# Chromaffin granule-cytoskeleton interaction

## Stabilization by F-actin of ATPase in purified chromaffin granule membranes

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The influence of cytoskeletal elements on the chromaffin granule function was studied using a model system consisting of purified granule membranes and F-actin. The membrane ATPase was partially inactivated by incubation at 37 °C, and this inactivation was prevented by adding F-actin. The stabilizing action of F-actin on the ATPase was abolished by adding DNase I. Detergent-solubilized ATPase was more rapidly and profoundly inactivated, but was not stabilized by F-actin. The stabilization of ATPase by F-actin may be due to the cross-linking of granule membranes with F-actin and the native structure of the granule membrane may be required for preserving the stability of membrane ATPase. These findings thus suggest the possibility that the interaction of microfilaments with chromaffin granules may influence the function of chromaffin granules within the cell.

*Chromaffin granule* *Cytoskeleton* *F-Actin* *ATPase*

## 1. INTRODUCTION

Chromaffin granule membranes bind F-actin and cause a large increase in the apparent viscosity of the membrane-actin mixture. This granule membrane–actin interaction is reversibly inhibited by raising the free calcium concentration [1], and the interaction of chromaffin granules with actin filaments may thus represent an aspect of the mechanism regulating the intracellular organelle movement, such as the translocation of chromaffin granules to the plasma membrane during the exocytotic secretion of catecholamines in adrenal chromaffin cells [2,3].

In addition to calcium, MgATP has also been shown to inhibit the granule membrane-actin interaction [4]. Although the function of this inhibitory action is not known, this finding suggests a possible relationship between the granule–actin interaction and MgATP-dependent function of chromaffin granules. Since the uptake of catecholamines into chromaffin granules requires MgATP and the membrane ATPase [5–9], it seems possible that the interaction of chromaffin granules with actin filaments may influence the uptake of catecholamines into the granules within the cell. However, the question of whether the cytoskeletal network is involved in the regulation of granule functions has not been approached, although the association of cytoskeletal elements with chromaffin granules has been reported [10–12]. To address this question, the influence of chromaffin granule–actin interaction on the granule membrane ATPase was studied using a model system consisting of purified chromaffin granule membranes and skeletal muscle F-actin in vitro.

*Abbreviations:* DCCD, dicyclohexylcarbodiimide; Pipes, 1,4-piperazinediethanesulfonic acid; BSA, bovine serum albumin; PMSF, phenylmethylsulfonyl fluoride

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## 2. EXPERIMENTAL

Chromaffin granules were prepared from fresh bovine adrenal medulla and the granule membranes were purified according to the procedures in [4]. Actin was prepared from an acetone powder of rabbit skeletal muscle by the method of Spudich and Watt [13]. To solubilize the ATPase activity from the granule membranes, the purified membranes were treated with 1.0% Triton X-100 for 30 min on ice, and the mixture was then centrifuged at 220000 × *g* for 60 min. The solubilized fraction and the residual membranes were extensively dialyzed prior to use. The solutions used for the purification contained 1.0 mM PMSF. Triton X-100 was purified by mixed bed resin, AG 501 × 8 (D).

Purified chromaffin granule membranes were incubated with or without F-actin under the cross-linking conditions as in [1,4]. Purified membranes (1.8–2.2 mg protein/ml) were mixed with F-actin (0.8 mg protein/ml) in the cross-linking medium consisting of 40 mM Pipes/NaOH (pH 6.8), 20 mM KCl, 1.0 mM $MgSO_4$, 5.0 mM EGTA and 0.25 mM $CaCl_2$, and then aliquots (20 $\mu$l) of this mixture were incubated at 37°C for various time periods.

ATPase activity in these aliquots was measured according to the method described by Lin and Morales [14]. The enzyme assay buffer (180 $\mu$l) consisting of 50 mM Tris–HCl (pH 7.5), 0.5 mM EDTA, 5.0 mM $MgSO_4$ and 1.0 mM ATP was added to these aliquots, and then the reaction was carried out at 37°C for 10 min. The amount of inorganic phosphate liberated from the enzymatic hydrolysis of ATP was determined.

Triton X-100 and AG 501 × 8 (D) were purchased from Bio-Rad. DNase I, DCCD, PMSF, Pipes and ATP were purchased from Sigma Chemicals. BSA was obtained from Calbiochem.

