SOME KINETIC PROPERTIES OF PHOSPHORYLATED ATPase OF SARCOPLASMIC RETICULUM FORMED IN THE ABSENCE OF ADDED ALKALI METAL SALTS

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SUMMARY: The decomposition of the Ca^{2+}-dependent phosphoprotein formed at the steady state in the presence of [γ-^{32}P]ATP and Ca^{2+} but in the absence of added alkali metal salts, is accelerated by KCl and ATP in a ATPase preparation of sarcoplasmic reticulum. In contrast to decomposition of phosphoprotein formed in the presence of KCl, that formed in the absence of added alkali metal salts is not accelerated by added ADP.

Sarcoplasmic reticulum vesicles isolated from skeletal (1-3) and cardiac (4) muscle accumulate calcium at a much slower rate in the absence of added alkali metal salts than when optimal concentrations of these salts are present. Concomitant with this slow rate of calcium accumulation, a slow rate of Ca^{2+}-dependent ATP hydrolysis is observed and ^{32}P is incorporated into the vesicles from [γ-^{32}P]ATP to form an acid-stable phosphoprotein (2,3). The Ca^{2+}-dependence and amount of the phosphoprotein formed during this slow ATP hydrolysis are comparable to those of the phosphoprotein formed in the presence of optimal alkali metal salt concentrations. However, the phosphoprotein formed in the absence of added alkali metal salts decomposes to yield P_i at a very slow rate (2), suggesting that alkali metal salts stimulate ATP hydrolysis and calcium transport by accelerating decomposition of the phosphoprotein intermediate of the Ca^{2+}-dependent ATPase.

The present study describes some kinetic properties of the Ca^{2+}-dependent phosphoprotein that is formed in the absence of alkali metal salts in partially purified preparations of the calcium pump ATPase of

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Abbreviations: EGTA, ethylene glycol bis (β-amino ethyl ether) N,N'-tetraacetic acid; EP, phosphoprotein.

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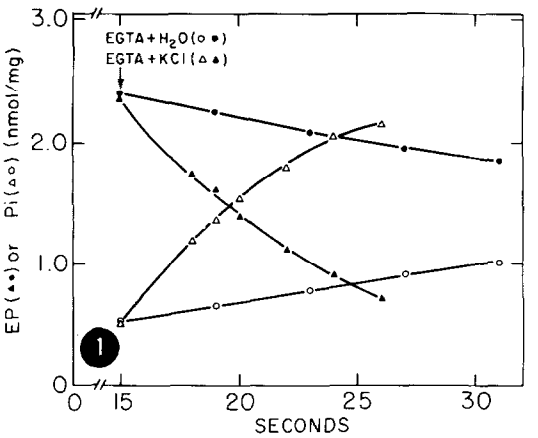

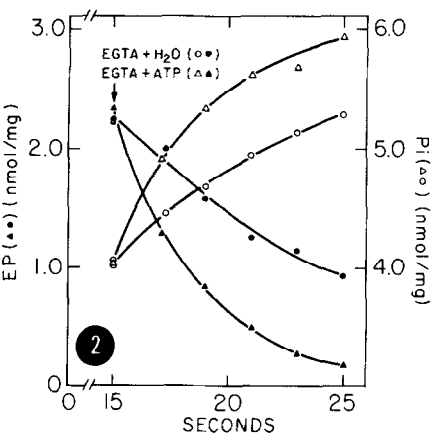

Figure 1. Effect of KCl on decomposition of the phosphoprotein formed in the absence of added alkali metal salts. The partially purified ATPase protein, 0.201 mg/ml, was phosphorylated at 0° C in 15 mM imidazole (pH 6.8), 4 mM $MgCl_2$, 10 μM [γ-^{32}P]ATP and 10 μM $CaCl_2$. Fifteen seconds after the reaction was started (↓), further phosphorylation was inhibited by chelation of Ca^{2+} with 2.86 mM EGTA or 2.86 mM EGTA + 48 mM KCl. In this and subsequent figures and the table the Ca^{2+}-dependent P_i liberation and phosphoprotein are shown.

Figure 2. Effect of ATP on decomposition of the phosphoprotein formed in the absence of added alkali metal salts. The partially purified ATPase protein, 0.196 mg/ml, was phosphorylated at 10° C in 15 mM imidazole (pH 6.8), 4 mM $MgCl_2$, 3.5 μM [γ-^{32}P]ATP and 10 μM $CaCl_2$. Fifteen seconds after the reaction was started (↓), further phosphorylation was inhibited by chelation of Ca^{2+} with 2.86 mM EGTA or 2.86 mM EGTA + 0.29 mM ATP.

sarcoplasmic reticulum. It is reported that the decomposition of this phosphoprotein is accelerated by KCl or ATP. In contrast to decomposition of the phosphoprotein formed in the presence of KCl, that formed in the absence of alkali metal salts is not accelerated by added ADP.

