Mild Proteolytic Digestion Restores Exocytotic Activity to N-Ethylmaleimide-inactivated Cell Surface Complex from Sea Urchin Eggs

ROBERT C. JACKSON, KAREN K. WARD, and JOHN G. HAGGERTY
Department of Biochemistry, Dartmouth Medical School, Hanover, New Hampshire 03756. Dr. Haggerty’s present address is Department of Human Genetics, Yale University School of Medicine, New Haven, Connecticut 06510. Address reprint requests to Dr. Jackson.

ABSTRACT The Ca\(^{2+}\)-stimulated release of cortical vesicle (cortical granule) contents from the cell surface complex (CSC) of the sea urchin egg is an in vitro model for exocytosis. To gain insight into the molecular mechanism of exocytosis we investigated the sensitivity of this model to sulfhydryl modification and proteolytic digestion. Our findings include the following: (a) Proteolytic treatment with trypsin or pronase of CSC prepared from the eggs of Strongylocentrotus purpuratus increased the free Ca\(^{2+}\) concentration required to elicit exocytosis. Although a small increase in the Ca\(^{2+}\) threshold was detected after mild proteolysis, high concentrations of trypsin (0.5 mg/ml) and prolonged incubation (3 h) were required to render the CSC unresponsive to high concentrations of Ca\(^{2+}\) (0.5 mM). Despite the severity of the proteolytic digestions required to inactivate the CSC, the individual cortical vesicles remained intact, as gauged by the latency of ovoperoxidase, a cortical vesicle enzyme. (b) As previously shown (Haggerty, J. C., and R. C. Jackson, 1983, J. Biol. Chem. 258:1819–1825), cortical exocytosis can be blocked by sulfhydryl-modifying reagents such as N-ethylmaleimide (NEM). In this report we demonstrate that NEM inhibits by increasing the Ca\(^{2+}\) threshold required for exocytosis. When CSC that had been completely inactivated by NEM modification was briefly digested, on ice, with a low concentration of trypsin (or several other proteases), exocytotic activity was restored. Although the Ca\(^{2+}\) threshold of the reactivated CSC was slightly higher than that of untreated CSC, it was nearly identical to that of control CSC, which was trypsinized but not treated with NEM. We discuss the significance of these results with regard to the molecular mechanism of exocytosis.

Cell surface complex (CSC)\(^1\) prepared from the eggs of the sea urchin Strongylocentrotus purpuratus is comprised of the vitelline layer, the plasma membrane and attached cortical secretory vesicles (CVs), also referred to as cortical granules (1). In response to physiologically relevant concentrations of free Ca\(^{2+}\) the CSC undergoes a reaction that morphologically approximates exocytosis (2–4). This response can be followed microscopically (2–5), enzymatically (6, 7), and turbidimetrically (6, 8). Recent investigations have shown that exocytosis in this in vitro model system can be inhibited by phenothiazine drugs (6, 7, 9) and by sulfhydryl-modifying reagents such as N-ethylmaleimide (NEM; reference 6). Inhibition by sulfhydryl-modifying reagents suggested that proteinaceous elements of the CSC were required for cortical exocytosis and prompted us to investigate the effect of proteolytic digestion of CSC and NEM-inhibited CSC on cortical exocytosis. We show that cortical exocytosis can be completely inhibited by prolonged proteolytic digestion. We also demonstrate that activity can be restored in NEM-inactivated CSC by mild proteolytic digestion. Preliminary results of this work have been published in abstract form (10).

\(^1\) Abbreviations used in this paper: CSC, cell surface complex; CV, cortical vesicle; DFP, diisopropylfluorophosphate; NEM, N-ethylmaleimide; PKME, buffer made with PIPES, KCl, MgCl\(_2\), and EGTA; SBTI, soybean trypsin inhibitor.
MATERIALS AND METHODS

Preparation of CSC: S. purpuratus were maintained at 12-15°C in Instant Ocean artificial seawater and spawned by intracoelomic injection of 0.5 M KCl. CSC was prepared from eggs that had been dejellied by two washes with 502 mM NaCl, 10 mM KCl, 2.5 mM NaHCO₃, 25 mM MgCl₂, pH 8.0. The eggs were washed once more with 50 mM piperase-N,N'-bis(2-ethanesulfonic acid) (PIPES), 425 mM KCl, 25 mM EGTA, pH 6.8, and resuspended in this buffer for homogenization. Subsequent steps performed were as previously described (6) except that protease inhibitors were not included and 50 mM Pipes (pKa 6.8) replaced 50 mM HEPES (N-2-hydroxyethyl piperezine-N'-2-ethanesulfonic acid, pKa 7.5) in all buffers.

