MYOSIN SUBFRAGMENT 1 MODULATES THE BINDING OF GLYCOLYTIC ENZYMES TO MUSCLE MYOFIBRILS

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SUMMARY

The binding of fructose bisphosphate aldolase and glyceraldehyde-3-phosphate dehydrogenase to rabbit muscle myofibrils as a function of the concentration of added enzyme has been examined in the presence and absence of myosin subfragment S-1. In the presence of S-1, the binding of aldolase is reduced by almost half at high aldolase concentration. By contrast, S-1 markedly increases both the affinity and stoichiometry of glyceraldehyde-3-phosphate dehydrogenase binding. The presence of Ca^{2+} increases binding of aldolase, but is without effect on glyceraldehyde-3-phosphate dehydrogenase binding. The effect of the non-metabolizable ATP-analogue adenylylimidodiphosphate is to increase the binding of aldolase. The results show that enzyme binding is influenced by effectors of myofibril organisation and activation and suggest that the mechanical signal of actin-myosin interaction could be utilized to signal changes in glycolytic enzyme organisation and control.

INTRODUCTION

Wilkie (1983) has recently shown that the onset of accelerated glycolysis induced by muscle contraction precedes any changes in the concentration of ATP, ADP or AMP as measured by nuclear magnetic resonance (NMR). He concluded that glycolytic activation in muscle is directly linked to the contractile cycle. On the face of it, these finding do not support the view that the work-induced stimulation of glycolysis is the result of ADP of AMP influencing the activity of phosphofructokinase as a consequence of ATP depletion.

It seems warranted then, to examine glycolytic events more closely related to the filament system of muscle and one such direct association is the absorption of glycolytic enzymes to the I-band of cardiac and skeletal muscle. The binding of glycolytic enzymes, notably fructose bisphosphate aldolase and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was first demonstrated by Arnold and Pette (1968). This enzyme binding has now been shown to vary with physiological manipulation such as electrical stimulation of skeletal muscle (Walsh et al. 1981) and ischemia of heart (Clarke et al. 1984). In general, increased glycolysis is associated with higher levels of binding and in some situations, notably ischemic sheep heart, up to 90% of the aldolase, GAPDH and phosphofructokinase are found in association with the particulate fraction of muscle homogenates.

In this report, we examine the influence of myosin of head/actin interactions on the binding of aldolase and GAPDH to rabbit myofibrils washed to remove endogenous enzyme. As there are pre-existing myosin-actin links in myofibrils in the overlap region of the A-I band, it is of interest to determine whether adenyl-imidodiphosphate (AMP-PNP) can effect the binding of aldolase. AMP-PNP is a non-hydrolyzable analogue of ATP which can change the effective sarcomere length of muscle fibres and reduce the affinity

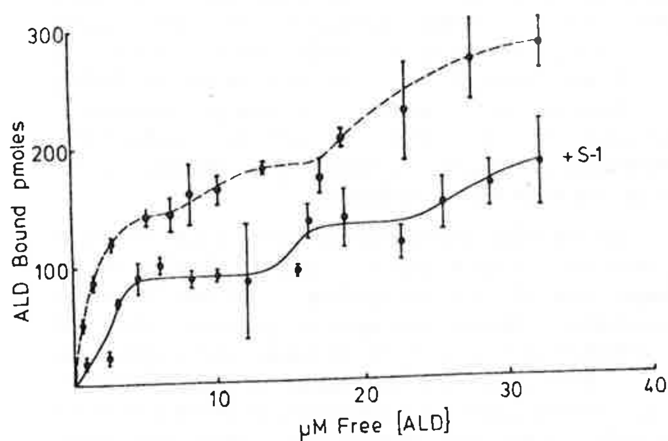

Figure 1. Binding of aldolase to rabbit skeletal myofibrils in the absence (dotted line) and presence (solid line) of S-1. Each point represents the mean of triplicate determination with S.D. shown.

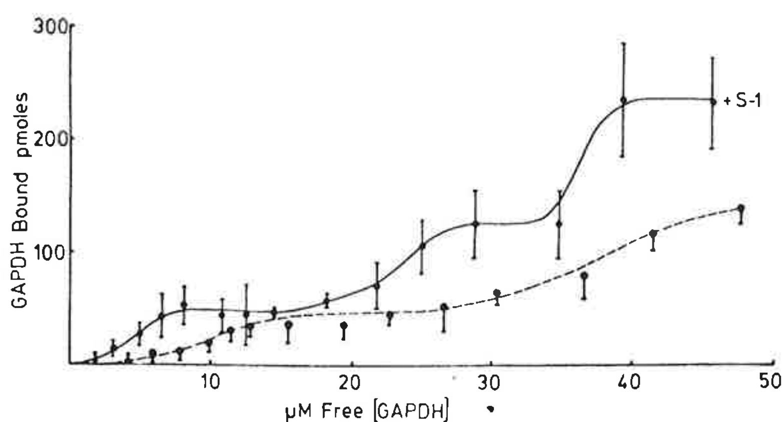

Figure 2. Binding of glyceraldehyde-3-phosphate dehydrogenase to rabbit skeletal myofibrils in the absence (dotted line) or presence (solid line) of S-1. Each point represents the mean of triplicate determination with the S.D. shown.

