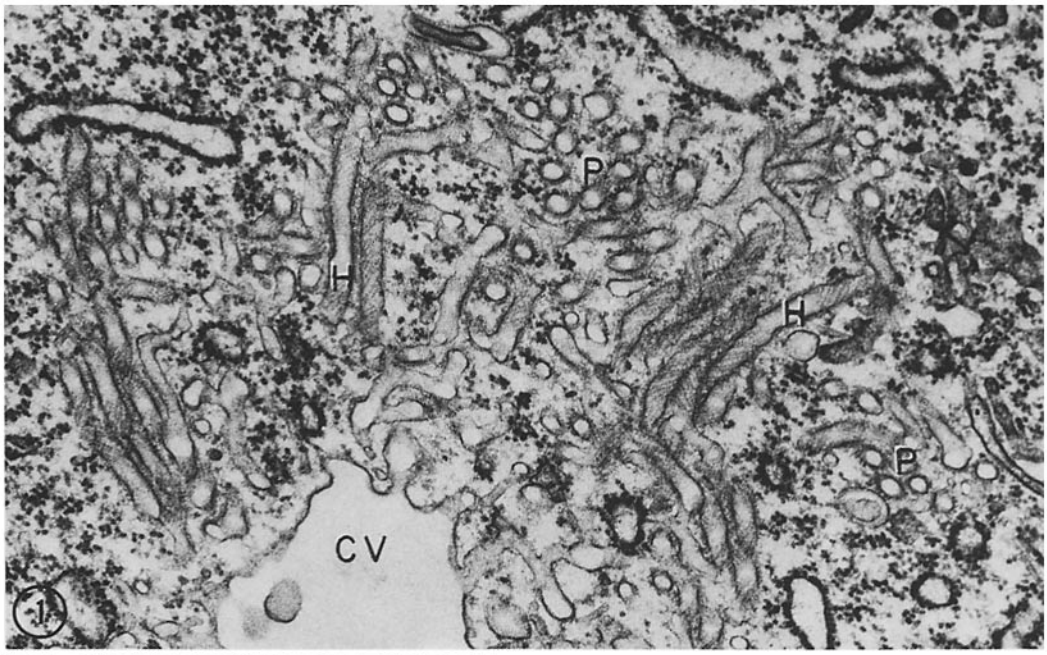
Accepting the basic assumption of comparative studies, i.e., that structures essential to a function will be present wherever such function is performed, we feel that the evidence cited above strongly implies the involvement of the PM coat in the common functional step of CVs. This common step is the extraction or segregation of a hypotonic solution from the cytoplasm, and it requires regulation of membrane permeability to water and ions. Additional support for this proposal arises from anatomy/physiology studies of morphologically similar coated membranes in other situations.

PM coated membrane composes the ubiquitous "spiny" coated vesicles which participate in uptake of extracellular materials (2, 36), intracellular transport of metabolic products (especially in the GERL [31]), and storage of substances for secretion (17). The comparative approach suggests that the membranes of these various vesicles are similarly modified (coated) because the coat is necessary to a certain physiological property that they have in common, and we propose that this property relates to the presence of an osmotic gradient or flux across the membrane. The vesicles in all cases appear to segregate either a hypotonic fluid or a highly concentrated, hypertonic substance from the cytoplasm (reviewed in reference 26).

In addition to vesicles, PM coated membrane composes tubules in teleost pseudobranch and gill chloride cells (14, 25, 38), and at neuronal nodes of Ranvier (6, 39). PM-coated membrane has been demonstrated forming planar structures and microvilli in insect osmoregulatory organs including salivary glands (32), midgut (3, 18), rectal papillae (7, 19, 29), rectal pads (33), anal sacs (30), Malpighian tubules (8), and chloride cells (22). Also, the "ruffled border" plasmalemma at the resorptive surface of osteoclasts exibits PM coat (20). Physiological data indicate that each of the above cells or organs is active in processes requiring active secretion, resorption, or regulation of water and electrolytes.

Coat Function

Two features of water/electrolyte control which are especially appearent in protozoan systems seem to shed light on the possible functional role of



