This lends support to our initial hypothesis that intramitochondrially synthesized phosphoenolpyruvate has additional functions besides being a precursor for glucose formation.


The Effect of Ionic Strength on the Magnesium Ion-Activated Adenosine Triphosphatase of Natural Actomyosin from Mammalian Skeletal Muscle

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The effect of ionic strength on the activity of the Mγ2+-activated ATPase† of natural actomyosin from fibres of skeletal muscles of the pig and ox was studied in relation to known differences in the rate of turnover of ATP in these tissues post mortem.

Semitendinosus muscle was removed from Piétrain and Landrace pigs immediately after death. The muscle contains visually distinct areas of predominantly red and white fibres (Tarrant et al., 1972), which were separated for the purpose of the investigation. Sections of longissimus dorsi muscle and the entire psoas major muscle were taken from cattle shortly post mortem. Natural actomyosin was prepared by the method of Heffron & Duggan (1971). The material was suspended finally in distilled deionized water and kept (at 2°C) for not more than 4 days. ATPase activity was measured by incubation (for 5min) at 37°C in 25mM-Tris-HCl buffer, pH7.2, containing either MgATP2- or CaATP2- (3.0mM). There was sufficient Ca2+ (0.1mM) present in the actomyosin to ensure activation of the Mg2+-dependent ATPase. The ionic strength of the assay medium (0.03M) was progressively increased by the addition to the solution of an appropriate concentration of KCl. The Pi liberated was determined by the method of Taussky & Shorr (1953), and ATPase activity is expressed as pmol of Pi/min per mg of protein.

The activity of various preparations of natural actomyosin and the effect of ionic strength on activity is shown in Fig. 1. Actomyosin from Piétrain white fibres split MgATP2- at a higher rate than did actomyosin from Landrace white fibres; and white-fibre actomyosin, irrespective of the breed of pig, was more active in the splitting of MgATP2- than was red-fibre actomyosin. The activity of the actomyosin from bovine muscle was similar to that of the actomyosin from pig red fibres. The differences in activity between the red fibres of the two breeds of pig and between the two muscles of the ox were relatively small, so in each case the results were combined for presentation in Fig. 1. White fibres and red fibres responded in a markedly different manner to an increase in the ionic strength of the assay medium. The white-fibre Mg2+-activated ATPase was activated initially by an increase but activity declined sharply above I = 0.15M. Red-fibre actomyosin showed a greater sensitivity to ionic strength than did white-fibre actomyosin, and the ATPase activity declined steadily as the concentration of KCl increased. The red-fibre enzyme was inhibited to 50% of its original activity at I = 0.15M. In the absence of KCl from the assay medium, pig red fibres exhibited about 70% the initial ATPase activity of that of the white fibres, but the results suggest that at intrafibre physiological ionic strength (≈ 0.15M) the activity of red-fibre actomyosin might be as little as 35% of that of white-fibre actomyosin.

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† Abbreviation: ATPase, adenosine triphosphatase.
Fig. 1. Effect of ionic strength on the hydrolysis of MgATP$_2^-$ (3.0 mm) at 37°C in 25 mm-Tris-HCl buffer, pH 7.2.

■, Pietrain pig semitendinosus-muscle white fibres; □, Landrace pig semitendinosus-muscle white fibres; ▲, Landrace and Pietrain pig semitendinosus-muscle red fibres (combined results); ●, ox longissimus dorsi and psoas muscles (combined results).

The hydrolyses of CaATP$_2^-$ and MgATP$_2^-$ by natural actomyosin from the ox muscles (combined results) are compared in Fig. 2. The Ca$^{2+}$-activated enzyme split ATP at a higher rate than did the Mg$^{2+}$-activated ATPase. The hydrolysis of CaATP$_2^-$ was stimulated by an increase in ionic strength to about 0.15 M, but above this value the activity declined rapidly. In contrast with Ca$^{2+}$-activated enzyme, the Mg$^{2+}$-activated ATPase was rapidly inhibited by an increase in ionic strength. On the premise that the hydrolysis of CaATP$_2^-$ by actomyosin reflects the activity of the myosin component of this material, the results appear to be in agreement with those of Bendall (1969), who found that an increase in ionic strength to 0.15 M stimulated the hydrolysis of ATP by myosin, and suggest that the inhibition of Mg$^{2+}$-activated actomyosin involves an interference with actin–myosin cross-linkages.

The results show that Mg$^{2+}$-activated actomyosin from white, or rapidly contracting fibres had a relatively high ATPase activity, which was not decreased until the ionic strength was raised above 0.15 M. Actomyosin from red, or slowly contracting fibres had a lower ATPase activity, which was sensitive to an increase in ionic strength. It is known that the white fibres of pig semitendinosus muscle, in contrast with the red fibres, have a high-energy phosphate potential in vivo and, under certain circumstances, a high rate of ATP turnover post mortem (Tarrant et al., 1972). Psoas major and longissimus dorsi muscles of the ox hydrolyse ATP at a very low rate post mortem (Mothersill & McLoughlin, 1974). We suggest that variations in the rate of ATP hydrolysis may be related to differences in the binding properties of actin and myosin and that such differ-
Fig. 2. Comparison of the effects of ionic strength on actomyosin ATPase activity of ox muscle

Combined results for psoas and longissimus dorsi muscles are shown. •, CaATP$^2$- as substrate; ○, MgATP$^2$- as substrate (in the presence of 0.1 mM-Ca$^{2+}$).

ences may be reflected in the sensitivity of the myofilament cross-linkages to changes in ionic strength. In this context it is noteworthy that myosin from white fibres contains C$_1$, C$_2$ and C$_3$ light chains whereas the C$_2$ chain is absent from red-fibre myosin (Taylor, 1972). However, the possible role of the light chains in the binding of actin and myosin has not yet been clarified.

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