N-ethylmaleimide-sensitive protein(s) involved in cortical exocytosis in the sea urchin egg: localization to both cortical vesicles and plasma membrane

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Summary

The exocytotic release of secretory products from fragments of sea urchin egg cortex has been shown to be inhibited by covalent modification of membrane sulfhydryl groups with N-ethylmaleimide (NEM). Exocytotically competent preparations of reconstituted cortex, formed by recombination of purified cortical vesicles (CVs) with fragments of egg plasma membrane (PM) were also inhibited by treatment with NEM. The cellular localization of sulfhydryl-containing constituent(s) responsible for inhibition was investigated by treating CVs and/or PM with NEM prior to reconstitution. Both native cortex and cortex reconstituted with NEM-treated components were challenged with calcium-containing buffers. Exocytosis was monitored by phase-contrast microscopy, and quantitated by light scattering. Evidence for CV-PM fusion was obtained with an immunofluorescence-based assay that permits visualization of the transport of CV content proteins across the PM. Cortex reconstituted by recombination of NEM-treated CVs with untreated PM was exocytotically competent, whereas cortex formed by recombination of NEM-treated CVs with NEM-treated PM was inactive. These results: (1) support the hypothesis that the mechanism of exocytosis in native and reconstituted cortex is the same; (2) provide evidence that both CV and plasma membranes participate in the release of CV contents from reconstituted cortex; and (3) suggest that sulfhydryl-containing protein(s) present on the surface of purified CVs and plasma membrane are involved in exocytosis.

Key words: exocytosis, secretion, N-ethylmaleimide.

Introduction

Regulated exocytosis, the release of stored secretory products in response to a stimulus at the cell surface, is a characteristic feature of complex multicellular organisms. The set of stimuli to which a secretory cell can respond is determined by the specific array of surface receptors and ion channels that it expresses. Activation of most, if not all, of these receptors triggers an increase in the intracellular concentration of calcium ion, which stimulates exocytosis (Penner and Neher, 1988).

The sea urchin egg is a particularly convenient system for studying the regulated form of exocytosis. In the mature egg, a specialized set of secretory vesicles known as cortical vesicles (CVs) are firmly attached to the cytoplasmic face of the plasma membrane (PM). Upon fertilization, an increase in the cytosolic concentration of Ca²⁺ (Steinhardt et al. 1977; Turner et al. 1986) triggers the fusion of the CVs with the PM. This process deposits proteins and mucopolysaccharides from the CVs onto the surface of the egg, where they contribute to the formation of the fertilization envelope and the hyaline layer (Kay and Shapiro, 1985).

Fragments of egg cortex, consisting of the PM, the CVs and the vitelline layer, comprise an exocytotically competent system. When bathed in calcium-containing buffers, egg cortex undergoes a reaction at physiologically relevant concentrations of Ca²⁺ (Moy et al. 1983; Whitaker and Baker, 1983) that results in the fusion of the CV and plasma membranes; and releases CV content proteins onto the extracytoplasmic surface of the PM (Whitaker and Baker, 1983; Chandler, 1984; Zimmerberg et al. 1985; Crabb and Jackson, 1985).

CV-free egg PM can be easily obtained by dislodging the CVs from cortical lawn (CL) preparations of egg cortex. This procedure produces a PM ‘lawn’, consisting of an array of PM fragments attached via their vitelline layer to a polylysine-coated microscope slide (Kopf et al. 1982; Crabb and Jackson, 1985). Fragments of egg cortex can be reconstituted by recombining purified CVs with a PM lawn (Crabb and Jackson, 1985; Whalley and Whitaker, 1988). The reconstituted cortical lawns (RLs) produced by this procedure appear to be exocytotically competent: Ca²⁺-containing buffers trigger a reaction that results in fusion of the CV and plasma membranes, and the deposition of CV contents onto the extracytoplasmic surface of the PM (Crabb and Jackson, 1985). While it remains to be determined whether reassociation correctly reconstitutes the CV-PM junction, the results of binding specificity and protease inhibition experiments suggest that reassociation may be a specific, protein-mediated event (Jackson and Modern, 1990).