Inactivation of CSC by Sulfhydryl Modification: CSC was inactivated by reaction with NEM as follows: enough NEM (100-200 mM in 0.5 M KCl) to achieve the desired reagent concentration was added to aliquots of the concentrated stock CSC suspension (A₄₀₀ ~ 20) in PKME buffer (50 mM Pipes, 425 mM KCl, 10 mM MgCl₂, 10 mM EGTA, pH 6.8) and allowed to react at 20-21°C. After the desired incubation period, the reaction mixtures were assayed for cortical exocytosis or were cooled by immersion in an ice bath for 5 min, treated with protease as specified, and then assayed for cortical exocytosis by the turbidimetric or peroxidase latency assays.

Assays for Cortical Exocytosis: We performed turbidity assays essentially as previously described (6). In brief, 50 µl of a CSC suspension was diluted into 950 µl of PKME buffer containing the desired concentration of CaCl₂ and H₂O₂, and assayed for cortical exocytosis. The final free Ca²⁺ concentration of 12 µM. Trypsin was inhibited by the inclusion of 50 µg/ml of SBTI in all assay buffers. Each data point is the mean of three determinations with an average deviation ≤ 0.05, except where indicated by error bars. In B, the effect of mild trypsin digestion on cortical exocytosis. In A, CSC was incubated with 50 µg/ml of trypsin. At the indicated times, CSC from both samples was assayed by the turbidimetric procedure at a final free Ca²⁺ concentration of 12 µM. Trypsin was inhibited by the inclusion of 50 µg/ml of SBTI in all assay buffers. Each data point is the mean of three determinations with an average deviation ≤ 0.05, except where indicated by error bars. In B, the effect of mild trypsin digestion on cortical exocytosis. In A, CSC was incubated with 50 µg/ml of trypsin. At the indicated times, CSC from both samples was assayed by the turbidimetric procedure at a final free Ca²⁺ concentration of 12 µM. Trypsin was inhibited by the inclusion of 50 µg/ml of SBTI in all assay buffers. Each data point is the mean of three determinations with an average deviation ≤ 0.05, except where indicated by error bars.
8 THE JOURNAL OF CELL BIOLOGY - VOLUME 101, 1985

Proteolytic Inactivation of Cortical Exocytosis

Since the cytoplasmic surface of CSC is accessible to the medium, this preparation provides a unique opportunity to investigate the effects of membrane impermeant, protein-specific reagents, such as proteases, on exocytosis. As shown in Fig. 1 A, cortical exocytosis at 12 μM free Ca\(^{2+}\) was completely inhibited by digestion of CSC with trypsin. DFP-inactivated trypsin was not inhibitory under these conditions (data not shown). Fig. 1 B shows that the threshold Ca\(^{2+}\) concentration required for exocytosis is related to the extent of trypsic digestion. In this experiment CSC was treated with 0, 50, 100, or 250 μg/ml of trypsin for 45 min on ice. Trypsin digestion was stopped with SBTI and cortical exocytosis was tested at the indicated free Ca\(^{2+}\) concentrations. As the severity of the trypsin digestion increased, the threshold free Ca\(^{2+}\) concentration required for exocytosis was progressively shifted to higher values. The only morphological change detected by phase contrast microscopy after mild trypsic digestions such as those of Fig. 1 was a small

![Diagram](image_url)

**Figure 2** The Ca\(^{2+}\) threshold for cortical exocytosis slowly increases during incubation. CSC was incubated on ice (A) or at 20-21°C (B). At the indicated times, 50-μl aliquots of CSC were removed and assayed for cortical exocytosis at the indicated free Ca\(^{2+}\) concentration by the turbidimetric procedure. In A, the times of incubation on ice were 0.0 (--●--), 2.75 (--■--), 4.75 (--△--), and 22.75 h (--□--). Duplicates deviated from the mean by <0.04, except where indicated by error bars. In B, the times of incubation at 20-21°C were 0.0 (--●--), 0.5 (--■--), 1.0 (--△--), 4.0 (--○--), and 7.0 h (--□--). The range of duplicates was <0.048.

