# CALCIUM EFFLUX DEPENDENT FORMATION OF ATP FROM ADP AND ORTHOPHOSPHATE BY THE MEMBRANES OF THE SARCOPLASMIC VESICLES

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## 1. Introduction

After the cessation of the ATP dependent calcium uptake the sarcoplasmic vesicles can maintain steep concentration gradients across their membranes. In assay media containing 5 mM oxalate or 20 mM phosphate in addition to 5 mM magnesium ATP the final concentration ratio calcium inside/calcium outside was found to be $\gtrsim 3000$. This calcium gradient is maintained as long as ATP is present whereby the calcium inside the vesicles is exchanged continuously with the calcium in the solution outside. The exchange proceeds considerably slower than the net uptake of calcium [1, 2]. In this report it is shown that during this exchange of calcium inorganic phosphate is steadily incorporated into the ATP fraction. The experimental conditions are given in the legends to the figures.

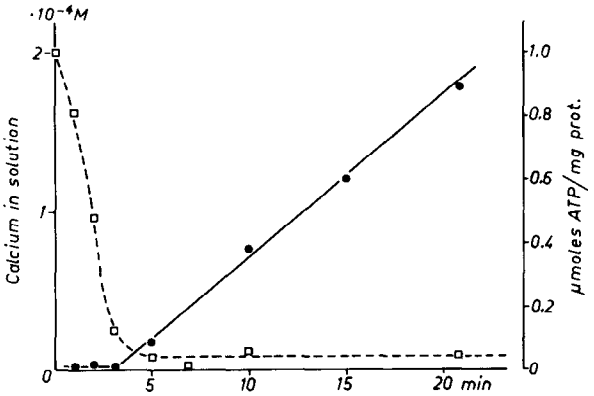


Fig. 1. Calcium uptake and ATP formation. The reactions were started by the addition of the vesicles at time 0. The composition of the medium is given in the text. *Solid line:* ATP formation. The reaction was stopped by the addition of 5% perchloric acid. After filtration and neutralisation with KOH, hexokinase and glucose were added to the supernatant. From the $^{32}$P-radioactivity in the glucose-6-phosphate fraction (isolated by paper chromatography), the amount of ATP formed was calculated (right ordinate). *Dotted line:* Decrease of the calcium concentration in the assay resulted from calcium uptake of the vesicles. The $^{45}$Ca-level was determined in aliquots filtered through Millipor filter (450 n) (left ordinate).

## 2. Results and remarks

The vesicles were loaded with calcium in media containing (mM) 20 orthophosphate ($^{32}$P), 5 ATP, 2 ADP, 7 $MgCl_2$, 0.2 $CaCl_2$ and 0.5 mg of vesicular protein. As soon as the net uptake of calcium ceases the formation of $\gamma$-$^{32}$P-ATP starts to occur. Under these conditions the rate of ATP formation (0.05–0.1 mmoles/mg of protein/min at 20°) approximates the calcium turnover rate. When the vesicular membranes are treated with ether or phospholipase A leaks are formed in the membranes and the calcium gradient is abolished. Simultaneously the vesicles loose the

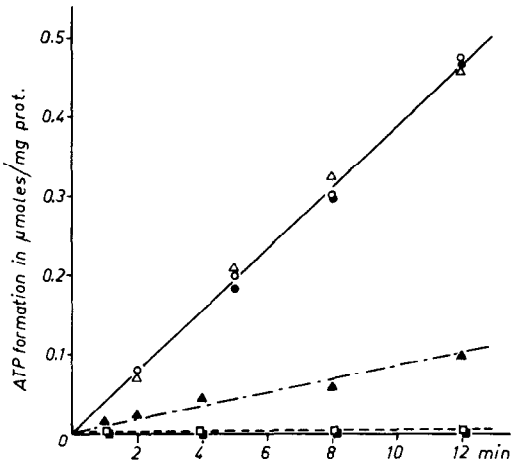


Fig. 2. ATP formation under varied conditions. The reaction mixtures contained 5 mM calcium and 7 mM EGTA in addition to the components given in the text. The high amount of calcium added on the one hand and the excess of EGTA on the other hand guarantee that a high concentration gradient of calcium ions across the vesicular membrane is generated in a short time whereby the calcium ion concentration in the solution declines only by less than 10% of the initial value ($4 \times 10^{-7}$ M). Reagents added (mM): • none, ○ 1 Na-azide, △ 0.2 DNP and ▲ 0.1 prenylamine. Pretreatment of the vesicles: □ phospholipase A digestion and ■ treatment with 5% diethyl ether. The ATP formation was measured in the same way as illustrated in fig. 1.

ability to form $\gamma^{32}$P-ATP completely although the transport ATPase is unimpaired. Prenylamine ($10^{-5}-10^{-4}$ M), inhibits the calcium turnover and the ATP formation to the same extent (fig. 2). Since neither 1 mM azide nor 0.1 mM 2,4-dinitrophenol do affect the calcium dependent formation of ATP (fig. 2) it cannot be caused by contamination of oxidative phosphorylation enzymes. The rate of $^{32}$P incorporation depends on the ADP and the orthophosphate concentration and its pH optimum lies between 6.7 and 6.9. The observed calcium gradient dependent $^{32}$P incorporation suggests a closed coupling between calcium efflux and ATP formation.

This work has been reported at the 18th meeting of the German Physiological Society in Erlangen, September 1970 [3].

## References

[1] W. Hasselbach and M. Makinose, Biochem. Z. 339 (1963) 94.
[2] M. Makinose and W. Hasselbach. Biochem. Z. 343 (1965) 360.
[3] M. Makinose, Arch. Ges. Physiol. 319 (1970) R116