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The question of the initial burst of ATP hydrolysis in myosin subfragments

The initial burst of P_i liberation of myosin ATPase (ATP phosphohydrolase, EC 3.6.1.3) has been studied extensively by TONOMURA AND KITAGAWA[1] and KANAZAWA AND TONOMURA[2], and the burst was also demonstrated with heavy meromyosin and a subfragment (S-1) (ref. 2) which resembles the subfragment-1 of MUELLER AND PERRY[4]. These investigators interpreted this phenomenon as a stoichiometric phosphorylation of myosin[1,2], although SARTORELLI *et al.*[5] claimed that no convincing evidence for the phosphorylation of myosin could be obtained in experiments using $H_2{}^{18}O$. The initial burst was examined in the enzymatically active subfragments of myosin, and it was found that the burst was partially or nearly completely lacking in the subfragments S-1 and S-n (ref. 6).

Myosin and heavy meromyosin were prepared from rabbit skeletal muscle according to the methods of PERRY[7] and SZENT-GYÖRGYI[8], respectively. S-1 was prepared from heavy meromyosin by tryptic digestion, and S-n was prepared from S-1 by Nagarse digestion[6]. The weight ratio of S-1 to Nagarse used in preparing S-n was 50:1–100:1. The tryptic digestion was stopped by adding an excess (1.3:1, w/w) of trypsin inhibitor, and the Nagarse digestion was terminated by DFP in a final concentration of $1 \cdot 10^{-4}$ M. DFP does not inhibit the initial burst, since heavy meromyosin preparations showed the same amount of initial burst whether they had been treated or not with DFP. Actin was extracted from acetone-treated rabbit skeletal muscle and purified according to MOMMAERTS[9]. Determination of the initial burst was made at pH 7.0 and 25° in the presence of 20 mM $MgCl_2$ and 1 M KCl, by measuring P_i following the MARTIN–DOTY[10] method with slight modifications. The amount of P_i released by the initial burst was determined from the difference between the concentration of P_i given at zero time and the intercept on the ordinate obtained by extrapolation of the ATPase activity at the steady state, the first measurement of which was made at 30 sec. The zero-time value was taken as the average of three determinations, each of which agreed within 3%. For the calculation of the amount of P_i per mole of enzyme, $3.5 \cdot 10^5$, $1.47 \cdot 10^5$ and $0.85 \cdot 10^5$ were used for the molecular weights of heavy meromyosin (ref. 10), S-1 (ref. 11) and S-n*, respectively. Specific ATPase activity (units) was measured at the steady state and is given as μmoles P_i liberated per min per mg protein.

Typical examples of the amount of initial burst of P_i liberation obtained with heavy meromyosin and S-1 are given in Table I. In good agreement with the findings of TOKUYAMA *et al.*[3], heavy meromyosin which had not been lyophilized showed an initial burst of P_i liberation of nearly 1 mole/mole of heavy meromyosin; with lyophilized heavy meromyosin the value was approx. 0.7 mole/mole of heavy meromyosin. Contrary to their finding with S-1, however, the amount of initially liberated P_i obtained with our S-1 preparations was not 1 mole/mole of S-1 but was in the range of 0.48 to 0.72 mole/mole (Table I). The average value of the amount and its standard deviation was 0.62 ± 0.09 mole of P_i per mole of S-1. The amounts of P_i initially liberated from S-1 preparations isolated from either lyophilized heavy meromyosin or nonlyophilized heavy meromyosin were not significantly different.

* The molecular weight of S-n was obtained from values of viscosity and sedimentation velocity using the equation of Scheraga and Mandelkern.

TABLE I

THE EFFECT OF LYOPHILIZATION ON THE Mg^{2+}-ATPase ACTIVITY AND THE AMOUNT OF P_i LIBERATED IN THE INITIAL BURST OF HEAVY MEROMYOSIN AND S-1

The period of exposure of heavy meromyosin to trypsin in preparation of S-1 was 3 min (f), 10 min (c, d, e and g) or 40 min (h). Determinations of the ATPase activity and the amount of P_i liberated in the initial burst were made at pH 7.0 and 25° in the presence of 20 mM $MgCl_2$, 1 M KCl, 0.4–0.5 mM ATP and an appropriate amount of the enzyme. 5 ml of the reaction mixture was used for each P_i measurement. a, nonlyophilized heavy meromyosin; b, lyophilized heavy meromyosin; c, preparation from nonlyophilized heavy meromyosin; d–h, preparations from lyophilized heavy meromyosin; f–h, preparations from the same heavy meromyosin preparation.