![Diagram](image_url)

**Figure 3** Effect of extensive trypsin digestion on cortical exocytosis. In A, CSC in PKM\(_{2}\)EGTA (A\(_{2000}=22.0\), 1.5 mg/ml protein) was incubated with (- - - - - - - -) or without (- - - - - - - -) 500 μg/ml of trypsin at 20°C. At the indicated times CSC from both samples was assayed in triplicate by a variation of the turbidimetric procedure (see Experimental Procedures) at a final free Ca\(^{2+}\) concentration of 0.5 mM. Trypsin was inhibited by the inclusion of SBTI (100 μg/ml) in the assay buffer. Average deviations were <0.03 except where indicated by error bars. In B, the effect of extensive trypsin digestion on the latency of the CV peroxidase was determined. CSC was treated with (columns 4-6) or without (columns 1-3 and 7-9) 0.5 mg/ml trypsin as in A. Digestion was initiated at t = 0 or 8 h by diluting triplicate 50-μl aliquots of both samples into 450 μl of PKM\(_{2}\)EGTA containing 100 μg/ml of SBTI. CVs were disrupted (columns 3, 6, 9, and 12) by the addition of sodium deoxycholate to a final concentration of 0.148% (wt/vol). Cortical exocytosis was initiated (columns 2, 5, 8, and 11) by the addition of 5 μl of 250 mM CaCl\(_2\) in PKM\(_{2}\)EGTA (final free Ca\(^{2+}\), 91 μM, pH 6.65). Peroxidase latency was determined by iodination as described in Experimental Procedures. Crosshatched bars, CSC, no CaCl\(_2\). Open bars, deoxycholate disrupted CSC, no CaCl\(_2\). Filled bars, CSC, 91 μM free Ca\(^{2+}\). The results presented are the average of two experiments (six determinations). Error bars represent the average deviation of the mean. Those samples receiving trypsin are designated 7. Hours of incubation (0 or 8) is also indicated.
decrease in the size of the CSC fragments. There was no significant accumulation of free CVs.

The data presented in Fig. 2A show that the threshold Ca\(^ {2+} \) concentration required for exocytosis is not altered by the incubation conditions used in Fig. 1. The Ca\(^ {2+} \) concentration required for half-maximal reaction of freshly prepared CSC (4.5 \( \mu \)M\(^2 \)) remained unchanged during 4 h of incubation on ice. After prolonged incubation (22.75 h), the free Ca\(^ {2+} \) concentration required for half-maximal reaction increased to 43 \( \mu \)M. This time-dependent increase in the Ca\(^ {2+} \) threshold occurred more rapidly at 20°C; however, even after 7 h of storage at 20°C the CSC could be maximally stimulated by 0.47 mM free Ca\(^ {2+} \). Note that the time-dependent inactivation process that we observed in our CSC preparations is much slower than the rapid "autoinactivation" of cortical lawn preparations (3, 7). Recent results from this laboratory (16) demonstrate that the rapid "autoinactivation" of cortical lawn preparations can be attributed to the inhibitory action of the polycationic substances (polysine or protamine) used to affix the cortical lawns to their coverslip or petri dish.

Since relatively mild tryptic digestion had no effect on exocytosis at millimolar free Ca\(^ {2+} \) levels (Fig. 1) we increased the severity of the digestion conditions. Fig. 3A shows that a CSC suspension slowly lost its ability to respond to 0.5 mM free Ca\(^ {2+} \) when treated with 0.5 mg/ml of trypsin at 20–21°C. At this concentration of trypsin (a 1:3 ratio of trypsin to endogenous CSC protein in this experiment) the CSC was >90% Ca\(^ {2+} \) insensitive by 3 h of digestion, whereas CSC incubated simultaneously without trypsin maintained its Ca\(^ {2+} \) sensitivity to 0.5 mM Ca\(^ {2+} \) for the entire 8 h of incubation (Fig. 3A). In a control experiment (data not shown) CSC treated with DFP-inactivated trypsin responded identically to untreated CSC, maintaining its sensitivity to Ca\(^ {2+} \) over 10 h of incubation, whereas trypsin-treated CSC was inactive after 3 h. Therefore, it was the proteolytic activity of the trypsin rather than a possible contaminant that was responsible for the observed loss of exocytotic activity. CSC was also inactivated by pronase digestion, which, like trypsin digestion, resulted in a progressive shift to higher Ca\(^ {2+} \) thresholds (data not shown).