The observation that cortical exocytosis can be inhibited by sulfhydrol-modifying agents such as N-ethylmaleimide (NEM; Haggerty and Jackson, 1983; Jackson et al. 1986) suggests that a sulfhydrol-containing protein may be a
part of the exocytic apparatus of the cell. As an additional measure of the authenticity of reconstitution, we have investigated the NEM sensitivity of reconstituted cortex. Our results suggest that the release of CV contents from reconstituted cortex and native cortex are mechanistically equivalent in that both can be inhibited with NEM. In addition, analysis of the exocytic capability of cortex reconstituted from NEM-treated components demonstrates that the NEM-sensitive component is present both in purified CVs and PM.

Materials and methods

Materials

Strongylocentrotus purpuratus were maintained at 9–12°C in a refrigerated aquarium containing Instant Ocean sea water from Aquarium Systems (Mentor, OH). Soybean trypsin inhibitor (SBTI), NEM, poly-L-lysine (molecular weight 2 x 10^6), Pipes, KCl, MgCl2, EGTA, di-thiobiotreitol (DTT), goat serum and fluorescein isothiocyanate (FITC)-conjugated rabbit anti-mouse IgG (whole molecule) were purchased from Sigma Chemical Co. (St Louis, MO). NH4Cl, NaCl, and NaFCl were from Fischer Scientific (Pittsburgh, PA). The mouse IgA monoclonal antibody to hyalin was prepared by Dr Carol Vater in this laboratory (Vater and Jackson, 1990).

Preparation of reconstituted cortical lawns (RLs)

PM lawns and cortical lawns (CLs) were prepared as soybean trypsin inhibitor (SBTI)-containing buffers, as previously described (Crabb and Jackson, 1985). CVs were dislodged from purified egg cell surface complex (CSC) by gentle homogenization in TKE buffer (50 mM Tris–HCl, 600 mM KCl, 5 mM EGTA, pH 8.0) containing 10 μg ml⁻¹ SBTI, and purified by two rounds of differential centrifugation (Crabb and Jackson, 1985). PM lawns and CVs were recombined to form RLs as follows (Crabb and Jackson, 1985): samples of a CV suspension (A400=10) were brought to pH 6.8 by the addition of 1,0 ml Piper, pH 6.1, and 150 μl samples were drawn (with a 2 cm x 2 cm cork of filter paper) into microscope slide chambers containing PM lawns. CVs were allowed to bind to the PM lawns for a period of 15 min. The coverglass was removed from the chamber and unbound CVs were washed away by dipping the slide five times into each of two 100 ml beakers containing PKME buffer, or RLs and PM lawns were incubated with 5 mM NEM in PKME buffer containing 10 μg ml⁻¹ SBTI and 1 μg ml⁻¹ leupeptin for the indicated period of time (usually 15 min) at 20°C. NEM was added from a freshly prepared 200 mM stock solution in 0.5 M KCl. Reactions were terminated by the addition of DTT (from a 1.0 M stock) to a final concentration of 10 mM. RLs and 10 mM DTT were treated at pH 6.8 with 5 mM NEM for the indicated period of time were washed once for 15 min with 5 mM NEM buffer containing 10 μg ml⁻¹ SBTI and 1 μg ml⁻¹ leupeptin for the indicated period of time (usually 30 min) at room temperature. Reactions were terminated by dipping each slide five times into a 100 ml beaker containing PKME buffer with 1 mM DTT, and five times into a beaker containing PKME buffer without DTT. The washed samples were flooded with PKME buffer containing 10 μg ml⁻¹ SBTI, 1 μg ml⁻¹ leupeptin, and a coverglass was placed on each chamber.