## 3. RESULTS AND DISCUSSION

The purified chromaffin granule membranes were incubated for different time periods under the cross-linking conditions which have been proposed to approximate the intracellular environment of resting cells [1,4], and the change in membrane ATPase activity during the incubation period was determined. As shown in fig.1, the granule membranes gradually lost a portion of their ATPase activity according to the incubation time. The same decrease in ATPase activity was observed by measuring the activity either in the presence or absence of 100 $\mu$M DCCD, this concentration of which caused an approx. 50% inhibition of the total enzyme activity. In fact, almost half of the DCCD-insensitive ATPase activity was lost over a 90-min incubation. Thus, these results indicate that only the DCCD-insensitive fraction of ATPase in the chromaffin granule membranes is inactivated merely by incubating the membranes at 37°C, but the mechanism of this inactivation is unknown. Although the granule membrane purification was performed in the presence of the irreversible protease inhibitor, PMSF, the possibility that a protease contained in the membrane preparation may be involved in the inactivation of membrane ATPase cannot be ruled out.

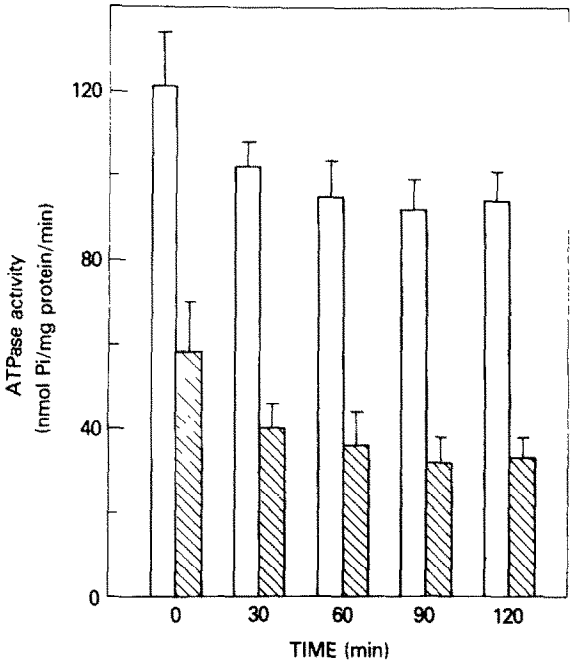


Fig.1. Partial inactivation of chromaffin granule ATPase during incubation at 37°C. Purified granule membranes were incubated at 37°C for various time periods in the cross-linking medium, and then ATPase activity in the preincubated mixture was measured in the presence (shaded bar) and absence (open bar) of 0.1 mM DCCD as described in the text. Values represent the mean ± SE of 3 experiments with triplicate determinations.

However, the fact that the inactivation of ATPase is selective to the DCCD-insensitive fraction seems to suggest that this inactivation may not be due to the nonspecific proteolysis. It therefore seems conceivable that the inactivation of membrane ATPase may reflect a thermal effect on the enzyme itself or its environment in the granule membrane. The partial inactivation of membrane ATPase as well as the different sensitivity to DCCD, a known inhibitor of mitochondrial ATPase [15], may indicate the possible existence of more than one type of ATPase present in chromaffin granules [16].

The thermal inactivation of granule membrane ATPase was almost completely prevented by adding F-actin, but this inactivation was not prevented by BSA (fig.2). Under the same conditions, a large increase in apparent viscosity of the membrane–actin mixture, but not the membrane–BSA mixture, was observed (not shown). These results indicate that F-actin binds to the granule membranes and then prevents the inactivation of membrane ATPase. Further study showed that DNase I, which is known to cause the depolymerization of F-actin [17], completely abolished the stabilizing action of F-actin on the membrane ATPase, without any direct effect on the enzyme (fig.3). This fact suggests that the filamentous structure of polymerized actin may be required for its stabilizing action. Thus, the stabilization of ATPase by F-actin seems to be due to the cross-linking between chromaffin granule membranes and actin filaments.

In view of these facts, it seems reasonable to presume that the membrane–actin interaction may primarily cause an alteration of the structure of chromaffin granule membrane which results in an increase in the stability of the membrane enzyme. To study the question whether the native structure of chromaffin granule membrane is required for preserving the stability of the membrane ATPase,

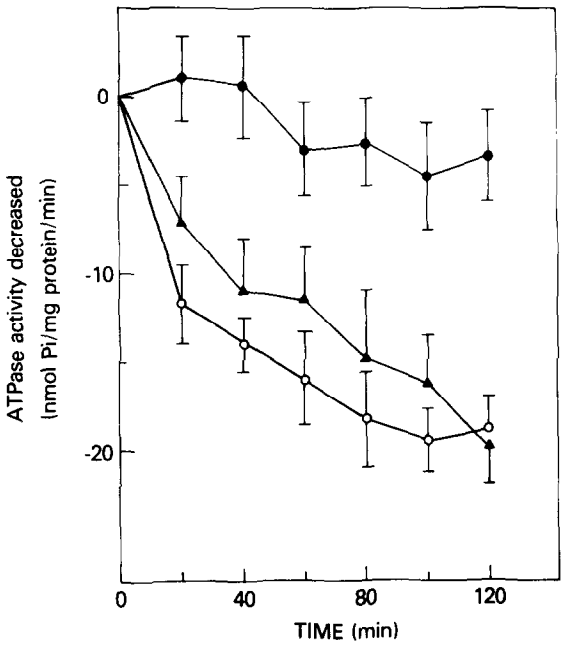


Fig.2. Effects of F-actin and BSA on the partial inactivation of chromaffin granule ATPase. Purified membranes were incubated at 37°C for various time periods in the presence (●) and absence (○) of F-actin, in the presence (▲) of BSA (4 mg/ml). Enzyme activity was measured as described in the text. Values are the mean ± SE of 3 experiments with triplicate determinations.

