Rapid Papers

(Pages 165–176)

Modification of the Kinetic Parameters of Aldolase on Binding to the Actin-Containing Filaments of Skeletal Muscle

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(Received 6 April 1977)

The kinetic parameters of fructose bisphosphate aldolase (EC 4.1.2.13) were shown to be modified on binding of the enzyme to the actin-containing filaments of skeletal muscle. Although binding to F-actin or F-actin-tropomyosin filaments results in relative minor changes in kinetic properties, binding to F-actin-tropomyosin-troponin filaments produces major alterations in the kinetic parameters, and, in addition, renders them Ca^{2+} -sensitive. These observations may be relevant to an understanding of the function of this enzyme within the muscle fibre.

The interaction of aldolase and other glycolytic enzymes with the structural proteins of the I-band of skeletal muscle is now well established (Clarke & Masters, 1976). The histochemical studies by Sigel & Pette (1969), for example, have located most of the glycolytic enzymes within the I-band, whereas biochemical studies (Arnold & Pette, 1968, 1970; Arnold *et al.*, 1971) have shown that glycolytic enzymes such as aldolase, pyruvate kinase, glyceraldehyde 3-phosphate dehydrogenase and others interact strongly with purified F-actin. More recently Clarke & Masters (1975) have pointed to the physiological feasibility of enzyme adsorption by demonstrating that enzyme binding can occur at ionic strengths and protein concentrations approximating to those found within the muscle. These studies also revealed that the regulatory proteins of the I-band filaments, tropomyosin and troponin, exert an important influence on enzyme adsorption on actin-containing filaments.

In spite of these studies the physiological significance of enzyme binding remains unclear. One suggestion is that adsorption may alter the kinetic parameters of an enzyme (Clarke & Masters, 1976), and indeed Arnold & Pette (1970) have reported a modification of aldolase kinetics on binding to purified F-actin. It is now widely recognized that the thin filaments of the I-band of skeletal muscle are not pure F-actin, however, but rather complexes of F-actin, tropomyosin and troponin; and, in recognition of this fact, it was decided to investigate the influence of these latter proteins on the binding and subsequent modification of the kinetic parameters of aldolase. This present paper deals with a pronounced change in the kinetic parameters of aldolase on binding to filaments reconstituted from F-actin, tropomyosin and troponin.

Materials and Methods

Ox muscle aldolase was purified by phosphocellulose chromatography (Penhoet *et al.*, 1969) to a specific activity of 18–22 $\mu\text{mol}/\text{min}$ per mg of protein at 26°C. F-actin and the tropomyosin-troponin complex were prepared from acetone-dried powders of ox skeletal muscle by the method of Spudich & Watt (1971). Tropomyosin and troponin were purified as described by Clarke *et al.* (1976). The F-actin-tropomyosin-troponin complexes were prepared by using a modification of the method of Ishiwata (1973). All preparations were examined by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis (Clarke *et al.*, 1976) to determine whether significant amounts of impurity were present.

Kinetic assays were performed in 5 mm-imidazole/HCl buffer, pH 6.8, containing 10 mM-KCl, 1 mM- MgCl_2 and 1 mM-dithiothreitol. Each 1 ml of assay mixture contained 100 μg of NADH and 16 μg of α -glycerol phosphate dehydrogenase/triose phosphate isomerase mixture (Sigma Chemical Co., St. Louis, MO, U.S.A.). Aldolase, filament preparations and fructose 1,6-bisphosphate were added at the desired concentrations indicated below. The aldolase and the α -glycerol phosphate dehydrogenase/triose phosphate isomerase mixture were completely freed of $(\text{NH}_4)_2\text{SO}_4$ by extensive dialysis against the above buffer. Fructose 1,6-bisphosphate (tetrasodium salt; Sigma) solutions were prepared in the assay buffer and the concentration was determined spectrophotometrically immediately before use, by using the aldolase and α -glycerol phosphate dehydrogenase/triose phosphate isomerase system. The assays were performed at 26°C by using a thermostatically controlled Unicam SP.800 spectrophotometer with scale expansion or a Cary 14 spectrophotometer.

Protein concentrations were generally determined by the method of Lowry *et al.* (1951) with bovine serum albumin as standard. Pure ox muscle aldolase concentration was determined by using an $A_{280}^{0.1\%}$ value of 0.95 determined refractometrically.

Results and Discussion

The investigation of the kinetic parameters of aldolase when bound to F-actin-containing filaments is made difficult experimentally by the influence of ionic strength and substrate concentrations on enzyme adsorption, particularly at the low enzyme concentrations used in kinetic analyses (Arnold & Pette, 1970). For these reasons a low-ionic-strength assay system with low fructose biphosphate concentrations was used, as described in the Materials and Methods section, a procedure that is similar to the assay system successfully used by Arnold & Pette (1970). With this assay system initial experiments were performed in which $2\mu\text{g}$ of aldolase was incubated with increasing concentrations of filament preparations. Centrifugation of these mixtures and assay of the resuspended pellets gave a measure of the amount of aldolase bound by this procedure. The minimum quantity of filament preparation required to ensure complete adsorption was $300\text{--}400\mu\text{g}$; consequently a quantity of $500\mu\text{g}$ was used in the following experiments. Such control centrifugation also revealed the complete adsorption of aldolase over the substrate range used and established that the linking enzymes were not bound under these conditions. In addition, inhibitory influences of the filaments on the linking enzymes were also examined, and found to be insignificant.

