# A CELL-FREE MODEL FOR PROTEIN-LIPID INTERACTIONS IN EXOCYTOSIS

## AGGREGATION AND FUSION OF CHROMAFFIN GRANULES IN THE PRESENCE OF CALCIUM, SYNEXIN, AND *CIS*-UNSATURATED FATTY ACIDS

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The release of hormones, neurotransmitters, and other secretory products by exocytosis requires the regulated interaction and fusion of two supramolecular structures, the secretory vesicle membrane and the plasma membrane. We have proposed that this process might be regulated by synexin, a $Ca^{2+}$-binding protein present in many secretory tissues (1–3). This report summarizes our attempts to reconstitute in vitro the membrane contact and fusion that occur during exocytosis. Rather than study the fusion of the secretory vesicle and the plasma membrane, we have found it more expedient to examine the fusion of secretory vesicles with one another, a process that occurs during compound exocytosis.

### RESULTS AND DISCUSSION

When activated by $Ca^{2+}$, synexin forms irreversible contacts between chromaffin granules, the secretory vesicles of the adrenal medulla (Fig. 1 *A*). The nature of these contacts is unknown, although the interaction is temperature dependent (1), enhanced by increased ionic strength (1), and has been reported to be sensitive to protease

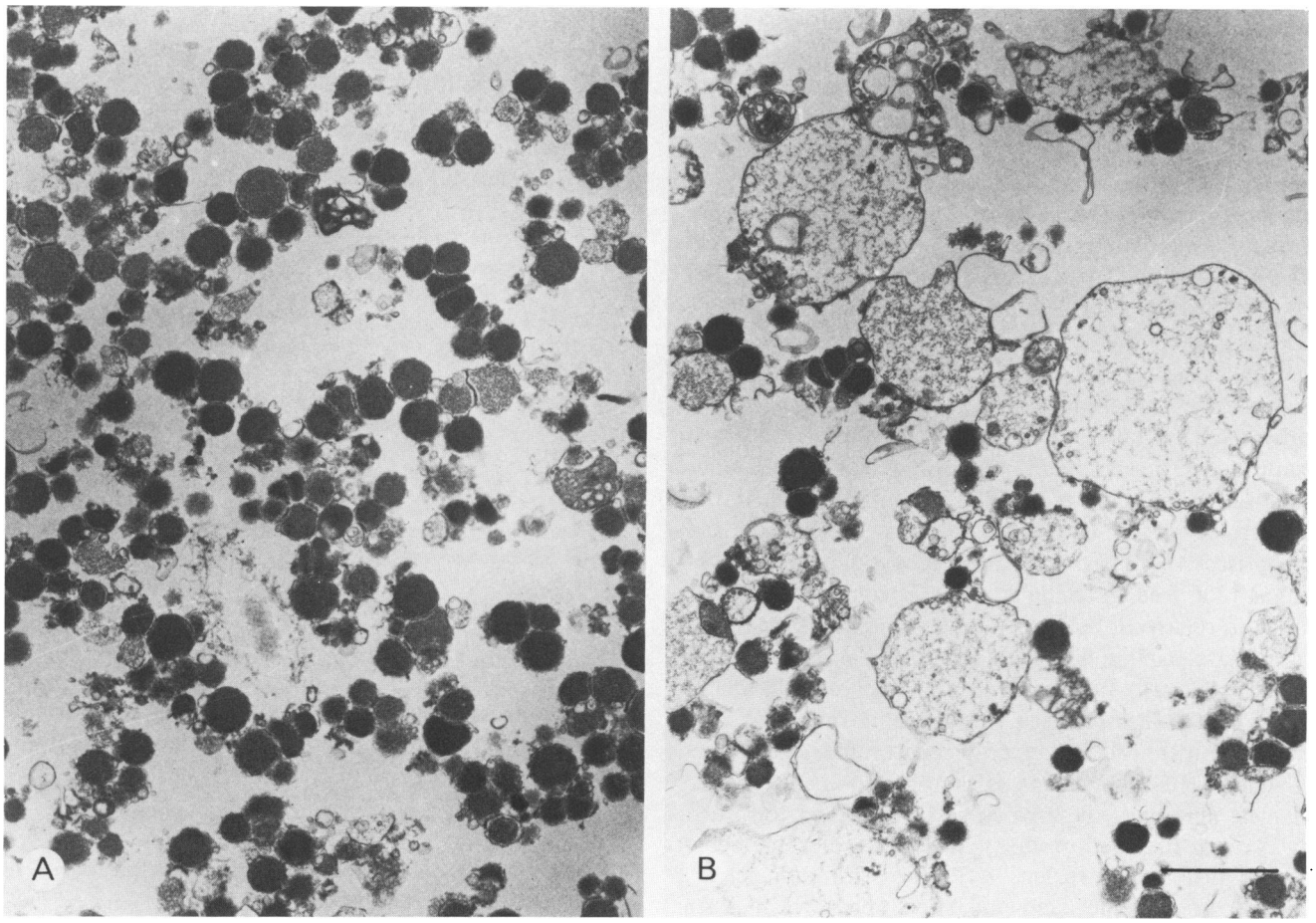


FIGURE 1 Electron micrographs of aggregated (*A*) and fused (*B*) chromaffin granules. Suspensions containing 80 μg/ml chromaffin granule protein, 15 μg/ml synexin, and 1 mM $Ca^{2+}$ were incubated for 20 min at 37°C, and in *B*, 4 μg/ml arachidonic acid was introduced at 15 min. Bar, 1 μm.