Due to the severity of the proteolytic digestions required to inactivate CSC, it became important to determine whether the integrity of individual CVs was maintained during prolonged incubation. During an 8 h incubation with trypsin the

\(^2\)The lower free Ca\(^ {2+} \) value for half-maximal reaction that we previously reported (6) was the result of inadequately buffered CaCl\(_2\) solutions.
morphology of individual CSC fragments as viewed by phasecontrast microscopy changed from large, nearly egg-sized, fragments to what appeared to be individual CVs. The morphology of CSC in a control suspension broke down from large to relatively small fragments, but we observed few free CVs (data not shown). To gauge the integrity of the CVs, we assayed the latency of ovoperoxidase, a CV enzyme (17, 18), in the presence and absence of 0.5 mM free Ca$^{2+}$ or 0.3% sodium deoxycholate (Fig. 3B). With control CSC the level of iodine incorporation induced by 0.5 mM free Ca$^{2+}$ was almost identical to that produced by deoxycholate disruption both initially (Fig. 3B, columns 2 and 3) and after 8 h of incubation (Fig. 3B, columns 8 and 9). Trypsin digestion (indicated by a T in Fig. 3B) completely abolished Ca$^{2+}$-induced $^{125}$I-iodine incorporation (Fig. 3, column 11) without decreasing the deoxycholate induced iodine incorporation (Fig. 4, column 12). This result confirmed the turbidity data presented above and demonstrated that after prolonged incubation with a substantial concentration of trypsin the bulk of the CVs remained intact. Hence, trypsin inactivation of cortical exocytosis occurred without permeabilizing the CV membrane or destroying CV contents.

**NEM Modification Increases the Threshold Ca$^{2+}$ Concentration Required for Exocytosis**

We previously showed that the sulfhydryl-modifying reagent NEM inhibits exocytosis in CSC prepared from the eggs of *S. purpuratus* (6). Fig. 4 demonstrates that NEM, like trypsin, inhibits by progressively shifting the Ca$^{2+}$ threshold to higher free Ca$^{2+}$ concentrations. In this experiment CSC was treated for 30 min at 20-21°C with 0, 0.1, 1.0, 5.0, or 10.0 mM NEM and then tested for cortical exocytosis by the turbidimetric assay. Under these conditions, 5 to 10 mM NEM was required to inhibit the response of CSC to millimolar levels of free Ca$^{2+}$, whereas lower concentrations of NEM were effectively inhibitory when assayed at submillimolar free Ca$^{2+}$. Comparison of the control sample that had been incubated at 20°C for 30 min (curve 0.0) with the sample that had remained on ice (curve I) illustrates the small extent of inactivation of the CSC that occurred during the 30-min incubation at 20°C. CSC morphology, as assessed by phasecontrast microscopy, was not altered by the NEM modification conditions used in this experiment. The size of the CSC fragments was similar to those in an untreated control sample, and the CVs were not released from the plasma membrane.

**Mild Protease Digestion Restores Ca$^{2+}$ Sensitivity to NEM-inactivated CSC**

Since proteins are the primary target of sulfhydryl-modifying reagents, we sought to relate the proteolysis results presented above to our NEM inactivation results. Specifically, we were interested in whether the observed proteolytic inactivation could be due to digestion of a sulfhydryl-containing protein. However, to our surprise, we discovered that mild proteolysis could restore activity to NEM-modified CSC.

The results of a typical reactivation experiment are shown in Fig. 5A. In this experiment CSC that had been treated for 30 min at 20°C, either with or without 10 mM NEM, was digested with 15 or 50 μg/ml of trypsin for 15 min on ice and assayed for exocytosis at the indicated free Ca$^{2+}$ concentrations. In Fig. 5A, curve N illustrates that the NEM-modification effectively inhibited cortical exocytosis. Curve C represents the control sample that was incubated for 30 min at 20°C without NEM and not treated with trypsin. Comparison of curve C with curves $T_{15}$ and $T_{30}$ illustrates that mild trypsinization (with 15 or 50 μg/ml trypsin) increased the Ca$^{2+}$ threshold only slightly. Comparison of curves $NT_{15}$ and $NT_{30}$ with curve N demonstrates that mild trypsinization could restore exocytotic activity to NEM-inhibited CSC. The Ca$^{2+}$ threshold of the reactivated CSC depended on the digestion conditions. Digestion of NEM-inhibited CSC with 50 μg/ml of trypsin for 15 min on ice (Fig. 5A, curve $N_{15}$) restored the reactivated CSC to nearly the same Ca$^{2+}$ threshold as control CSC that had been treated with trypsin only (Fig. 5A, curve $T_{15}$). Digestion with 15 μg/ml trypsin was not as effective in this respect (compare curves $NT_{15}$ and $T_{15}$, Fig. 5A).

