Paramecium Secretory Granule Content: Quantitative Studies on In Vitro Expansion and Its Regulation by Calcium and pH

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ABSTRACT Ca²⁺-dependent secretion in Paramecium involves the exocytic release of a para-crystalline secretory product, the trichocyst matrix, which undergoes a characteristic structural change from a highly condensed storage form (Stage I) to an extended needle-like structure (Stage III) during release. We studied trichocyst matrix expansion in vitro to examine factors regulating the state of secretory organelle content. A new method for the isolation of membrane-free, condensed (Stage I) trichocyst matrices is described. These highly purified, condensed matrices were used to develop a rapid quantitative, spectrophotometric assay for matrix expansion to examine factors regulating the Stage I to Stage III transition. Expansion from Stages I to III was elicited in vitro by addition of Ca²⁺ and we found that at neutral pH, expansion required a Ca²⁺ concentration slightly above 10⁻⁶ M. Previous studies indicate that calmodulin (CaM) antagonists inhibit matrix expansion in vivo. However, in vitro matrix expansion is normal even when trichocyst matrices are preincubated in CaM antagonists before stimulation. Thus, matrix components themselves are unlikely to be the site of CaM antagonist action in vivo. In vitro matrix expansion is also modulated by pH. Decreasing pH to 6.0 inhibits expansion, i.e., expansion requires higher Ca²⁺ concentration. Conversely, increasing pH to >7.0 promotes expansion, allowing it to occur at a lower Ca²⁺ concentration. The pH sensitivity of the Ca²⁺ binding sites of the matrix suggests that, in vivo, the interior of the trichocyst vesicle may be maintained at an acidic pH. Exposure of cells to acridine orange, a fluorescent amine that accumulates in acidic intracellular compartments, leads to its uptake and concentration within trichocysts. Thus intratrichocyst pH appears to be acidic in vivo and may serve as a regulatory or “safety” mechanism to inhibit premature expansion.

The involvement of the Ca²⁺-dependent regulatory protein, calmodulin (CaM), in stimulus-secretion coupling has been suggested in studies of various secretory systems. The precise role of CaM, however, has been difficult to assess. The ciliated protozoan, Paramecium, was recently used to investigate the role of CaM in exocytosis (10). In these cells, thousands of membrane-bounded secretory organelles known as trichocysts are positioned in the cell cortex at defined secretory sites. Release of the secretory product, the trichocyst matrix (tmx), follows stimulation and involves two separable Ca²⁺-dependent steps: the fusion of trichocyst and plasma membranes to create the exocytic opening, and the expansion of the tmx from its highly condensed resting form (Stage I) to an elongated, needle-like secreted form (Stage III).

Earlier studies demonstrated that two structurally different CaM antagonists reversibly inhibit secretion (10). Ultrastructural examination of these cells revealed that a specific Ca²⁺-dependent step in the release process, expansion of the tmx, is inhibited. We suggested that matrix expansion is blocked in vivo because CaM antagonists limit the access of Ca²⁺ to the matrix.

However, a possible mechanism for CaM antagonist action that had not been ruled out is the direct interaction of these agents with the tmx itself. In this study, we examined the mechanism of tmx expansion and its regulation. Matrix expansion was examined in vitro using preparations of isolated, membrane-free condensed trichocysts. We describe a novel...
purification scheme for tmx that yields highly purified Stage I matrices; these matrices were free of subcellular particles or cell fragments. This purified tmx preparation was used to develop a rapid, quantitative, spectrophotometric assay for expansion based on the turbidity change that accompanies the Stage I-Stage III (condensed-expanded) transition. Using this assay, we have made the first accurate determination of the Ca\(^{2+}\) concentration necessary to induce expansion. We have demonstrated that matrix components per se are unaffected by CaM antagonists and can therefore be eliminated as a site of action for these agents in vivo. In addition, the effect of alterations in pH on Ca\(^{2+}\)-induced in vitro expansion were examined. We show that at low pH (~ 6.0) expansion requires higher Ca\(^{2+}\) concentration whereas at pH > 7.0, expansion is facilitated (i.e., it occurs at a lower Ca\(^{2+}\) concentration). The pKa of pH in regulating expansion in vivo was examined using acridine orange, a fluorescent amine that accumulates in acidic intracellular compartments (18). We found that brief incubation of Paramecium in acridine orange led to its uptake and concentration within trichocysts. This suggests that, in vivo, intrachocyst pH is acidic, and that a primary function of low intragranule pH may be to maintain secretory products in their storage form.