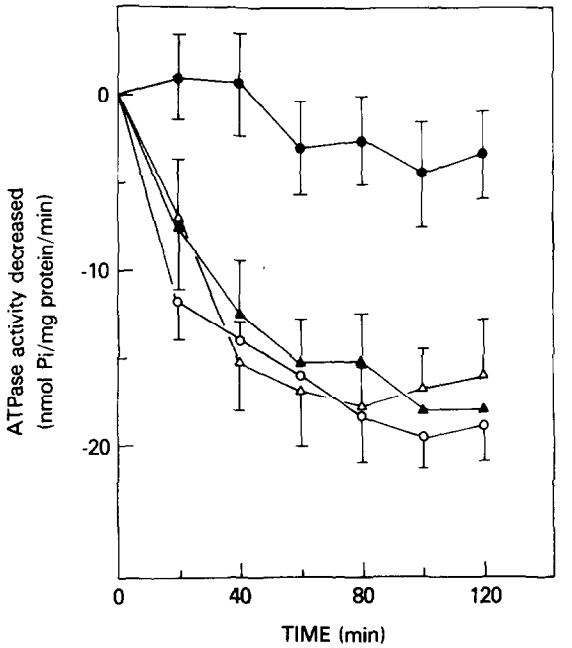


Fig.3. Effect of DNase I on the protecting action of F-actin against the partial inactivation of chromaffin granule ATPase. Purified membranes were incubated at 37°C for various time periods in the presence of F-actin (●), DNase I (40 μg/ml) plus F-actin (▲), DNase I (△), or in their absence (○). Enzyme activity was measured as described in the text. Values are the mean ± SE of 4 experiments with triplicate determinations.

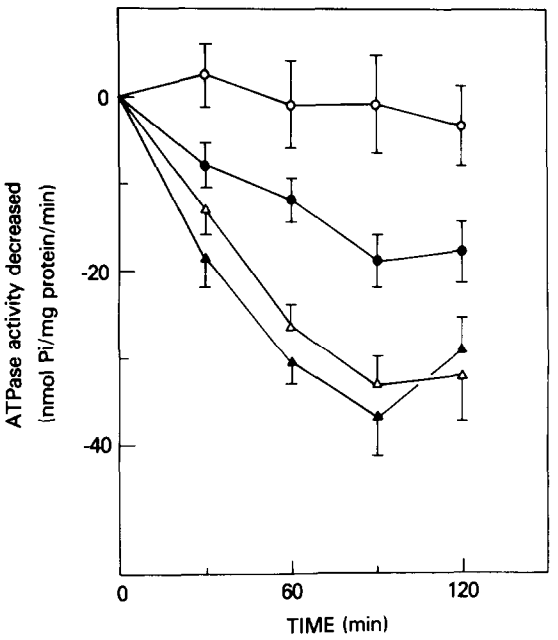


Fig.4. Difference in the stability of intact granule membrane, solubilized and membrane-bound ATPase. Purified membranes were solubilized according to the procedure described in the text. Intact membranes (●), residual membranes (○), and the solubilized fraction with (▲) or without (△) F-actin were incubated at 37°C for various time periods. Enzyme activity was measured as described in the text. Values are the mean ± SE of 3 experiments with duplicate determinations.

the purified membranes were treated with Triton X-100 and then the inactivation of ATPase in the solubilized and residual membrane fraction was examined. Approx. 40% of total enzyme activity was solubilized by detergent treatment. As shown in fig.4, the solubilized enzyme was inactivated more rapidly and profoundly than that of the intact membrane during incubation at 37°C, and this inactivation was not prevented by F-actin. On the other hand, the ATPase activity of the residual membranes did not change under the same conditions. These findings suggest that the native membrane structure seems to be required for preserving the membrane ATPase activity and the inactivation of ATPase therefore may be due to the disturbance of rigid structure of the granule membranes. The cross-linking of chromaffin granule membranes with actin filaments thus seems to protect the membrane ATPase against thermal inactivation as a result of the stabilization of granule membrane structure. These findings suggest a possible role of the microfilament network in the preservation of chromaffin granule functions as well as the position of granules within the chromaffin cell.

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