THE EFFECT OF GLYCOLYTIC ENZYMES ON THE STRUCTURE AND PHYSICAL PROPERTIES OF ACTIN

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SUMMARY

The addition of glycolytic enzymes, notably aldolase and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), to actin filaments results in marked increase in the viscosity of the preparations. At higher aldolase concentrations, however, this viscosity falls abruptly as large filament bundles are formed. This crosslinking property is modified by the presence of enzyme substrate. Morphological evidence is presented that glycolytic enzymes are involved in actin bundling, nucleation and filament severing activity and, as such, have sequence homology with actin and the other actin severing proteins gelosolin, severin and fragmin.

A role for glycolytic enzymes as structural components of the cell cytoskeleton is proposed. As such they would be responsive to the metabolic demands of the cell.

INTRODUCTION

Actin and glycolytic enzymes can be regarded as conserved universal proteins in all eucaryotic cells: actin is the principal cytoskeletal protein, while the fundamental metabolic role of the glycolytic enzymes is well known. It is also well established that actin acts as an absorbent for these enzymes in both muscle and non-muscle cells providing organisation to the metabolic system (Clarke et al. 1985). In skeletal muscle, the actin filament based organisation of these enzymes within the I-band is envisaged as providing an efficient enzyme system for the direct delivery of ATP to its site of utilisation at the actin-myosin interface. While this function is undoubtedly an important feature of enzyme-actin associations it may not be the only one.

It has long been recognised that glycolytic enzymes occur in muscle and other cells and tissues in very considerable quantities, which more often than not, are far in excess of the amounts required to catalyse even the maximal glycolytic rates observed (Ottaway and Mowbray 1977). As a consequence there has been a belief that at least some of these enzymes may fulfil other rules within the cell, and indeed there is now growing evidence to suggest this belief is well founded, as exemplified by the recent demonstration of a structural role for GAPDH in the formation of the muscle triad function (Caswell and Corbett 1985). In this paper we extend the concept by demonstrating that studies on enzyme-actin associations also suggest that these enzymes should be considered as structural elements of the cytoskeleton, specifically as actin modulating proteins contributing to, and participating in the regulation of the structure of actin based cytoskeletal systems.

EXPERIMENTAL METHODS

Protein preparations: Rabbit muscle actin was prepared by the method of Spudick and Watt. Rabbit muscle aldolase and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were purified as detailed elsewhere (Clarke et al. 1984).

Low shear viscosity measurements: Mixtures of F-actin (200 g) and the desired concentrations of enzyme(s) were prepared in a final volume of 100 µl m 10 mM Imidazole, 40 mM KCl, 1 mM MgCl, 0.5 mM DTT (I KMD) buffer. The mixtures were immediately drawn into 100 µl glass capillary micropipette tubes, sealed at one end and incubated at room temperature for 1 h. Low shear viscosity was then determined using the capillaries as part of a falling ball viscometer which was constructed, operated, and calibrated essentially as described by McLean and Pollard (1980).

Electron microscopy: Negative staining was done with 1% uranyl-acetate. For SEM samples were placed on polylysine coated glass coverslips, fixed by immersion in 1% glutaraldehyde, dehydrated through graded alcohols, stained with 1% uranyl acetate in ethanol, critically point dried, rotary coated with carbon and examined in a Joel 35 µl SEM.

RESULTS

Enzyme modify F-actin structure

During biochemical studies on the binding of enzymes to F-actin we observed that the addition of enzymes to actin solution caused dramatic changes in the physical properties of these solutions. The actual changes induced were dependent on the enzyme or combination of enzymes, the amount of enzyme added, and the

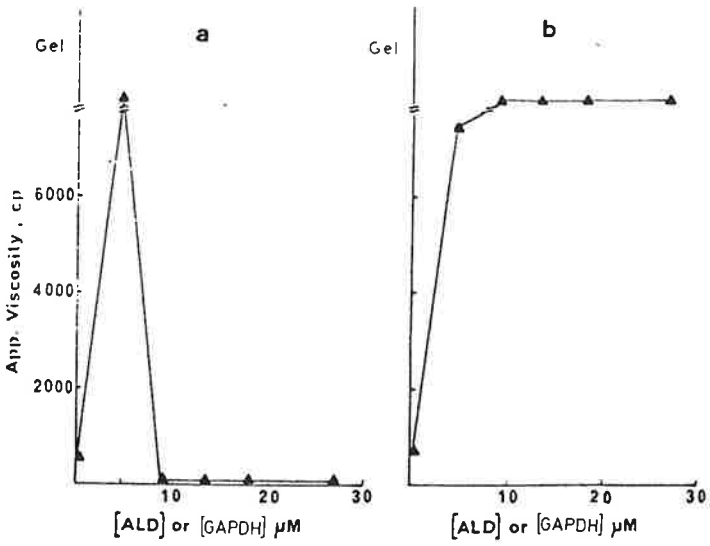

Figure 1. Low-shear viscosities of F-actin-enzyme mixtures. F-actin (2 mg/ml) was mixed with enzymes at the indicated concentration in IKMD-buffer pH 6.8 and the low-shear viscosity measured after 60-120 minutes at room temperature.

a: F-actin/aldolase; glyceraldehyde-3-phosphate dehydrogenase
b: F-actin/glyceraldehyde-3-phosphate dehydrogenase/aldolase + 100 µM fructose bisphosphate.