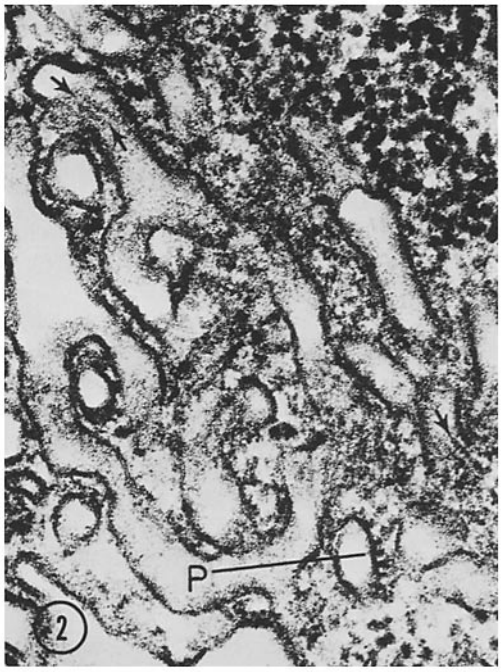


FIGURE 1 Straight fluid segregation tubules are confluent with the contractile vacuole (*CV*) through a network of irregular anastomosing tubules (*A*). Fluid segregation tubules in transverse section reveal the paired PM coat elements in profile (*P*), while longitudinal views show the helical array of the coat (*H*). × 39,000.

FIGURE 2 PM coat is composed of 6 × 14-nm peg-shaped elements (*P*) which, *en face*, appear organized into pairs in linear array (arrows). × 130,000.

the membrane coat. First, in ameboid movement a fluid hyaline cap is formed by syneresis at the leading edge of an advancing pseudopodium. Anderson (4) has presented evidence that the hyaline cap fluid has a high Na^+/K^+ ratio, exactly opposite the ratio of these ions in the cytoplasm but similar to the ratio in the contractile vacuole fluid (35). In the syneresis which forms the cap, the hyaline fluid is exuded from the cytoplasmic gel, but is not separated from the gel by a membrane.

We propose that a similar syneresis, on a microscopic scale in the vicinity of the fluid segregation tubules or vesicles, could separate the fluid from the cytoplasm, and that subsequently the fluid would be segregated by the membranous organelles without the intervention of a membrane "pump".

The second feature concerns the nature of fluid segregation. Since biological membranes are highly permeable to water, it cannot be expected that once a water molecular has entered a fluid segregation tubule or vesicle it is destined to be expelled from the cell. In fact, assuming that permeability of the tubule membrane is similar to the permeability of living cells and artificial membranes (10^{-3} cm s^{-1}), we can predict that a water molecule inside a 50-nm diameter membranous tubule at any instant would be statistically outside the tubule less than 10 μs later. Thus, segregation in this context is not the isolation of a given set of molecules, but rather the segregation of a given concentration of fluid.

Regardless of the frequency with which "pumps" are discussed, the mechanism whereby apparent concentration gradients exist at equilibrium is unknown (28); however, we feel that two anatomical features of the fluid segregation organelles of contractile vacuoles give clues to the importance of physical parameters in determining chemical activities in biological systems. The first feature is the small diameter (35–85 nm) of the vesicles and tubules, which may impart to them some of the water-structuring properties apparent in microcapillaries (15). This feature has been considered elsewhere (26).

Physical studies of water at interfaces and studies of membrane permeability have conceptually implicated unstirred layers and/or anomalous structured water adjacent to the membrane as important factors in the passage of materials from the bulk solution on one side to the other side. The other anatomical feature of fluid segregation organelles, the PM coat, may relate directly to these concepts. Briefly, most of the resistance to the osmotic flux of water through an artificial membrane appears to arise not from the lipid bilayer but from concentration gradients in the layer of solution immediately adjacent to the membrane (5, 13). In most cases, if the water in the unstirred layer is assumed to have diffusion properties similar to those of bulk water, it is calculated to be several micrometers thick.

The concept of vicinal water, a layer of water situated at an interface and therefore having physical properties different from those of bulk solution, has arisen from comparative correlation of physical and biological data (16). Although not necessarily related to unstirred layer phenomena, structured vicinal water could account for the anomalous osmotic flow by means of a layer which was nanometers rather than micrometers thick. Whether the layer of anamalous water is pictured as a discrete layer with unique bonding and dimensions or as a gradient of more stable clusters, it is clear that like the oriented water responsible for surface tension at an air/water interface, water at biological interfaces seems to exhibit physical properties different from those of bulk solution, and these properties seem to influence the passage of materials across the interface.

Regardless of the choice of water-structuring models, however, we propose that the electron microscope demonstration of peg-shaped elements on the surface of diverse membranes similar only in their highly active transport of materials or resistance to osmotic gradients is no mere coincidence. The peg-shaped elements of the PM coat are perfectly situated to modulate the properties of the water layer immediately adjacent to the membrane. Simply by their presence, these elements would either inhibit the stirring or stabilize the water structuring. In addition, although our model of coat function depends only on some type of physical interaction between the peg-shaped elements and the vicinal water layer, it may be recognized that if the peg-shaped elements had enzymatic activity and were capable, for instance, of hydrolyzing adenosine triphosphate, the liberation of highly charged species within the vicinal layer would profoundly affect the bonding, and thus the physical properties of the water in this layer.

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