Results

NEM inhibits exocytosis in CLs

We have previously shown that treatment of suspensions of egg cortex (CSC preparation) with NEM inhibits exocytosis by increasing the threshold Ca²⁺ concentration required to elicit reaction (Jackson et al., 1985). At pH 8.0, complete inactivation of CSC to a challenge by buffers containing 1 mM free Ca²⁺ required 15 min with 5 mM NEM (Fig. 1A). Similar results were obtained with fragments of egg cortex attached to poly-L-lysine-coated glass slides (CL preparations, Fig. 1B). CLs that had been treated at pH 6.8 with 5 mM NEM for the indicated period of time were challenged with a buffer containing 1 mM free Ca²⁺ and exocytosis was assessed by light-scattering analysis. Under these conditions, complete inactivation was achieved within 30 min. The control (Fig. 1B, open symbol) showed that a 30 min incubation in the absence of NEM is not inhibitory. Though it appears from the data presented in Fig. 1 that there is a difference in the rates of inactivation of the CSC (Fig. 1A) and CL (Fig. 1B) samples, this difference is due to the higher pH used in the CSC inactivation experiment (thiolate anions are more readily modified than thiols). At pH 6.8, the rate of inactivation of CSC is comparable to that of CLs (compare Fig. 1B with the NEM inactivation data of Jackson et al., 1985). The lag in the CSC and CL inhibition curves (Fig. 1A and 1B) reflects the fact that mild inactivation (low NEM concentration or short reaction time) increases the Ca²⁺ threshold, but does not prevent 100 % release in response to a strong stimulus (1 mM Ca²⁺). As previously noted (Jackson et al., 1985), this behavior suggests that a small fraction of the total number of NEM-sensitive proteins may be sufficient to support 100 % exocytosis.

The use of higher than physiological concentrations of Ca²⁺ in these and subsequent experiments was necessitated by the characteristics of the CL and reconstituted
NEM inhibits exocytosis in CLs and RLs

We were interested in the susceptibility of RLs to NEM inhibition because it provides an additional criterion by which the authenticity of reconstitution can be judged. If reconstitution correctly reassembles a functional CV—PM junction, it should be possible to inhibit RLs with NEM. To test this possibility, RLs and CLs were prepared as described in Materials and methods, incubated with or without 5 mM NEM for 30 min, and challenged with PKME buffer containing 1 mM free Ca^{2+}. The extent of reaction was quantitated by light-scattering analysis and is expressed in terms of percentage of reaction in order to facilitate comparison of CLs that contain many CVs with RLs that contain fewer. The results of this experiment (Fig. 2A, columns 1–4) demonstrate that both RLs and CLs are susceptible to inhibition by NEM, and lend support to the hypothesis that the mechanism of exocytosis in native and reconstituted cortex is the same.

Activity of RLs prepared from NEM-treated components

RLs are prepared by recombination of purified CVs with PM lawns. By pretreating each of these components with NEM prior to reconstitution it should be possible to determine whether the NEM-sensitive protein is located on the CVs, the PM, or both. To this end, the exocytotic capability of RLs that had been prepared with the four possible combinations of NEM-treated and untreated components was tested. In order to be sure that the NEM treatment was sufficient to inactivate the purified components, we chose conditions known to be sufficient to

**Fig. 1.** NEM inhibits exocytosis in CSC and CLs. A. CSC (○—○) in TKME buffer, pH 6.8, was treated with 5 mM NEM at 20°C for the indicated times, as described in Materials and methods. Control CSC (•••) was incubated in the same buffer without NEM. Exocytosis was initiated by diluting samples of the NEM-treated CSC into cuvettes containing a buffer with 1 mM free Ca^{2+}. Percentage reaction was determined by the turbidimetric procedure referred to in Materials and methods, with the turbidity change of the untreated control assigned a value of 100% reaction. Results are the mean±S.D. of triplicate samples, and are representative of three similar experiments. B. Cortical lawns (○—○) were treated with 5 mM NEM in PKME, pH 6.8, buffer for the indicated time at room temperature. Control lawns ( •••) were incubated in the same buffer without NEM. Exocytosis was initiated by drawing a buffer containing 1 mM free Ca^{2+} into the slide chamber. Percentage reaction was determined by light-scattering analysis. Each data point represents the mean±S.D. of triplicate samples. In this and subsequent figures, data points without error bars indicate that the S.D. was less than the size of the data point. Results are representative of three similar experiments.