Results of in vivo (10) and in vitro studies on the effects of CaM antagonists, Ca\(^{2+}\), and pH on the regulation of matrix expansion and release in Paramecium are synthesized into a working hypothesis. This model suggests that similar strategies for secretory product storage and release have been maintained through evolution from progenitor cells such as Paramecium to more specialized secretory cells in higher organisms.

MATERIALS AND METHODS

Culture Conditions: Cell cultures of Paramecium tetraurelia, wild type, were grown at 27°C in bacterized monoxenic (Enterobacter aerogenes) Cerophyle medium (31) (Cerophyl Laboratories, Inc., Kansas City, MO), and generally harvested at late log phase (3,000-4,000 cells/ml).

Purification of Stage I tmx: Purification of tmx was carried out using a modification of a procedure of Matt et al. (20). Late log-phase cells (1 liter) were harvested, washed twice in homogenization medium (HM) (20 mM Tris, 100 mM KCI, 5 mM EGTA [pH 7.0]), and the washed pellet of cells was resuspended to 3 ml in HM. Cells were allowed to stand in HM at room temperature for 15 min, and then homogenized on ice (50 to 100 strokes) in a tight-fitting glass Dounce homogenizer. The homogenate was diluted to 6 ml with HM, and centrifuged for 5 min at 1.500 g in a Sorvall HB-4 rotor. The resulting supernatant was discarded and the pellet resuspended to 3 ml in HM. The homogenization and centrifugation steps were repeated, the supernatant again discarded, and the pellet resuspended to 1 ml in HM. This was layered on 24 ml of 70% Percoll, and centrifuged for 15 min at 35,000 g in a Sorvall SS-34 rotor. A band was formed 10-15 mm from the tube bottom that contained Stage I tmx. Tmx were collected and washed by diluting at least 10-fold in either HM or wash buffer (20 mM KH\(_2\)PO\(_4\)-K\(_2\)HPO\(_4\), 100 mM KCI, 5 mM EGTA [pH 7.0]), and centrifuged for 18 min at 1,500 g in HB-4 rotor. The resulting pellet of washed Stage I tmx was resuspended to 1-2 ml in HM.

Calibration of Percoll gradients was done using density marker beads (Pharmacia Fine Chemicals) of known density. The tmx band spans a density range from 1.099 to 1.108 g/ml, placing Stage I tmx among the denser organelles such as lysosomes and mitochondria (25).

Ca\(^{2+}\) Effects on tmx Expansion

The Ca\(^{2+}\) sensitivity of condensed tmx was maintained. Stage I tmx preparations exposed to 10\(^{-8}\) M (pCa 8.0) Ca\(^{2+}\) remained largely condensed (Fig. 1a), while increasing the Ca\(^{2+}\) concentration to >10\(^{-6}\) M (Fig. 1b) causes expansion of the tmx to Stage III.