MATERIALS AND METHODS: Sarcoplasmic reticulum vesicles, prepared from rabbit white skeletal muscle as described previously (5), were further purified according to Meissner and Fleischer (6) with the following modifications: the vesicles (11 mg/ml) were incubated for 5 min at 0° C in a medium contained deoxycholate (2.8 mg/ml), 0.25 M sucrose, 0.45 M KCl, 1 mM EDTA, 1.5 mM $MgCl_2$, 0.1 mM $CaCl_2$ and 10 mM Tris-HCl buffer (pH 8.0), and centrifuged for 60 min at 50,000 rpm in a Spinco Type 65 rotor. The membraneous pellet was suspended in the above sucrose/KCl/ histidine solution and stored on ice. The calcium pump ATPase accounted for approximately 90% of the total protein in these partially purified preparations as estimated by SDS gel electrophoresis (7).

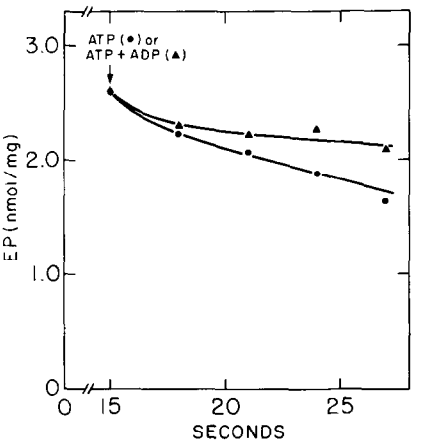

Figure 3. Effect of ADP on decomposition of the phosphoprotein formed in the absence of added alkali metal salts. The partially purified ATPase protein, 0.2 mg/ml, was phosphorylated at 0° C in 15 mM imidazole (pH 6.8), 4 mM $MgCl_2$, 3.0 μM [γ-^{32}P]ATP and 20 μM $CaCl_2$. Fifteen seconds after the reaction was started (↓), further phosphorylation was interrupted by a chase of 0.147 mM non-radioactive ATP or 0.147 mM non-radioactive ATP + 0.53 mM ADP.

Alkali metal salts were removed from the vesicles as described previously (2). Protein concentration, ATPase activity and phosphoprotein level were assayed as described previously (5) with slight modification. Disodium ATP and disodium ADP were converted to imidazole forms by passage through a cation exchange resin (2).

RESULTS AND DISCUSSION: The Ca^{2+}-dependent phosphoprotein formed in the absence of added alkali metal salts decomposed spontaneously to yield P_i after a steady state level was reached and further phosphorylation prevented by EGTA (Figures 1 and 2) or excess non-radioactive ATP (Figure 3). The initial rate of phosphoprotein decomposition was accelerated approximately 5-fold by addition of 48 mM KCl, and was accompanied by liberation of a stoichiometric amount of P_i (Figure 1). Under similar conditions, LiCl at concentrations up to 100 mM had no effect on phosphoprotein decomposition.

The initial rate of decomposition of the phosphoprotein formed at the steady state in the absence of added alkali metal salts in 3.5 μM

Table 1: Effect of ADP on decomposition of phosphoprotein formed in the presence of 80 mM KCl. The partially purified ATPase protein, 0.12 mg/ml, was phosphorylated at 0° in 15 mM imidazole (pH 6.8), 4 mM $MgCl_2$, 80 mM KCl, 3.0 μM [γ-^{32}P]ATP and 15 μM $CaCl_2$. Fifteen seconds after the reaction was started, further phosphorylation was inhibited by chelation of Ca^{2+} with 2.2 mM EGTA + 0.48 mM ADP. Time of addition of EGTA + ADP is zero time in this table. A similar result was obtained when phosphorylation was interrupted by a chase of excess non-radioactive ATP + ADP instead of EGTA + ADP.