In a control experiment, NEM-modified CSC, which had been incubated for 15 min on ice with no additions, or with 50 μg/ml trypsin or 50 μg/ml DFP-activated trypsin, was tested for exocytosis. The trypsin digested sample was reactivated, as in Fig. 5A, whereas the untreated and DFP-trypsin treated samples were inactive throughout the entire range of free Ca$^{2+}$ concentrations tested (data not shown). The ability of trypsin to reactivate NEM-modified CSC was confirmed with the peroxidase latency assay. The level of iodine incorporated into control and trypsin reactivated CSC was nearly identical when assayed with 1.4 mM free Ca$^{2+}$ (Fig. 5B). Phase-contrast microscopy of trypsin reactivated CSC revealed that trypsin digestion decreased the size of the CSC fragments but did not release CVs from the plasma membrane.

**DISCUSSION**

Inhibition of cortical exocytosis by NEM modification (Fig. 4) or by trypsin digestion (Figs. 1 and 3) strongly suggests that proteinaceous elements are involved in the exocytotic process. The mode of inhibition by trypsin and NEM is noteworthy. Under mild conditions, both NEM (Fig. 4) and trypsin (Fig. 1) inhibit cortical exocytosis by increasing the threshold free Ca$^{2+}$ concentration required for exocytosis, without affecting the magnitude of the response at higher Ca$^{2+}$ concentrations. When the NEM modification or trypsin digestion conditions are more severe, exocytosis is blocked even at high Ca$^{2+}$ concentrations (Figs. 3 and 4).

This mode of inactivation suggests that the protein(s) and sulfhydryl group(s) responsible for inhibition are involved in the mechanism that imparts micromolar Ca$^{2+}$ sensitivity to the exocytotic apparatus. It also suggests that inactivation is not a single-hit phenomenon, in which each hit completely inactivates a single exocytotic unit (presumably a CV). If inactivation were a single hit phenomenon, it should proceed by a decrease in the magnitude of exocytosis, with no change in the Ca$^{2+}$ threshold. The observed mode of inactivation suggests that each exocytotic unit (or CV) must be modified several times before it is rendered totally inactive. At the molecular level this implies that only a small fraction of the total number of Ca$^{2+}$-sensitizing proteins of each exocytotic unit are required for exocytosis. Inactivation of a fraction of the total number of Ca$^{2+}$-sensitizing proteins increases the proportion of active Ca$^{2+}$-sensitizing proteins that must be
recruited to promote exocytosis. The net result is an increase in the Ca\(^{2+}\) threshold without a concomitant decrease in the magnitude of exocytosis at high Ca\(^{2+}\) concentrations. There are two significant aspects of this observation. First, it indicates that there are many copies of the target protein per CV (an important consideration for investigators interested in identifying and purifying this component), and, second, it suggests a convenient mechanism by which the Ca\(^{2+}\) sensitivity of the exocytotic apparatus may be modulated (i.e., by altering the fraction of active proteins per vesicle, perhaps through a reversible covalent modification such as phosphorylation).

These observations are consistent with the recent report by Sasaki (19) that cortical lawns prepared from the eggs of *Hemicentrotus pulcherremitus* contain a KCl-extractable proteinaceous factor that is required for exocytosis at micromolar free Ca\(^{2+}\) concentrations (the sensitivity of this factor to NEM modification was not determined). Although the Ca\(^{2+}\) threshold of CSC prepared from *S. purpuratus* eggs was not altered by brief extraction with KCl, (Jackson, R. C., unpublished observation), the fact that trypsin shifts the Ca\(^{2+}\) threshold to higher values suggests that a proteinaceous element(s) confers micromolar Ca\(^{2+}\) sensitivity in this species as well.