To determine whether the inhibition of matrix expansion by CaM antagonists in vivo (10) is due to a direct effect of these agents on the matrix, we exposed tmx preparations to CaM antagonists (14, 19, 34), and monitored expansion in response to a series of Ca\(^{2+}\)/EGTA buffers calculated to yield free Ca\(^{2+}\) ranging from 10\(^{-8}\) M to 10\(^{-12}\) M (pCa 8.0 to pCa 3.0). Information on size and shape of macromolecules can be gained from their light scattering properties (6). We exploited this phenomenon to monitor the structural transition from the compact, highly condensed Stage I matrix to the extended rod-shaped Stage III matrix. Matrix expansion led to a decrease in the optical density when the turbidity of a tmx suspension was monitored using a spectrophotometer, measuring at 320 nm (OD\(_{320}\)). As described in Materials and Methods, a small volume (~40 μl) of a tmx suspension that contained ~95% Stage I matrices in 5 mM EGTA buffer with no added Ca\(^{2+}\) was added to a cuvette that contained 720 μl of a Ca\(^{2+}\)/EGTA buffer adjusted to a desired free Ca\(^{2+}\) concentration. The results of such a turbidity assay are shown in Fig. 2. The Ca\(^{2+}\)
concentration is expressed as its pCa \((\text{pCa} = -\log[\text{Ca}^{2+}])\) equivalent. The pH of tmx suspension and Ca\(^{2+}\)/EGTA buffers in this experiment was 7.0. The OD\(_{320}\) of an unstimulated tmx suspension (5 mM EGTA buffer in cuvettes) (as indicated on the Y-axis in Fig. 2) was \(-0.215\). There was little change in the OD\(_{320}\) with increasing Ca\(^{2+}\) concentration (decreasing pCa) up to pCa 6.0. Below pCa 6.0, a rapid drop in OD\(_{320}\) occurred, which was essentially complete by pCa 5.5. Increasing Ca\(^{2+}\) concentration further had little effect. Light microscope examination of samples at different pCa’s indicated that the drop in OD\(_{320}\) corresponded to tmx expansion. Preincubation of tmx in CaM antagonists at the indicated concentrations had no effect on matrix expansion, whether or not stimulation buffers contained CaM antagonists at the same concentration. Similar results were obtained when expansion was monitored by differential counts of Stage I versus Stage III tmx via phase-contrast microscopy: CaM antagonists did not affect matrix expansion in vitro.

A parameter that was found to influence Ca\(^{2+}\)-induced matrix expansion using this assay was pH. Fig. 3a is representative of an experiment showing Ca\(^{2+}\)-induced expansion as a function of pH. It is immediately obvious that the drop in OD\(_{320}\) corresponding to matrix expansion was shifted to the right or left, depending on pH of stimulation buffers. At pH 6.0, half-maximal expansion occurred at pCa 4.8 (pCa\(_{50}\)). Increasing the pH to 6.6–7.0 raised the pCa\(_{50}\) to 5.5–5.7. At pH 7.4, pCa\(_{50}\) was 6.2, and at pH 8.0, pCa\(_{50}\) was 6.7 (Fig. 3b). Therefore, increasing pH allowed expansion to occur at lower Ca\(^{2+}\) concentration. This was the expected result since H\(^{+}\) ions are presumably acting to displace or interfere with Ca\(^{2+}\) binding to sites on the tmx.

Some expansion appeared to occur at pH 8.0 even at low Ca\(^{2+}\) concentration (Fig. 3a). At pCa 7.0, OD\(_{320}\) is reduced from 0.195 to 0.174, a decrease of \(-10\%\). A similar decrease in OD\(_{320}\) at pH 8.0 was also observed in another experiment at pCa 7.0. However, at lower Ca\(^{2+}\) concentration (pCa 8.0), OD\(_{320}\) at pH 8.0 was similar to that at pH 7.0 (data not shown), suggesting that an increase in pH alone is not suffi-
FIGURE 2  In vitro expansion of isolated tmx: effects of CaM antagonists (turbidity assay). Ca\(^2+\) concentration is expressed as its pCa equivalent (pCa = -log\([Ca^{2+}]\)). Control preparations (no drug addition) (A) exhibit a sharp drop in OD\(_{320}\) corresponding to matrix expansion (Stage I to Stage III) below pCa 6.0. Preincubation of tmx in 30 \(\mu\)M trifluoperazine (A), 5 \(\mu\)M R24571 (O), or 40 \(\mu\)M N-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide (△) does not inhibit this expansion.

FIGURE 3  In vitro expansion of isolated tmx: effect of pH (turbidity assay). (a) In vitro expansion induced by Ca\(^2+\)/EGTA buffers of different pH: pH 8.0 (O); pH 7.4 (●); pH 7.0 (△); pH 6.6 (□); pH 6.0 (△). (b) pCa\(_{50}\) for matrix expansion vs. pH. Data from three experiments.