Time (seconds)	Phosphoprotein level (nmol/mg)	P_i liberated (nmol/mg)
0	2.38	1.52
1	0.53	1.59
1.9	0.52	1.61
3	0.24	1.67
4	0.23	1.75
5	0.11	1.70

[γ-^{32}P]ATP was accelerated approximately 3-fold by addition of 0.29 mM ATP, and was accompanied by liberation of a stoichiometric amount of P_i (Figure 2). However, the decomposition of this phosphoprotein was not accelerated by added ADP (Figure 3) up to 1.0 mM or in 100-fold excess over ATP present in the reaction medium. Instead, the high ADP concentration was found to decrease slightly the rate of phosphoprotein decomposition (Figure 3). In contrast to this result, the decomposition of the phosphoprotein formed at the steady state in the presence of 80 mM KCl was greatly accelerated by addition of ADP without being accompanied by the corresponding increase of P_i liberation (Table 1), suggesting that ATP was synthesized under these conditions (8, 9, 12).

The present studies indicate that the Ca^{2+}-dependent phosphoprotein which accumulates in the absence of added alkali metal salts can be distinguished by its sensitivity to ADP from that which accumulates in the presence of KCl. The major fraction of the latter, which has been identified as a reaction intermediate of the Ca^{2+}-dependent ATPase of

sarcoplasmic reticulum (8-13), is sensitive to ADP (Table 1 and references 8, 9, 12). However, the effects of ATP on the decomposition of this phosphoprotein are controversial; the failure of high ATP concentrations to accelerate decomposition of the phosphoprotein formed in the presence of KCl has been reported (8, 9) while others have found that phosphoprotein decomposition under these conditions is stimulated by ATP (14, 15).

We previously observed that the phosphoprotein formed in the absence of added alkali metal salts is Ca^{2+}-dependent, and that this Ca^{2+}-dependence is similar to that of the phosphoprotein formed in the presence of KCl (2). The levels of both Ca^{2+}-dependent phosphoproteins were also comparable (Figures 1-3 and Table I, see also reference 2). It has been found that KCl stimulates both Ca^{2+}-dependent ATPase activity (1-3) and calcium transport (1-4) by sarcoplasmic reticulum vesicles and that KCl accelerates decomposition of the phosphoprotein formed in the absence of alkali metal salts (Figure 1). These findings thus suggest that the Ca^{2+}-dependent phosphoprotein formed at the steady state in the absence of alkali metal salts represents a reaction intermediate of the Ca^{2+}-dependent ATPase. It appears possible that the Ca^{2+}-dependent phosphoproteins that are formed in the absence and presence of alkali metal salts appear sequentially during the normal Ca^{2+}-dependent ATPase reaction of the sarcoplasmic reticulum and that removal of alkali metal salts, by inhibiting one of the steps in this reaction sequence, yields a phosphoprotein intermediate with properties different from that which accumulates when KCl is present.

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REFERENCES

1. Rubin, B.B. and Katz, A.M. (1967) Science 158, 1189-1190.
2. Shigekawa, M. and Pearl, L.J. (1976) J. Biol. Chem. 251, 6947-6952.
3. Duggan, P.F. (1977) J. Biol. Chem. 252, 1620-1627.

4. Katz, A.M. and Repke, D.I. (1967) Circulation Res. 21, 767-775.
5. Shigekawa, M., Finegan, J.M. and Katz, A.M. (1976) J. Biol. Chem. 251, 6894-6900.
6. Meissner, G. and Fleischer, S. (1974) in Methods in Enzymology (Fleischer, S. and Packer, L., eds.), vol. 32, pp. 475-481, Academic Press, New York.
7. Weber, C. and Osborn, M. (1969) J. Biol. Chem. 244, 4406-4412.
8. Kanazawa, T., Yamada, S., Yamamoto, T. and Tonomura, Y. (1971) J. Biochem. (Tokyo) 70, 95-123.
9. Martonosi, A., Lagwinska, E. and Oliver, M. (1974) Ann. N.Y. Acad. Sci. 227, 549-567.
10. Makinose, M. (1969) Eur. J. Biochem. 10, 74-82.
11. Inesi, G., Maring, E., Murphy, A.J. and MacFarland, B.H. (1970) Arch. Biochem. Biophys. 138, 285-294.
12. Panet, R., Pick, U. and Selinger, Z. (1971) J. Biol. Chem. 246, 7349-7356.
13. Hasselbach, W. (1972) in Molecular Bioenergetics and Macromolecular Biochemistry (Weber, H.H., ed.), pp. 149-171, Springer-Verlag, Berlin.
14. de Meis, L. and Fialho de Mello, M.C. (1973) J. Biol. Chem. 248, 3691-3701.
15. Froehlich, J.P. and Taylor, E.W. (1975) J. Biol. Chem. 250, 2013-2021.