We have demonstrated that cortical exocytosis can be inhibited by pretreating CSC with a variety of sulphydryl-modifying reagents (6), and have shown that NEM treated eggs are exocytotically incompetent, i.e., they fail to raise fertilization envelopes when incubated with Ca\(^{2+}\) ionophore (A23187) in Ca\(^{2+}\)-containing seawater (Jackson, R. C., and K. Ward, unpublished results). These results raised the possibility that an essential sulphydryl group may be required for exocytosis. Recent reports that the Ca\(^{2+}\) stimulated exocytosis release of catecholamines from adrenal chromaffin cells that had been permeabilized by electric shock or digitonin treatment is also inhibited by NEM (20, 21), suggests that our observations may not be unique to invertebrate exocytosis.

Insight into the role of sulphydryls in cortical exocytosis is provided by the observation that NEM-inactivated CSC can be reactivated by mild proteolysis (Fig. 5). The Ca\(^{2+}\) threshold of the reactivated CSC depends on the degree of proteolytic digestion but under optimal conditions approaches that of control CSC (Fig. 5A). These results may be interpreted in at least two ways: (a) a sulphydryl-dependent, NEM-sensitive step that can be mimicked or circumvented by mild proteolysis is required for cortical exocytosis; or (b) the sulphydryl group(s) is not itself required for exocytosis, but its modification by NEM renders it inhibitory due to steric interference with the exocytotic reaction. Mild tryptic digestion could restore exocytotic activity to NEM-treated CSC by removing this putative NEM-modified inhibitory domain. The second interpretation is interesting, because it is quite possible that the protein that bears the inhibitory domain is a functional element of the exocytic machinery. The possibility that proteolysis mimics the NEM-sensitive element (presumably a Ca\(^{2+}\)-activated sulphydryl protease) seems unlikely since several unrelated proteases (trypsin, chymotrypsin, prionase, and subtilisin) can reactivate NEM-modified CSC, and since cortical exocytosis is not inhibited by a variety of protease inhibitors, including 1,10-o-phenanthroline, leupeptin, pepstatin, DFP, antipain, and Ep-475 (Jackson, R. C., and K. Ward, unpublished results).

Although these results suggest that trypsin does not mimic an endogenous CSC protease, we have not ruled out the possibility that proteolytic digestion may nonspecifically circumvent a required step. For instance, proteolysis may nonspecifically reanimate an NEM-inhibitable CSC enzyme that is required for exocytosis and normally activated by micromolar free Ca\(^{2+}\). Precedents for the nonspecific proteolytic activation of enzymes are available. In fact, many calmodulin regulated enzymes, e.g., myosin light chain kinase (22), cyclic nucleotide phosphodiesterase (23), and calcineurin (24), can be activated and rendered independent of Ca\(^{2+}\) by the nonspecific proteolytic digestion of their calmodulin binding domains. Though it is not regulated by calmodulin, protein kinase C is another Ca\(^{2+}\)-requiring enzyme that can be nonspecifically activated by mild proteolysis (25) and is potentially relevant to exocytosis.

We thank Dr. Bill Culp for allowing us to use his Zeiss universal microscope, Dr. Stan Froehner, Carol Vater, and Joe Crabb for critically reading a preliminary form of this manuscript, and Suzanne Bogle for typing the manuscript.

This research was supported by grant GM26763 from the National Institutes of Health.

Received for publication 12 November 1984, and in revised form 14 March 1985.