The effect of changes in pH at constant pCa is illustrated in Fig. 4. At pCa 7.0, raising the pH from 6.0 to 8.0 caused a slight drop in OD\(_{320}\), indicating that a small fraction of the tmx undergo expansion at this low Ca\(^2+\) concentration at high pH. At pCa 6.0, alterations in pH between 6.0 and 7.0 did not promote expansion, but expansion occurred when pH was >7.0. At pCa 5.0, the change in OD\(_{320}\) was approximately linear with increasing pH. Therefore, at a subthreshold pH (pCa 7.0), changing pH had little effect on expansion. At pCa 6.0, expansion occurred above a critical pH value, indicating a requirement for both pH and pCa to be in the correct range for expansion to occur. At pCa 5.0, Ca\(^2+\) did not appear to be limiting, and expansion occurred primarily as a function of pH.

The modulation of Ca\(^2+\)-induced in vitro expansion by changes in pH suggests that pH may play a role in vivo in regulating the state of the matrix. We examined this possibility by incubating Paramecium in acridine orange, a fluorescent amine that accumulates in acidic intracellular compartments (18). Figure 5a shows a phase-contrast micrograph of a cell after a 60-s exposure to acridine orange (10 \(\mu\)g/ml). One can see trichocysts docked beneath the plasma membrane along the cell periphery (arrows). Figure 5b shows the fluorescence image of the same cell. Elongate, fluorescent bodies within the cell cortex, lining the entire perimeter of the cell (arrows), are clearly visible. These correspond in shape, location, and number to trichocysts. This observation strongly suggests that in vivo, intratrichocyst pH is acidic.

In summary, in vitro studies of tmx indicate that: (a) at neutral pH, matrix expansion occurred at a Ca\(^2+\) concentration >10\(^{-6}\) M; (b) matrix expansion in isolated Stage I tmx preparations was not inhibited by CaM antagonists; and (c) matrix expansion is dependent on pH as well as Ca\(^2+\), showing inhibition by acidic pH and potentiation by alkaline pH. In addition, the fluorescent amine acridine orange accumulated within trichocysts in vivo, suggesting that pH may serve to modulate Ca\(^{2+}\)-induced expansion in vivo in a manner similar to that demonstrated in vitro.

DISCUSSION

Stage I tmx isolated according to the methods outlined in this report were devoid of their surrounding membranes (Fig. 2) yet maintained their characteristic paracrystalline appearance and exhibited the Ca\(^{2+}\)-dependent expansion reaction that normally accompanies release. We have examined this com-
ponent of the release reaction in isolation to determine if it is the site of CaM antagonist action in vivo. We found that CaM antagonists did not inhibit expansion of the isolated, functional secretory granule content. Therefore, inhibition of matrix expansion and secretion by CaM antagonists in vivo (10) was not due to a direct interaction of these agents with the tmx itself. Inhibition in vivo is due to an effect of CaM antagonists outside the matrix.

The influx of extracellular Ca$^{2+}$ that follows stimulation in vivo is probably sensed initially by an intracellular Ca$^{2+}$ receptor, most likely cytoplasmic CaM. The route of Ca$^{2+}$ into the secretory vesicle seems to be via the cytoplasm since mutants incapable of membrane fusion (3, 5) exhibit matrix expansion when stimulated (11). This matrix expansion is also inhibitable by CaM antagonists (11). Although we consider it likely that the primary target of the CaM antagonists is cytoplasmic Ca$^{2+}$-CaM complexes (10), we cannot yet rule out an effect on other cellular targets, including Ca$^{2+}$ and phospholipid-dependent protein kinase (protein kinase C) (22). Keeping this caveat in mind, we can discuss a potential role for Ca$^{2+}$-CaM complexes in initiating matrix expansion in vivo that is consistent with the data presented here. Cytoplasmic Ca$^{2+}$-CaM complexes that are formed after stimulation appear to act at the trichocyst membrane to initiate ionic changes that permit and/or activate matrix expansion. CaM antagonists do not block the influx of Ca$^{2+}$ into the cytoplasm (23), but prevent the initiation at the trichocyst membrane of the critical changes leading to Ca$^{2+}$ access to the trichocyst matrix. Our working hypothesis is depicted in Fig. 6.