**Fig. 2.** Activity of RLs prepared from NEM-treated components: comparison with CLs, RLs and NEM-treated RLs. A. The following cortex preparations were tested for exocytotic capability as described in the legend to Fig. 1B: (1) untreated CLs; (2) CLs that had been NEM-treated for 30 min at room temperature; (3) untreated RLs; (4) RLs that had been NEM-treated for 30 min at room temperature; (5) RLs prepared from NEM-treated PM; (6) RLs prepared from NEM-treated CVs; (7) RLs prepared from NEM-treated CVs and NEM-treated PM. B. In addition to their reactivity, the RL samples (A, 3–7) were also analyzed for CV binding by the light-scattering technique described in Materials and methods. Column 1, Untreated RLs; 2, RLs that had been NEM-treated for 30 min at room temperature; 3, RLs prepared from NEM-treated PM; 4, RLs prepared from NEM-treated CVs; 5, RLs prepared from NEM-treated CVs and NEM-treated PM. The results presented in A, and in columns 1–5 of B are the mean±S.D. of six to eight determinations from three independent experiments. For comparison, column 6 of B shows that the ΔmV for un-reconstituted PM lawns in CV binding experiments is negligible (1.0±2.6 mV; n=13 experiments). Under these same conditions the ΔmV for native cortical lawns is approximately 4000 mV (see Fig. 1 of Jackson and Modern, 1990).
inactivate cortex preparations. Thus, purified CVs were NEM-treated under conditions (5 mM NEM for 15 min at pH 8.0) that completely inactivate a suspension of cell surface complex (Fig. 1A), and PM lawns were NEM-treated under conditions (5 mM NEM for 30 min at pH 6.8) that completely inactivate CLs (Fig. 1B).

Surprisingly, RLs in which either the PM or the CVs had been NEM-treated retained activity to 1 mM Ca²⁺ (Fig. 2A, columns 5 and 6), whereas RLs in which both the CVs and PM had been NEM-treated were inactive (Fig. 2A, column 7). Controls showed that untreated CLs and RLs were active (Fig. 2A, columns 1 and 3), and that the NEM treatment was sufficient to inhibit CLs and RLs (Fig. 2A, columns 2 and 4). This result suggests that NEM-sensitive protein(s) are present in both purified CV and PM preparations.

Analysis of CV binding revealed that NEM treatment did not inhibit the binding of CVs to PM (Fig. 2B). In fact, in two of three experiments, NEM-treated CVs bound somewhat better than untreated CVs. Untreated CVs bound poorly to NEM-treated PM lawns (Fig. 2A, column 6). However, the few samples that were tested were observed to undergo exocytosis in response to 1 mM Ca²⁺ (data not shown).

In early work with this system (before routine determination of CV turbidity) we compared the binding of CVs prepared from NEM-inactivated CSC. This variation produced CV suspensions that were significantly more dilute than those produced by the standard procedure. RLs formed from these (acute CV suspensions had a small extent of CV binding, and were not thoroughly examined. However, the few samples that were tested were observed to undergo exocytosis in response to 1 mM Ca²⁺ (data not shown).

