ATP SYNTHESIS BY THE REVERSE OF THE SARCOPLASMIC CALCIUM PUMP

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1. Introduction

The result of the release studies described in the preceeding report [1] prompted the search for net ATP synthesis by the sarcoplasmic calcium pump. It is shown that under the conditions where a fast ADP and inorganic phosphate dependent calcium release takes place from calcium loaded sarcoplasmic vesicles, the net outward movement of calcium is stoichiometrically related to a net formation of ATP. The experimental conditions are given in the legend to fig. 1.

2. Results and remarks

Fig. 1 illustrates that for every two calcium ions that leave the vesicles one phosphate group is transferred to ATP. The rate of ATP formation reaches the approximately 20% of the initial rate of ATP consumption during active calcium uptake. The net ATP formation depends on the tightness of the vesicular membranes for calcium ions as it emerges from the complete abolition of ATP formation after the vesicular membranes have been made permeable for calcium by treating them with ether or phospholipase A. On the other hand, when the passage of calcium through membranes is retarded by prenylamine [2] ATP formation is retarded also. To exclude the participation of mitochondrial impurities in the calcium dependent phosphorylation reaction azide and 2,4-dinitrophenol were applied. Both agents were found to be ineffective.

The coupling between calcium outward movement

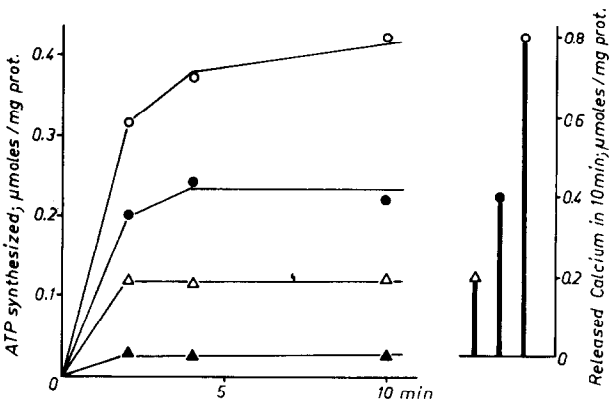

Fig. 1. Calcium release and net ATP synthesis. During an incubation period of 15 min in media containing (mM) 2 acetyl phosphate, 7 $MgCl_2$, 40 KCl, 0.1 glucose, 20 Na orthophosphate (^{32}P) and 0.5mg/ml of vesicular protein at pH 7, the vesicles (one mg protein) were loaded with different amount of calcium (nmoles): ○ 800, ● 400, △ 200 and ▲ no calcium. Subsequently the calcium release was started by the addition of 2 mM ADP, hexokinase* (0.02 mg/ml) and 1 mM to 2 mM EGTA at time 0. The amount of EGTA was at least 5 times higher than the total calcium in the system. The synthesis of ATP was measured as glucose-6-phosphate (^{32}P) formation (left ordinate). The amounts of calcium released after 10 min are represented by the column at the right side of the graph.

* Hexokinase was obtained from Boehringer, Mannheim, Germany.

and ATP synthesis is constant as revealed by the constant Ca/P ratio of two, although the calcium load is varied by a factor of four obviously the calcium ratio inside to outside does not fall below a critical level until the vesicles are nearly empty. As a consequence of the calcium phosphate store inside the vesicles, the decline of the ratio is only the result of the rising calcium concentration in the solution outside. The minimum energy which has to be provided by the calcium gradient under the experimental conditions that establish a very low ATP concentration by the hexokinase reaction can be estimated to be about 2000–3000 cal. Since the translocation of two calcium ions gives rise to the incorporation of one phosphate group, a calcium ratio calcium inside to calcium outside ~ 20–30 is sufficiently high to provide the required energy. However, for a medium containing phosphate instead of oxalate as calcium precipitating agent it is difficult to estimate the existing ratio calcium inside/calcium outside. Assuming the same free calcium concentration in the solution outside, the ratio is certainly higher than in the oxalate system because the ionized calcium in equilibrium with calcium phosphate crystals inside the vesicles is higher than that in equilibrium with calcium oxalate crystals. In oxalate systems ratios up to 3000 have been found. These ratios are sufficiently high to drive even the reaction when ATP is accumulated (~ 0.05 mM) in the absence of the hexokinase reaction.

These results show that the calcium translocation across the sarcoplasmic membranes is reversibly connected with the phosphoryl transfer reaction giving rise to a splitting of ATP when calcium moves inward and to an ATP synthesis when calcium moves outward. This calcium gradient dependent ATP synthesis takes place with high efficiency under most simple conditions. Thereby the sarcoplasmic membrane system is superior to most other membranes used for the attempt to gain support for the conversion of osmotic into chemical energy [3, 4].

References

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