The Effect of pH on Matrix Expansion

At low pH (6.0), matrix expansion required a higher Ca$^{2+}$ concentration than at neutral pH (Fig. 3). Thus, tmx Ca$^{2+}$-binding sites are likely to resemble those of troponin C and CaM, which exhibit pH-modulated Ca$^{2+}$ binding (7, 32).

The influence of pH on expansion might suggest that it is not Ca$^{2+}$ alone that regulates this process in vivo. If pH within the trichocyst vesicle was maintained at a low value, expansion would not occur unless intratrichocyst Ca$^{2+}$ became very high. This would provide an additional regulatory or "safety" mechanism; both pH and Ca$^{2+}$ concentration would have to be in the correct range for expansion to occur. Preliminary evidence shown here (Fig. 5) indicates that the intratrichocyst pH is indeed acidic. Brief exposure of Paramecium to acridine orange leads to uptake and concentration of the dye within organelles corresponding in shape, location, and number to the trichocysts. Further work by Busch and Satir (8) has shown that Paramecium mutants lacking trichocysts also lack the elongate fluorescent bodies described here, and that proton ionophores eliminate the trichocyst fluorescence, indicating that the acidic orange distribution is dependent on an existing pH gradient across the trichocyst membrane.

Low intragranular pH may be a common feature of secretory granules. The pH of the interior of neurosecretory gran-
ules from the posterior pituitary (29) and chromaffin granules (17, 26) has been determined to be acidic (pH 5.7–5.8). The low pH is maintained by a Mg$^{2+}$/ATP-driven proton pump ATPase in the chromaffin granule (4, 15, 26, 30) and is also Mg$^{2+}$- and ATP-dependent in neurosecretory granules of the posterior pituitary (29). The function of low intragranular pH is unclear; however, the observation reported here, that low pH inhibited matrix expansion, suggests that a primary function of low pH is to maintain the condensed state, as demonstrated for matrix expansion in vitro. After stimulation, Ca$^{2+}$, rises above 10$^{-6}$ M and cytoplasmic Ca$^{2+}$-CaM complexes are formed. These complexes are thought to be the primary target for CaM antagonists, although other cellular targets cannot be ruled out. Data from previous studies (10) suggests that Ca$^{2+}$-CaM complexes act at the trichocyst membrane to control access of Ca$^{2+}$ to the matrix. Whether CaM acts via direct binding to trichocyst membrane components, or via CaM-activated regulatory enzymes such as kinases or phosphatases, is not known (indicated by the black box). Transport of Ca$^{2+}$ into the trichocyst may occur through a gate or channel in the membrane, or may be coupled to the outward movement of protons by an antiport mechanism. In this manner, stored energy in the form of a chemiosmotic gradient would be used to promote matrix expansion, while at the same time removing Ca$^{2+}$ from the cytoplasm and thus terminating the signal for release. Ca$^{2+}$ within the vesicle can then bind to sites on the tmx and lead to expansion. Membrane fusion must occur in a coordinated fashion with matrix expansion to allow release of secretory products to the extracellular space. (Alv, alveolar sacs; PM, plasma membrane).

![Diagram](https://example.com/diagram.png)

**Figure 6** Model of regulation of secretion in Paramecium. (a) In the unstimulated condition, Ca$^{2+}$ is low (<10$^{-7}$ M), CaM is largely Ca$^{2+}$-free, and the tmx is condensed (Stage I). The interior of the trichocyst vesicle may be maintained at a low pH by a proton pump in the trichocyst membrane. Within the acidic secretory granule, the interaction of H$^+$ with the matrix will help to maintain the condensed state, as demonstrated for matrix expansion in vitro. (b) After stimulation, Ca$^{2+}$ rises above 10$^{-6}$ M and cytoplasmic Ca$^{2+}$-CaM complexes are formed. These complexes are thought to be the primary target for CaM antagonists, although other cellular targets cannot be ruled out. Data from previous studies (10) suggests that Ca$^{2+}$-CaM complexes act at the trichocyst membrane to control access of Ca$^{2+}$ to the matrix. Whether CaM acts via direct binding to trichocyst membrane components, or via CaM-activated regulatory enzymes such as kinases or phosphatases, is not known (indicated by the black box). Transport of Ca$^{2+}$ into the trichocyst may occur through a gate or channel in the membrane, or may be coupled to the outward movement of protons by an antiport mechanism. In this manner, stored energy in the form of a chemiosmotic gradient would be used to promote matrix expansion, while at the same time removing Ca$^{2+}$ from the cytoplasm and thus terminating the signal for release. Ca$^{2+}$ within the vesicle can then bind to sites on the tmx and lead to expansion. Membrane fusion must occur in a coordinated fashion with matrix expansion to allow release of secretory products to the extracellular space. (Alv, alveolar sacs; PM, plasma membrane).