Table 1

An homologous and conserved sequence in aldolase and actin.

α-Actin	359	Q	E	Y	D	E	A	G	P	S	I	V	H	R	K	C	F	374
β,γ-Actin	359	Q	E	Y	D	E	S	G	P	S	I	V	H	R	K	C	F	374
ALD-A	30	L	A	A	D	E	S	T	G	S	I	A	K	R	L	Q	S	45
ALD-B	30	L	A	A	D	E	S	V	G	T	N	G	Q	R	L	N	K	45
ALD-C	30	L	A	A	D	E	S	V	G	S	M	A	K	R	L	S	Q	45
Dros	30	L	A	A	D	E	S	G	P	T	H	S	K	R	L	Q	D	45
Maize	27	L	A	A	D	E	S	T	G	T	I	G	K	R	L	S	S	42
Trypanos	41	L	A	A	D	E	S	T	G	S	G	S	K	R	L	A	G	56

Standard one-letter code for amino acids where A=Ala R=Arg D=Asp C=Cys Q=Gln E=Glu G=Gly H=His I=Ile L=Leu K=Lys M=Met F=Phe P=Pro S=Ser T=Thr Y=Tyr V=Val. Numbers refer to order in sequence. Dros is Drosophila aldolase, Trypanos is Trypanosome aldolase.

presence or absence of specific glycolytic metabolites which are either substrates or modifiers of the enzymes. We could monitor these physical changes by using low shear viscosity measurements and at the same time visualise the structures formed by using electron microscopy techniques. Some of the results obtained are shown below. Figures 1a and b show for example the changes in low shear viscosity observed on adding increasing concentrations of an equimolar mixture of aldolase and GAPDH to F-actin solutions in the absence (Fig.1a) and presence (Fig.1b) of the aldolase substrate fructose-1,6-bisphosphate (FBP). In the absence of FBP, the addition (and binding) of low levels of aldolase and GAPDH induce gel formation as these enzymes crosslink the actin filaments. However as more enzymes are added a precipitous change in physical properties occurs (Fig.1a). The solution becomes very turbid and the gel structure breaks down as the mixtures assume the properties of a sol, with low shear viscosities well below that of actin solutions alone. Under these conditions electron microscopy reveals that as more enzymes bind, the actin filaments are organised into large diameter filament bundles due to the crosslinking properties of the enzymes. These bundles however are relatively short as the actin filaments are also reduced in length by the enzymes expressing an actin severing activity.

When FBP is added (Fig.1b) the physical changes are quite different. In the presence of this enzyme modifier a gel structure is formed at low enzyme concentrations and maintained over the entire range tested.

In this situation electron microscopy reveals that the enzymes have organised the actin into a network of small diameter, anastomosing actin filament bundles. The ability of FBP to so dramatically change the properties of these enzyme actin mixtures is not due to it inhibiting the binding of one enzyme or the other, as binding studies reveal that both bind quite effectively in its presence. Rather FBP changes the way in which the enzymes bind and so the physical structures formed. It appears to limit the crosslinking ability of aldolase and also its severing activity and these changes may well account for the different structures which are formed.

Aldolase shares sequence homology with actin

In examining the sequences of aldolase and the actin capping/severing protein gelsalin, severin and fragmin, we have found that their putative actin binding domains contain regions of conserved homologous sequence. Moreover these regions share homology well a conserved C-terminal segment of actin itself which is believed to be in the vicinity of the actin-actin interactive site involved in F-actin polymer formation. Table 1 shows part of the conserved segment of aldolases and the homologous region of actin. The homology in this segment is quite striking and centres around a "DESG" segment also found in the putative actin binding sites of the other actin severing proteins mentioned above.

DISCUSSION

The present data suggest that some glycolytic enzymes may express both catalytic and structural functions within the cell. Aldolase and GAPDH have been shown to be actin-binding proteins which may exhibit actin bundling, severing and nucleating activities to control the physical properties of actin solutions. In this regard they are shown to share functional properties with other well-established actin-modulating proteins such as gelsalin, severin and fragmin. Moreover aldolase has been found to share some conserved sequence homology with these proteins and with actin itself, raising the interesting possibility of a common evolutionary origin. Functionally the most interesting observation was that the actin modulating properties of these enzymes could be regulated by specific glycolytic metabolites. Consequently the expression of this functional duality by some glycolytic enzymes could provide the means by which the cell integrates the structure and function of its cytoskeleton with the metabolic activity which provides that cytoskeleton with its immediate energy supply.

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