treatment of the granule membrane (4). We presumed that these contacts were similar to those occurring between membranes during exocytosis in vivo since they were pentalaminar in structure, although complete fusion was not observed. However, we recently found that when a small amount of *cis*-unsaturated fatty acid was added to a suspension of granules aggregated as shown in Fig. 1 *A*, the granules rapidly fused to form large vesicular structures (Fig. 1 *B*, reference 5) that were morphologically similar to the vacuoles that form in the cytoplasm of the chromaffin cell following extensive compound exocytosis (6). This transition began immediately and was complete in a few minutes. The clusters of fusing granules performed a dramatic dance that could be observed in the phase microscope as the large vesicles swelled in sudden and discrete steps from the clusters. The process could also be monitored by recording the decline in turbidity (A540) of the suspension which occurred as fusion took place (Fig. 2).

The fusion reaction was temperature dependent, occurring at 37°C but not at 0°C. During fusion the soluble proteins stored inside the granules were retained, although ~ 50% of the epinephrine escaped (above control levels of leakage). The rate of fusion could be inhibited 10-fold by raising the pH of the suspension from 6.0 to 7.3. The formation of the large vesicles could be suppressed by increasing the osmotic strength of the medium with sucrose. However, the formation of the vesicles did not appear to depend on the chemiosmotic properties of the granule membrane: it was not influenced by ATP, a proton ionophore, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone, or an anion transport inhibitor, 4-acetamido-4′-isothiocyanostilbene-2,2′ disulfonic acid.

The fatty acids and other lipids used at high concentrations by Ahkong et al. (7) to induce fusion of red blood cells were tested for fusogenic activity in this system. Chromaffin granule fusion was found to require specifically those *cis*-unsaturated fatty acids that are common components of mammalian phospholipids. Arachidonic acid was the most effective fusogen, causing vesicle formation at 2 μg/ml (6 μM), at which concentration it comprised 4% of the total lipid in the suspension. This amount of free arachidonic acid is comparable to the amount, relative to total membrane lipid, that is released from platelet phospholipids during secretion of serotonin by exocytosis (8). We have recently observed that arachidonic acid is also released from chromaffin cells during exocytosis (9). *Trans*-unsaturated, saturated, or esterified *cis*-unsaturated fatty acids did not induce fusion. Isomers of oleic acid with the *cis*-double bond moved toward or away from the head group (petroselenic, vaccenic acids) caused little or no fusion. Studies with radioactively labeled arachidonic acid indicated that when fusion occurred, 93% of the added fatty acid was bound to the granule, but none was incorporated covalently into phospholipids.

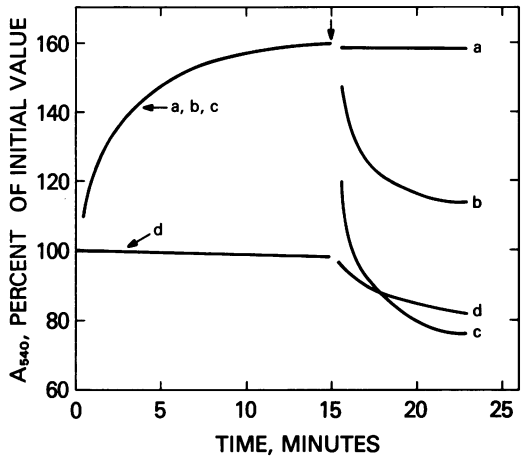


FIGURE 2 Turbidity (A540) of chromaffin granule suspensions undergoing aggregation and fusion. From 0–15 min a suspension (A540 ~ 0.3, 80 μg/ml protein) is incubated with (traces *a*, *b*, *c*) or without (trace *d*) 15 μg/ml of synexin. At 15 min 4 μg/ml of a fatty acid is introduced: trace *a*, elaidic acid (*trans* 18:1); trace *b*, oleic acid (*cis* 18:1); traces *c* and *d*, arachidonic acid (*cis* 20:4).

The factors regulating this in vitro process of secretory granule aggregation and fusion—$Ca^{2+}$, synexin, and unesterified, *cis*-unsaturated fatty acids—may be available in the cytoplasm of a secretory cell when it is stimulated. Therefore, this fusion may be the same as the one that occurs between secretory vesicles undergoing compound exocytosis.

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