**REFERENCES**

1. Abrahamsson, H., and E. Gylfe. 1980. Demonstration of a proton gradient across the insulin granule membrane. Acta Physiol. Scand. 109:113-114.
2. Anderer, R., and K. Hausmann. 1977. Properties and structure of isolated extrusive organelles. J. Ultrastruct. Res. 60:21–26.
3. Ausieler, K. J. 1978. The effective site of some mutations affecting exocytosis in *Paramecium tetraurelia*. Mol. Gen. Genet. 165:199–205.
4. Bashford, C. L., G. K. Rudda, and G. A. Ritchie. 1973. Energy linked activities of the chromaffin granule membrane. FEBS Lett. 304:21–24.
5. Bashford, C. L., G. K. Rudda, and G. A. Ritchie. 1973. Energy linked activities of the chromaffin granule membrane. FEBS Lett. 304:21–24.
6. Blanehard, E. M., B.-S. Pan, and R. J. Solaro. 1984. The effect of acidic pH on the ATPase activity and troponin Ca$^{2+}$-binding of rabbit skeletal myofilaments. J. Biol. Chem. 259:3181–3186.
7. Bier, M. 1957. Light scattering measurements. Methods Enzymol. 147–146.
8. Black, D. E., B.-S. Pan, and R. J. Solaro. 1984. The effect of acidic pH on the ATPase activity and troponin Ca$^{2+}$-binding of rabbit skeletal myofilaments. J. Biol. Chem. 259:3181–3186.
9. Busch, G., and Satir, B. H. 1982. Mature secretory organelles in Paramecium are acidic compartments. J. Cell Biol. 9904, Pt: 23:383a (Abstr.).
10. Coore, H. G., B. Hellman, E. Pihl, and I. B. Toljadal. 1969. Physiological characteristics of islet secretion granules. Biochem. J. 110:107–113.
11. Faragol, R., S. M. Gilligan, and B. H. Satir. 1983. Calmodulin antagonists inhibit secretion in Paramecium. J. Cell Biol. 96:1072–1081.
12. Faragol, R., S. M. Gilligan, and B. H. Satir. 1983. A role for calmodulin in the regulation of secretion in *Paramecium tetraurelia*. Ph.D. thesis. Albert Einstein College of Medicine, Ann Arbor, MI. 222 pp.
13. Gilligan, D. M., and B. H. Satir. 1983. Stimulation and inhibition of secretion in Paramecium: role of divalent cations. J. Cell Biol. 67:224–234.
14. Hellman, B., E. Gylfe, P. Berggren, T. Andersson, H. Abrahamsson, P. Rosman, and C. Benzbuhl. 1980. Ca$^{2+}$ transport in pancreatic β-cells during glucose stimulation of insulin secretion. Ups. J. Med. Sci. 85:321–329.
15. Hirata, H., T. Inoue, M. Naka, T. Tanaka, H. Hayashi, and R. Kobayashi. 1980. Calcium regulated modulator protein interacting agents inhibit smooth muscle calcium-stimulated protein kinase and ATPase. Mol. Pharmacol. 17:66–72.
16. Holtz, R. C. 1980. Evidence that calcium pump transport into chromaffin vesicles is coupled to vesicle membrane potential. Proc. Natl. Acad. Sci. USA 75:5190–5104.
17. Johnson, R. G., and A. Scarpa. 1976. Ion permeability of isolated chromaffin granules.
18. Lee, H. C., J. G. Forte, and D. Epel. 1982. The use of fluorescent amines for the measurement of pH in applications in liposomes, gastric microsomes, and sea urchin gametes. In Intracellular pH: Its Measurement, Regulation and Utilization in Cellular Functions. R. Nuccitelli and D. W. Deamer, editors. Alan R. Liss, New York. 135-160.