In the membrane-intact sample (Fig. 3A) hyalin contained in the domes in the center portion of the PM fragment (Fig. 3A, arrows in phase-contrast micrograph) was protected and did not combine with the anti-hyalin antibody. An imperfect seal between the PM and the coverglass allowed labelling of domes at the periphery of the PM fragment (Fig. 3A, arrows in fluorescence micrograph). Most free CVs (i.e., those bound to the coverglass rather than the PM) remained intact, and were not labelled with the anti-hyalin antibody. Detergent disruption of the membrane resulted in heavy labelling of all domes and free CVs (Fig. 3B), thereby confirming the presence of immunoreactive hyalin within these structures. Similar results were obtained with RL samples prepared by recombination of NEM-treated CVs with untreated PM lawns (Fig. 4); hyalin within intact domes was not labelled (Fig. 4A); hyalin within trypsin-digested disrupted domes was heavily labelled (Fig. 4B).

These results are comparable to those obtained with CL (Fig. 5) and untreated RL (Fig. 6) samples that served as positive controls for transfer of CV contents across the PM. The large domes formed from the densely packed CVs of CL samples (Fig. 5A, arrows in phase-contrast micrograph) present a different image than the small domes of proteolized samples (Fig. 5A, arrows in phase-contrast micrograph). Notice, however, that intact domes immediately adjacent to the those that ruptured remained unlabelled. In the detergent-disrupted sample (Fig. 5B) all domes were labelled.

These results provide strong evidence that RLs prepared protein(s) from both membranes probably participate in the release of CV contents from untreated RLs and CLs.

Evidence for transfer of CV contents across the PM

Although untreated CVs and PM seemed to be capable of rescuing their NEM-inactivated counterparts, it was necessary to demonstrate that CV contents were released via exocytosis. The mechanism of release was investigated with an immunofluorescence-based assay that permits visualization of the transfer of CV content proteins across the PM (Crabb and Jackson, 1985). We have previously used this technique to demonstrate that Ca²⁺ stimulation of CLs and RLs results in the transfer of CV contents across the PM (Crabb and Jackson, 1985). When a CV in a CL or RL fuses with the PM, its contents are transferred across the PM into a dome-shaped compartment that is bounded on one side by the egg PM and on the other by the coverglass to which the lawn is attached. Within these compartments, secreted CV components (e.g. hyalin) are protected from exogenously added probes (e.g. antibodies). Immunofluorescence analysis of samples whose membranes have been disrupted by detergent is used to confirm the presence of CV contents within the domes (Crabb and Jackson, 1985).

Analysis of RLs prepared from NEM-treated components by this technique revealed that RLs in which either the CVs or PM had been NEM-treated reacted via exocytosis. Fig. 3 presents paired phase-contrast and fluorescence micrographs obtained with RLs prepared by recombination of NEM-treated PM lawns with untreated CVs. In the membrane-intact sample (Fig. 3A) hyalin contained in the domes in the center portion of the PM fragment (Fig. 3A, arrows in phase-contrast micrograph) was protected and did not combine with the anti-hyalin antibody. An imperfect seal between the PM and the coverglass allowed labelling of domes at the periphery of the PM fragment (Fig. 3A, arrows in fluorescence micrograph). Most free CVs (i.e. those bound to the coverglass rather than the PM) remained intact, and were not labelled with the anti-hyalin antibody. Detergent disruption of the membrane resulted in heavy labelling of all domes and free CVs (Fig. 3B), thereby confirming the presence of immunoreactive hyalin within these structures. Similar results were obtained with RL samples prepared by recombination of NEM-treated CVs with untreated PM lawns (Fig. 4); hyalin within intact domes was not labelled (Fig. 4A); hyalin within trypsin-digested disrupted domes was heavily labelled (Fig. 4B).
Fig. 3. CV content proteins are vectorially transferred across the PM in RLs prepared by recombination of NEM-treated PM with untreated CVs. Reconstituted lawns (RLs) were prepared from NEM-treated PM lawns and untreated CVs. Exocytosis was initiated by dipping the RLs into a buffer containing 1 mM free Ca²⁺. At t=15 s, the reaction was terminated by dipping the samples into a buffer containing 1% glutaraldehyde. The fixed samples were probed for anti-hyalin immunofluorescence with an anti-hyalin monoclonal (1/250 dilution of the anti-hyalin ascites fluid). A. Paired phase-contrast and immunofluorescent images of a reconstituted cortical fragment with intact membranes. Arrows in the phase-contrast image designate hyalin-containing domes that are not labelled with the anti-hyalin antibody. Labelled domes (denoted by arrows in the fluorescence image) are often seen at the circumference of the fragment. These apparently result from an imperfect seal between the coverglass and the membrane fragment. B. Paired phase-contrast and immunofluorescent images of a reconstituted cortical fragment with detergent-disrupted membranes. Results presented in Figs 3–6 are representative samples from two independent experiments. Bar, 10 μm.