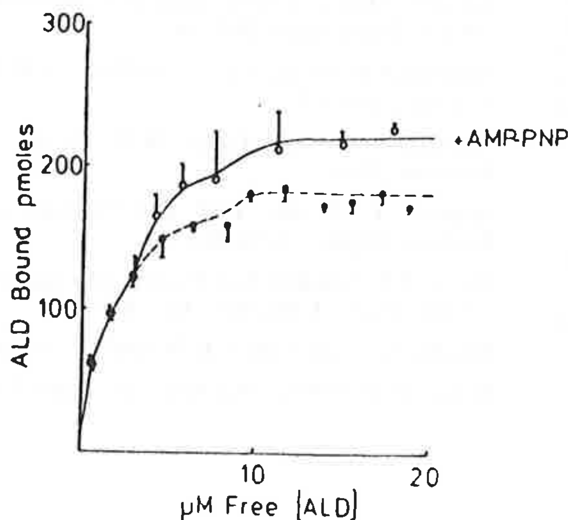

Figure 3. Binding of aldolase to rabbit skeletal myofibrils in the absence (closed circles) and presence (open circles) of 200 μM AMP-PNP. Each point represents the mean of triplicate determinations.

of heavy meromyosin for actin by 100-fold (Marston et al. 1976).

METHODS AND MATERIAL

Myofibrils: Rabbit skeletal muscle myofibrils were prepared at 4°C by a modification of the method of Goodno et al. (1978). Myosin S-1 was prepared by the procedure of Weeds and Taylor (1975) and used at a weight ratio of S-1:actin of 2:1. Enzyme binding was measured using enzymes radio-iodinated by the method of Bolton-Hunter (see Clarke et al. 1984)).

RESULTS

A comparison of aldolase binding to myofibrils in the presence and absence of S-1 is shown in Fig.1. The effect of S-1 is to reduce the affinity and apparent stoichiometry of aldolase binding over the entire range of aldolase concentrations tested which included the levels of aldolase normally found intracellularly. Furthermore, the nature of the binding appears as sigmoidal indicating that S-1 has changed the character of the enzyme-filament interaction. Under identical conditions, there is no detectable interaction between S-1 and the [125I]-labelled enzymes.

Glyceraldehyde-3-phosphate dehydrogenase binding to myofibrils is also influenced by the interaction of S-1 with the myofibrils and the results are shown in Fig.2. It is evident that S-1 binding results in a marked increase in the affinity of glyceraldehyde-3-phosphate dehydrogenase for its binding site on the myofibril. Three discrete binding maxima are now apparent.

Figure 3 shows a comparison of aldolase binding to myofibrils in the presence and absence of 200 m AMP-PNP. In the presence of the ATP analogue there is no effect on early phase of binding but there is an increase in the affinity and/or stoichiometry at higher aldolase concentration.

DISCUSSION

In terms of the amount of enzyme bound Aldolase and GAPDH are quantitatively the most significant of the glycolytic enzymes bound to the I-band of muscles. While GAPDH is well recognised as a control point in glycolysis, aldolase on the basis of a relatively low total enzyme activity be considered as having regulatory potential (Scrutton and Utter 1968). Changes in the binding of both these enzymes as a consequence of a myosin-actin interaction have the potential to influence the glycolytic behaviour of muscle. There are certainly changes in the kinetic parameters of aldolase activity as a consequence of binding with marked increases of Vmax and Km. Such differences between the catalytic capabilities of free and bound enzyme commend themselves as being of regulatory significance. As no interaction of glycolytic enzymes with myosin or its subunits are found, it must be concluded that the changes in binding must be attributed to the structural rearrangements known to occur when myosin interacts with the I-band filament. There is evidence that there is a displacement of tropomyosin with respect to the F-actin helix during the contraction cycle and this may explain alterations to the site of enzyme binding after S1-actin interactions. Studies on ordered paracrystals show that F-actin alone, F-actin with tropomyosin and F-actin-tropomyosin-troponin all offer different sites for aldolase binding and the binding curves in Figure 1 provide some indication that more than one site is affected in the presence of S-1.

We have recently presented evidence that triosephosphate isomerase is capable of forming a minicomplex by binding to aldolase and glyceraldehyde-3-phosphate dehydrogenase when these latter enzymes are bound to the actin filament of the I-band (Stephan et al. 1986). It follows then that any effect of myosin on the binding of aldolase and GAPDH also has the potential to influence the indirect binding of triose phosphate isomerase. Thus myosin may be more influential in the behaviour and organisation of a rather broader range of glycolytic enzymes than those directly studied here.

AMP-PNP is known as an effective competitive inhibitor for ATP since it binds to the enzymic site of myosin without being susceptible to enzymic cleavage. X-ray diffraction and electron microscopy studies suggest that the addition of AMP-PNP causes a structural change in muscle fibres with respect to the cross-bridge reaction (Marston et al. 1976). In the present experiments the effect of AMP-PNP in favouring aldolase binding can be attributed to the effect of the inhibitor in loosening actin-myosin head interactions. Clearly the binding of glycolytic enzymes in the overlap A-I band can vary from that seen in the I-band itself, particularly in highly contracted muscle.

Recent studies of the binding of phosphofructokinase to actin have shown that phosphorylation of the enzyme leads both to increase enzyme binding and freedom from

allosteric control (Kuo et al. 1986). It remains to be determined whether S-1 influences phosphofructokinase binding, but the presumption of alterations to the configuration of the actin filament do not rule out the possibility of an effect. Thus binding, whether influenced by myosin, phosphorylation or Ca^{2+} may contribute to the mechanochemical coupling between contraction and glycolysis.

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