19. Levin, R. M., and B. Weiss. 1977. Binding of trifluoperazine to the Ca2+-dependent activator of cyclic nucleotide phosphodiesterase. Mol. Pharmacol. 13:690-697.
20. Matt, H., M. Bilinski, and H. Plattner. 1978. Adenosinetriphosphate, calcium and temperature requirements for the final steps of exocytosis in Paramecium cells. J. Cell Sci. 32:67-86.
21. Matt, H., H. Plattner, K. Reichel, M. Lefort-Tran, and J. Beisson. 1980. Genetic dissection of the final exocytosis steps in Paramecium tetraurelia cells: trigger analyses. J. Cell Sci. 46:41-60.
22. Mori, T., Y. Takai, R. Minakuchi, B. Yu, and Y. Nishizuka. 1980. Inhibitory action of chlorpromazine dibucaine and other phospholipid-interacting drugs on calcium-activated, phospholipid-dependent protein kinase. J. Biol. Chem. 255:8378-8380.
23. Otter, T., B. H. Satir, and P. Satir. 1984. Trifluoperazine-induced changes in swimming behavior of Paramecium: evidence for two sites of drug action. Cell Motility. 4:249-267.
24. Pace, C. S., J. T. Tarvin, and J. S. Smith. 1982. The role of protons in glucose-induced stimulus-secretion coupling in pancreatic islet β-cells. In Intracellular pH: its measurement, regulation and utilization in cellular functions. R. Nuccitelli and D. W. Deamer, editors. Alan R. Liss, New York. 483-512.
25. Pertoff, H., T. C. Laurent, K. Seljeld, G. Akreutrom, L. Kajdal, and M. Hirtenstein. 1979. The use of density gradients of Percoll for the separation of biological particles. In Separation of Cells and Subcellular Elements. H. Peeters, editor. Pergamon Press, New York. 67-72.
26. Pollard, H. B., H. Shindo, C. E. Creutz, C. J. Pazoies, and J. S. Cohen. 1979. Internal pH and state of ATP in adrenergic chromaffin granules determined by 31P nuclear magnetic resonance spectroscopy. J. Biol. Chem. 256:1170-1177.
27. Portzehl, H., P. C. Caldwell, and J. C. Rüegg. 1964. The dependence of contraction and relaxation of muscle fibers from the crab Maia squinado on the internal concentration of free calcium ions. Biochem. Biophys. Acta. 79:391-591.
28. Rothman, S. S., S. Burwen, and C. Liebow. 1974. The zymogen granule: intracellular organization and its functional significance. Adv. Cytomorphol. 2:342-348.
29. Russell, J. T., and R. W. Holz. 1981. Measurement of ApH and membrane potential in isolated neurosecretory vesicles from bovine neurohypophyses. J. Biol. Chem. 256:5950-5953.
30. Saltama, G., R. G. Johnson, and A. Scarpa. 1980. Spectrophotometric measurement of transmembrane potential and pH gradients in chromaffin granules. J. Gen. Physiol. 25:109-140.
31. Soneborn, T. M. 1970. Methods in Paramecium research. Methods Cell Physiol. 4:241-339.
32. Tkachuk, V. A., and M.-Y. Mens’nikov. 1981. Effect of pH on Ca-binding properties of calmodulin and its interaction with the Ca-dependent form of cyclic nucleotide phosphodiesterase. Biochemistry (Engl. Transl. Biokhimia). 46:779-788.
33. Zanini, A., and G. Gianattasio. 1974. Molecular organization of rat prolactin secretory granules. Adv. Cytomorphol. 2:329-339.
34. Van Belle, H. 1981 R24571: a potent inhibitor of calmodulin-activated enzymes. Cell Calcium. 2:483-494.