Mechanical coupling of zymogen granule membrane with the granule core

In a recent paper, Miyamoto and Fujime (1990) describe experiments to determine the area expansion or elastic modulus of zymogen granule membranes. The principle of the method is to subject an osmotically active compartment to solutions of an impermeant solute of decreasing osmolality and, after equilibration, to determine the extent to which the actual volume of the compartment falls below that expected from an ideal osmometer. The difference in volume is attributed to an elastic resistance to expansion by the compartment membrane.

Problems with interpretation in this kind of experiment are essentially of two kinds: practical and theoretical. Practical problems include the permeability of the compartment membrane to solute (Rivers and Williams, 1990), and active transport of solute (Miyamoto et al., 1988). These can be dealt with, in principle, by appropriate attention to experimental detail.

The other source of problems are those of a theoretical kind, and that is where, I suggest, the paper by Miyamoto and Fujime (1990) faces difficulties. Implicit in the interpretation of their experiments are the assumptions (*a*) that the zymogen granule membrane is not coupled mechanically to the granule core, and (*b*) that the granule membrane is not stressed at normal osmolalities. Evidence suggests that these assumptions are wrong.

Zymogen granules possess a coherent core (Jamieson and Palade, 1967, 1971; Burwen and Rothman, 1972; Ermak and Rothman, 1978) whose boundary is contiguous with the granule membrane. In hyper-osmotic conditions, these granules should therefore resist contraction because of the need to compress this core. There is evidence that this is indeed the case (Warashina, 1981). Under hypo-osmotic conditions, the granules should swell. They do so less than would have been expected if they were ideal osmometers (Warashina, 1981). The discrepancy between actual and expected size can be attributed either to the elastic nature of the granule membrane (the explanation implicitly put forward by Miyamoto and Fujime, 1990), or to its attachment to an elastic granule core. Morphological evidence suggests that granule membranes are attached to their cores (Ermak and Rothman, 1978). If that is the case, then the measurements reported by Miyamoto and Fujime (1990) need have nothing to do with the elastic modulus of the granule membrane.

That is not the only criticism that one can make of their experiments, however. Evidence suggests that isolated zymogen granules are unstable (Jamieson and Palade, 1971; Ermak and Rothman, 1978). This instability is reflected morphologically in an irreversible transition from a homogeneous to a reticulated core structure (Jamieson and Palade 1971; Ermak and Rothman, 1978). The change is particularly widespread after extended incubation in 0.3 M sucrose (Ermak and Rothman, 1978). Given that Miyamoto and Fujime (1990) keep their isolated zymogen granules in isotonic sucrose for up to 48 h before use, there must be some concern that their granules are substantially in the reticulated form, and not the form in which they exist inside intact acinar cells. If that is the case, then the connection between the osmotic behavior of reticulated-core granules and normal granules needs to be established because it is by no means obvious. For example, it is unclear from the literature whether granules with reticulated cores are larger than normal granules. In other words, are the cavities a result of granule core expansion, or are they the result of selective dissolution? Either way, the phenomenon suggests that granules have become permeable to external solute. If the cores have swollen, then external solute has probably entered the granule and permitted gel swelling or colloid osmotic pressures to cavitate its core, as happens in guinea pig sperm and sea urchin cortical granules (Green, 1978, 1982; Whitaker and Zimmerberg, 1987). If they have not swollen, then the dissolved material must have left the granule following localized membrane rupture. Work on secretion over the past decade indicates that in a number of systems (sperm acrosome, amebocytes, cortical granules, mast cell granules), granule swelling follows membrane fusion (Green, 1978, 1982; Ornberg and Reese, 1981; Whitaker and Zimmerberg, 1987; Breckenridge and Almers, 1987; Zimmerberg et al., 1987). *Prima facie,* therefore, granule cores have osmotic properties and are kept in a condensed, metastable state before the loss of granule membrane integrity. This evidence suggests, by analogy, that zymogen granules have swollen during reticulation of their cores.

The second assumption implicit in Miyamoto and Fujime's work is that the granule membrane is unstressed under isotonic conditions. This is an important assumption, for if the granule core is already compressed in isotonic solution because, for example, of an osmotic deficit within the granule, then decreasing the external osmolality is merely going to relieve that compression. The swelling that ensues will, once again, reflect core elasticity rather than that of the membrane and this will be the case regardless of whether the core is physically attached to the membrane or not. The authors only measure the diameters of their zymogen granules over a narrow osmotic range, and this range may lie wholly within the compressive response of the core.

Where does this leave the osmotic properties of zymogen granules? It should be clear by now that their properties almost certainly reflect the mechanical coupling of their membrane to the granule core. Whether this is of any significance in exocytosis is not clear. The suggestion that bulk granule swelling occurs before membrane fusion in exocytosis and is the source of granule membrane fusigenicity (Cohen et al., 1982; Finkelstein et al., 1986; Holz, 1986) is not the case in a number of cells (Green, 1978, 1982; Ornberg and Reese, 1981; Whitaker and Zimmerberg, 1987; Breckenridge and Almers, 1987; Zimmerberg et al., 1987). Indeed, bulk granule swelling during exocytosis may not occur in any cell. However, this does not eliminate *local* swelling as a cause of secretory granule fusigenicity (Green, 1987). Fusion "hot-spots" caused by local blistering of membranes could allow osmotic forces to play a role in membrane fusion. For the generation of these localized forces, the granule membrane must either be held onto the core by an osmotic deficit, or by mechanical attachment. If that were not the case, then bulk swelling of the whole granule would occur instead, and the evidence is that this does not occur. Membrane blisters can be generated in erythrocytes and are fusigenic

(Ahkong and Lucy, 1988; Lucy, 1989), showing that the mechanism is, at the very least, plausible. However, it remains possible that no osmotic event occurs before secretory granule fusion with the plasma membrane. On this model, membrane fusion involves activation of a fusion protein, and the osmotic expansion of the granule core is simply a means of making patent the initial exocytotic pore.

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