	Heavy meromyosin		*S-1*					
	a	*b*	*c*	*d*	*e*	*f*	*g*	*h*
Concn. in reaction mixture (mg/ml)	3.0	3.0	1.4	0.8	1.4	1.5	1.5	1.5
Mg^{2+}-ATPase activity $\times 10^3$ (units)	3.6	4.1	6.8	9.5	7.0	10.1	10.4	10.4
Amount of P_i initially liberated (mole/mole)	0.90	0.69	0.69	0.48	0.61	0.72	0.58	0.58

MUELLER[13] has pointed out that the degree of heterogeneity of S-1 preparations may depend on the period of exposure of heavy meromyosin to trypsin. On the other hand, YOUNG *et al.*[14] reported the same value for the molecular weights of S-1 obtained after varying times of proteolysis. Therefore in our experiments, the same heavy meromyosin preparation was exposed to trypsin for three different periods of time (3, 10 and 40 min), and the three resulting S-1 preparations were isolated from the digests. Homogeneity was shown in each preparation by column chromatography using DEAE-cellulose at pH 7.6 with a KCl gradient, by gel filtration using Sephadex G-200 at pH 7.6 and 0.15 M KCl, and by ultracentrifugation. As shown in Table I, the same ATPase activity was obtained for the three preparations (f, g, h), and the amount of P_i initially liberated was about the same for all three within the limits of experimental accuracy. Therefore, the property of S-1 preparation does not appear to vary with time of tryptic digestion within certain limits.

Although our S-1 preparations were apparently homogeneous and also identical, the amount of P_i initially liberated with these preparations was nearly 0.5 mole/mole S-1 (0.62 ± 0.09); this marked departure from a 1:1 molar ratio raises the question of their purity.

In ATP hydrolysis by the S-n preparations, P_i appeared to be liberated linearly from time zero, and there was no significant initial burst of P_i liberation as shown in Table II. Homogeneity of these preparations was shown by gel filtration using Sephadex G-100 or G-200, and by ultracentrifugation. In the presence of 5 mM $CaCl_2$ and 0.5 M KCl, specific ATPase activity of S-n was 1.3–1.4 units and that of S-1 was 1.0–1.1 units (ref. 5), but the ATPase activity of S-n in the presence of 20 mM $MgCl_2$ and 1 M KCl was nearly the same as that of S-1 (Tables I and II). The results clearly show that ATPase activity at steady state was preserved in S-n, which showed almost no initial burst. This indicates that the ATPase activity at the steady state is independent of the phenomenon of initial burst of P_i liberation in heavy meromyosin ATPase. Recently MORITA[15] has proposed that the difference spectrum of heavy meromyosin induced by ATP depends on the formation of a Michaelis–Menten com-

TABLE II

THE Mg^{2+}-ATPase ACTIVITY AND THE AMOUNT OF P_i LIBERATED IN THE INITIAL BURST OF S-n PREPARATIONS

Conditions for the measurement of ATPase activity and amount of initial burst were as in Table I.

Concn. in reaction mixture (mg/ml)	0.9	1.0	1.2	1.2
Mg^{2+}-ATPase activity $\times 10^3$ (units)	8.0	9.8	9.8	6.7
Amounts of P_i initially liberated (mole/mole)	None	<0.14	<0.08	<0.07

plex. She found that decay of this difference spectrum (which accompanies steady-state ATP hydrolysis) was not seen during the initial burst of P_i liberation. Thus the site of steady-state ATPase may be different from the site of initial burst in the myosin molecule.

In the presence of $MgCl_2$, myosin ATPase is activated by F-actin at low ionic strength, and an intimate connection between F-actin activation and the initial burst was emphasized by TONOMURA AND KITAGAWA[1] and KANAZAWA AND TONOMURA[2]. This phenomenon was examined with heavy meromyosin and S-n. Concentrations of heavy meromyosin and S-n were both 0.1 mg/ml and that of F-actin was 1.0 mg/ml in the presence of 1 mM $MgCl_2$ and 2 mM ATP at pH 7.0 and 25°. In spite of the absence of an initial burst, the S-n ATPase was activated by F-actin. The extent of the activation was about 2–3-fold under conditions where heavy meromyosin ATPase activity is enhanced 11–15-fold by F-actin. Although ATP might be expected to dissociate F-actin and S-n more strongly than F-actin and heavy meromyosin, possible contamination of S-n preparations by other components responsible for the F-actin-activated ATPase has not yet been completely excluded.

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