REFERENCES

1. Detering, N. K., G. L. Decker, E. D. Schmell, and W. J. Lennarz. 1977. Isolation and characterization of plasma membrane-associated cortical granules from sea urchin eggs. *J. Cell Biol.* 75:899-914.
2. Vacquier, V. D. 1975. The isolation of intact cortical granules from sea urchin eggs. *Dev. Biol.* 45:327-329.
3. Baker, P. F., and M. J. Whitaker. 1978. Influence of ATP and calcium on the cortical reaction in sea urchin eggs. *Nature (Lond.)* 276:513-515.
4. Decker, G. L., and W. J. Lennarz. 1979. Sperm binding and fertilization envelope formation in a cell surface complex isolated from sea urchin eggs. *J. Cell Biol.* 81:92-103.
5. Steinhardt, R. A., and J. A. Alderton. 1982. Calmodulin confers calcium sensitivity on secretory exocytosis. *Nature (Lond.)* 295:154-155.
6. Haggerty, J. G., and R. C. Jackson. 1983. Release of granule contents from sea urchin egg cortex: new assay procedures and inhibition by sulphydryl-modifying reagents. *J. Biol. Chem.* 258:1819-1825.
7. Moy, G. W., G. S. Kopf, C. Gache, and V. D. Vacquier. 1983. Calcium-mediated release of glucanase activity from cortical granules of sea urchin eggs. *Dev. Biol.* 100:267-274.
8. Sasaki, H., and D. E. Yel. 1983. Cortical vesicle exocytosis in isolated cortical granules of sea urchin eggs: description of a turbidimetric assay and its utilization in studying effects of different media on discharge. *Dev. Biol.* 98:327-337.
9. Baker, P. F., and M. J. Whitaker. 1979. Trifluoperazine inhibits exocytosis in sea urchin eggs. *J. Physiol. (Lond.)* 298:55P. (Abstr.)
10. Haggerty, J. G., and R. C. Jackson. 1983. Effects of proteolysis on the calcium-stimulated release of granule contents from the cell surface complex of sea urchin eggs. *J. Cell Biol.* 95:5, Pt. 21:25a. (Abstr.)
11. Wahlb, D. A., 1979. Trypsinogenes and trypsin of various species. Methods Enzymol. N.XIX:41-63.
12. Raper, T. J. 1978. Refinement of the Coomassie Blue method of protein quantitation. *Anal. Biochem.* 86:142-146.
13. Sasaki, H., and D. E. Yel. 1983. Cortical vesicle exocytosis in isolated cortical granules of sea urchin eggs. *Dev. Biol.* 98:327-337.
14. Knight, D. E., and P. F. Baker. 1982. Calcium-dependence of catecholamine release from bovine adrenal medullary cells after exposure to intense electric fields. *J. Membr. Biol.* 68:107-140.
15. Wilson, S. P., and M. Kirschner. 1983. Calcium-evoked secretion from digitonin-permeabilized adrenal medullary chromaffin cells. *J. Biol. Chem.* 258:4994-5000.
16. Bremel, R. D., and M. E. Shaw. 1978. Conversion of calcium-sensitive myosin light chain kinase to a calcium-insensitive form. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 88:242-246.
17. Kneitz, M. H., J. H. Huiech, A. Rhoads, and C. B. Klee. 1984. Reversible and irreversible activation of cyclic nucleotide phosphodiesterase: separation of the regulator and catalytic domains by limited proteolysis. *J. Biol. Membr.* 149:938-953.
18. Katsura, S., and A. Tominga. 1974. Peroxidatic activity of catalase in the cortical granules of sea urchin eggs. *Dev. Biol.* 40:292-297.
19. Sasaki, H. 1984. Modulation of calcium sensitivity by a specific cortical protein during sea urchin egg cortical vesicle exocytosis. *Dev. Biol.* 101:125-135.
20. Night, D. E., and P. F. Baker. 1982. Calcium-dependence of catalase release from bovine adrenal medullary cells after exposure to intense electric fields. *J. Membr. Biol.* 68:107-140.
21. Wilson, S. P., and M. Kirschner. 1983. Calcium-evoked secretion from digitonin-permeabilized adrenal medullary chromaffin cells. *J. Biol. Chem.* 258:4994-5000.
22. Bremel, R. D., and M. E. Shaw. 1978. Conversion of calcium-sensitive myosin light chain kinase to a calcium-insensitive form. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 88:242-246.
23. Kneitz, M. H., J. H. Huiech, A. Rhoads, and C. B. Klee. 1984. Reversible and irreversible activation of cyclic nucleotide phosphodiesterase: separation of the regulatory and catalytic domains by limited proteolysis. *Adv. Cyclic Nucleotide Res.* 16:31-47.
24. Manalan, A. S., and C. B. Klee. 1984. Activation of calmodulin by limited proteolysis. *Proc. Natl. Acad. Sci. USA.* 81:491-495.
25. Mori, T., Y. Takai, R. Minakuchi, B. Yu, and Y. Nishizuka. 1980. Inhibitory action of cholorpromazine, dibucaine and other phospholipid interacting drugs on calcium-activated phospholipid-dependent protein kinase. *J. Biol. Chem.* 255:8374-8380.