Discussion

The ability to prepare reconstituted egg cortex (RLs) by recombination of purified CVs with PM (Crabb and Jackson, 1985; Whalley and Whitaker, 1988) provides a potentially powerful tool for investigation of the molecular mechanism of cortical exocytosis. To make full use of this technology it is important to demonstrate that the mechanism of reaction in RLs is equivalent to that of CLs, CSC and eggs. Several pieces of data support this hypothesis. (1) Binding specificity experiments suggest that reassociation of CVs with PM may be specific, protein-mediated event (Jackson and Modern, 1990). (2) Reassociation has been shown to be a prerequisite for the Ca²⁺-triggered release reaction (Crabb and Jackson, 1985). (3) The Ca²⁺-triggered release reaction results in the vectorial transfer of NEM-treated components, like their untreated CL and RL counterparts, react via an exocytotic mechanism.
CV content proteins are vectorially transferred across the PM in RLs prepared by recombination of NEM-treated CVs with untreated PM. Reconstituted lawns (RLs) were prepared from NEM-treated CVs and untreated PM lawns, as described in Materials and methods. Exocytotic transfer of CV contents across the PM was assessed by anti-hyalin immunofluorescence, as described in the legend to Fig. 3. A. Paired phase-contrast and immunofluorescent images of a reconstituted cortical fragment with intact membranes. Arrows in the phase-contrast image designate hyalin-containing domes that are not labelled with the anti-hyalin antibody. B. Paired phase-contrast and immunofluorescent images of a reconstituted cortical fragment with detergent-disrupted membranes. Bar, 10 μm.

Despite the potentially detrimental dissociative procedures used to prepare RLs, their Ca²⁺ threshold (36 μM), though higher than that of CLs (5 μM), is low enough to be compatible with an exocytotic mechanism of release. To these we can now add the observation that RLs, CLs, CSC and eggs are all susceptible to inhibition by NEM (Figs 1 and 2; and Jackson et al. 1985). Thus, in each case, at least one sulphydryl-containing protein must be involved in the reaction. This finding is consistent with the hypothesis that the reactions are all mechanistically equivalent.
Fig. 5. CV content proteins are vectorially transferred across the PM in cortical lawns (CLs). Exocytotic transfer of CV contents across the PM of CLs was assessed by anti-hyalin immunofluorescence, as described in the legend to Fig. 3. A. Paired phase-contrast and immunofluorescent images of a cortical fragment with intact membranes. Arrows in the phase-contrast image designate large, hyalin-containing domes that are not labelled with the anti-hyalin antibody. Arrows in the fluorescence image designate ruptured domes that are heavily labelled with the anti-hyalin antibody. B. Paired phase-contrast and immunofluorescent images of a cortical fragment with detergent-disrupted membranes. Bar, 10 μm.