The kinetic data obtained with this assay system gave rise to linear double-reciprocal plots from which the numerical values of K_m and $V_{\max}$, shown in

Table 1. *Kinetic parameters of aldolase in the presence and in the absence of filaments*

Values are given as means $\pm$ S.E.M. The numbers of determinations are indicated in parentheses.

Assay mixture	K_m (μM)	$V_{\max}$ ($\mu\text{mol}/$ min per mg of aldolase)
Aldolase	1.0 ± 0.15	20.1 ± 0.9 (8)
Aldolase/F-actin	8.0 ± 0.7	26.1 ± 2.0 (4)
Aldolase/F-actin-tropomyosin	12.0 ± 1.0	28.1 ± 3.7 (4)
Aldolase/F-actin-tropomyosin-troponin/ 0.1 mM- CaCl_2	20.0 ± 2.4	56.2 ± 4.2 (10)
Aldolase/F-actin-tropomyosin-troponin/ 0.2 mM-EGTA	100.0 ± 11.8	80.4 ± 9.8 (10)

Table 1 were obtained by least-squares analysis. The K_m value for pure soluble aldolase compares favourably with previously reported values, and the increase in magnitude of both K_m and $V_{\max}$ on binding to pure F-actin were similar to the previous observations by Arnold & Pette (1970). Binding to F-actin-tropomyosin produced changes in $V_{\max}$ and K_m that were very similar to those observed on binding to pure F-actin. In neither case were these parameters affected by the concentration of Ca^{2+} ions.

In contrast, binding of aldolase to F-actin-tropomyosin-troponin filaments caused a marked change in kinetic parameters when compared with either the soluble or the F-actin-bound enzyme. By comparison with the soluble enzyme, for example, the apparent K_m was increased from 1 to $100\mu\text{M}$ (cf. 1 to $8\mu\text{M}$ for F-actin), whereas the apparent $V_{\max}$ was increased 4-fold (cf. 1.4-fold for F-actin) in the absence of Ca^{2+} . Most interestingly, these parameters were significantly influenced by the presence of Ca^{2+} , when the apparent K_m was substantially lowered (from 100 to $20\mu\text{M}$), whereas the maximal catalytic capacity was only slightly affected and was still 3-fold greater than that of the soluble enzyme.

In view of the fact that F-actin-tropomyosin-troponin provides a substantially more accurate analogue *in vitro* of the thin filament of skeletal muscle than does F-actin, the present results serve to indicate that there may exist within muscle the

Fig. 1. *Dependence of aldolase activity on fructose biphosphate concentration*

Curve (a), soluble enzyme; curve (b), 50% enzyme bound to thin filament in the absence of Ca^{2+} ; curve (c), 100% enzyme bound to thin filament in the absence of Ca^{2+} ; curve (d), 100% enzyme bound to thin filament in the presence of Ca^{2+} . Activity curves were calculated from data in Table 1; the $V_{\max}$ values are expressed relative to that of the enzyme bound to thin filaments in the absence of Ca^{2+} .

means for a far more significant modification of aldolase kinetic parameters than previously expected (Arnold & Pette, 1970). Taken together with the earlier cited evidence in favour of the physiological reality of enzyme adsorption, the present data make it clear that a substantial divergence may exist between the kinetic properties of aldolase *in vivo* and those determined for the isolated soluble enzyme.

The effect of these modifications is illustrated in Fig. 1, which compares the catalytic capacity of aldolase as a function of fructose biphosphate concentration on the assumption that the enzyme is completely soluble (curve *a*), 50% bound (curve *b*) on totally bound (curve *c*). Comparison of these curves indicates that increasing enzyme adsorption results in a decreased catalytic capacity at low concentration of fructose biphosphate; by contrast, at higher concentrations ($>30\ \mu\text{M}$), enzyme adsorption leads to a higher activity than would be expected from the soluble enzyme at an equivalent fructose biphosphate concentration. Such an increased catalytic capacity may be most appropriate during rapid glycolysis when fructose biphosphate concentrations are markedly raised, e.g. in muscular contraction (Ozand & Narahara, 1964).

A second important feature of this system can be noted by comparing curves (*c*) and (*d*), which depict the effects of the presence and of the absence respectively of Ca^{2+} . As the prime role of this ion on the initiation of muscle contraction is well documented, its effect on the catalytic capacity of aldolase in this system is noteworthy, and may be considered in relation to the co-ordination of energy production and contraction in this tissue. A coupling of muscle mechanics and metabolism has been suggested previously in the case of the phosphorylase system, for example (Heilmeyer *et al.*, 1970). In the present instance, lowering of the apparent K_m with minimal

decrease in V_{max} in the presence of Ca^{2+} may be conceived as a means of facilitating the attainment of increased catalytic capacity after initiation of contraction.

In conclusion, the present data provide evidence that binding of aldolase to thin filaments causes a significant alteration in the enzyme's catalytic properties and delineate a definite role for tropomyosin-troponin in this phenomenon. These observations should be taken to stress the need for continuing studies at this level of organization to understand more fully the means by which enzyme adsorption may be utilized in the regulation of metabolic processes.

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