Investigation of the activity of RLs prepared from NEM-treated components demonstrated that untreated PM can rescue NEM-treated CVs and that untreated CVs can rescue NEM-treated PM. This surprising finding suggests that functionally equivalent NEM-sensitive protein(s) are present in both the CV and PM lawn preparations. At present it is not clear whether this shared activity is the result of a single protein located on both organelles, or of different yet functionally equivalent proteins. It is also not clear whether these protein(s) are located on both CV membranes and plasma membranes in the intact egg; however, an interesting precedent for colocalization is provided by the sec4 protein of yeast. sec4 mutants are defective in constitutive secretion at a post-Golgi stage (Novick et al. 1980). The sec4 protein has been found to be tightly associated with both PM and secretory vesicles (Goud et al. 1988). Mutational analysis suggests that it regulates vesicular traffic by cycling between the PM and secretory vesicles (Walworth et al. 1989); thus, sec4 is located on both secretory vesicles and PM and is required
Fig. 6. CV content proteins are vectorially transferred across the PM in RLs prepared by recombination of untreated CVs and PM. Exocytic transfer of CV contents across the PM of an RL prepared by recombination of untreated CVs with PM lawns was assessed by anti-hyalin immunofluorescence, as described in the legend to Fig. 3. A. Paired phase-contrast and immunofluorescent images of a reconstituted cortical fragment with intact membranes. Arrows in the phase-contrast image designate hyalin-containing domes that are not labelled with the anti-hyalin antibody. B. Paired phase-contrast and immunofluorescent images of a reconstituted cortical fragment with detergent-disrupted membranes. Bar, 10 μm.

for constitutive exocytosis. Another possibility is that the NEM-sensitive protein(s) may reside at the CV–PM junction. Dislodgement of CVs from the PM could then result in the distribution of the protein(s) to both membranes. Alternatively, in the intact egg, the protein(s) could be exclusively located on one membrane and become redistributed to the other during fractionation.

The rescue experiments also suggest that NEM inhibition does not result from steric inhibition of CV–PM contact. Steric inhibition should be dominant, i.e. the steric constraints preventing membrane contact should not be removed by supplying an unmodified partner. The fact that unmodified fractions can restore function implies that the unmodified fraction is capable of actively promoting exocytosis, i.e. it supplies an essential function. On the other hand, RLs prepared from NEM-treated components could react via a qualitatively different mechanism than CLs and untreated RLs, but the results of the vectorial
transfer experiments (Figs 3–6) suggest that this is not the case. Rather, it seems that both CVs and the PM carry proteins capable of promoting exocytosis. This is consistent with the observation that large aggregates of CVs can fuse with each other, even in the absence of exogenously added PM (Crabb and Jackson, 1985). It is also consistent with the phenomenon of compound exocytosis, in which secretory vesicles fuse with each other, as well as with the PM, in cells that undergo massive and concerted exocytic reactions, e.g. mast cells (Rohlch et al. 1971), parotid acinar cells (Amsterdam et al. 1989) and eggs (Chandler, 1984).

The precise role of NEM-sensitive protein(s) in cortical exocytosis cannot be determined from the data that are currently available. The simplest hypothesis that accommodates the data suggests that the NEM-sensitive protein(s) may provide an essential function; however, the observation that mild proteolysis can reverse NEM inhibition of CSC (Jackson et al. 1985), CL and RL samples (Jackson and Modern, unpublished results) indicates that the sensitive sulphydryl group is not at the active site. It is likely that it is located on a regulatory domain that is non-functional (i.e. inhibitory) when modified. The putative regulatory domain could be either covalently or non-covalently associated with the domain that is essential for exocytosis. Thus the NEM-sensitive protein(s) must either supply an essential function or be so closely associated with an essential protein that modification of the NEM-sensitive protein(s) interferes with the activity of the essential protein. It is tempting to speculate that the NEM-sensitive protein(s) of egg cortex may be related to 78000 M_r NEM-sensitive factor (NSF) identified by Rothman and his colleagues (Block et al. 1989). NSF has been shown to be required for membrane fusion steps at several stages of the secretory (Glick and Rothman, 1987; Beckers et al. 1989) and endocytic pathways (Diaz et al. 1989), but its precise role in these events is also unknown. In any case, identification and characterization of the NEM-sensitive protein(s) of egg cortex is clearly essential for the understanding of the molecular mechanism of regulated exocytosis in the egg, and perhaps in other cell types as well.

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References


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