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Antigenic probes locate binding sites for the glycolytic enzymes glyceraldehyde-3-phosphate dehydrogenase, aldolase and phosphofructokinase on the actin monomer in microfilaments

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The topology of the interfaces between actin monomers in microfilaments and three glycolytic enzymes (glyceraldehyde-3-phosphate dehydrogenase, aldolase and phosphofructokinase) was investigated using several specific antibodies directed against precisely located sequences in actin. A major contact area for glyceraldehyde-3-phosphate dehydrogenase was characterized in a region near residue 103. This interaction altered, by long-range conformational changes, the reactivity of antigenic epitopes in the *C*-terminal part of actin. The interface between actin and aldolase appeared to involve a sequence around residue 299 in the *C*-terminal region of actin. The interaction of phosphofructokinase, in contrast, modified the reactivity of all antibodies tested. Finally, the phosphagen kinases arginine kinase and creatine kinase showed no interaction with the microfilament.

INTRODUCTION

A considerable number of cytoplasmic enzymes can interact (under physiological conditions of ionic strength and pH) with each other and with various cellular structures (membrane, cytoskeleton, myofibrils, etc.) (Masters, 1981). The binding of glycolytic enzymes to contractile components is especially well documented (for reviews, see Clarke & Masters, 1976; Masters, 1984). Their cyto-localization by histochemistry has revealed compartmentalization within the I band of the muscle fibre (Siegel & Pette, 1969). Most of the glycolytic enzymes appear to be associated with myofibril components, but three of them [phosphofructokinase (PFK), glyceraldehyde-3-phosphate dehydrogenase (GPDH) and aldolase] have been used in a number of studies because of their high affinity for microfilaments and because their adsorption affects catalytic functions. The compartmentalization of another enzyme group, the phosphagen kinases, has also been extensively studied in relation to myofibril topology (Walliman *et al.*, 1977; Lang *et al.*, 1980). The analysis of the compartmentalization of these two groups of enzymes is important for understanding the role of ATP flux in muscular contraction. The adsorption of the enzymes on to cellular structures alters their kinetic and regulatory properties (Clarke & Masters, 1976; Masters, 1984). For instance, the binding of glycolytic enzymes, in conjunction with creatine kinase, to contractile elements keeps intermediary metabolites and ATP at hand for the next enzymic step or for myosin activity, via the phenomenon of metabolite channelling (Arrio-Dupont *et al.* 1985; Arrio-Dupont, 1988). Furthermore, the equilibrium of these enzymes between soluble and bound fractions and their versatility may have a physiological significance. For example, changes in the amounts of aldolase or PFK bound to myofibres in the heart are related to the drop in pH following a limited experimental ischaemia (Masters, 1981; Clarke *et al.*, 1984). Similarly, electrical stimulation of skeletal muscle increases the interaction of PFK, GPDH and aldolase with contractile proteins (Masters, 1981; Walsh *et al.*, 1981).

These interactions have been shown (Masters, 1984) to concern essentially the components of the microfilament, and notably filamentous F-actin, at least in reconstituted systems (Arnold *et al.*, 1971). The strong dependence of these complexes on ionic strength and pH, and their lability, require that high enzyme and actin concentrations such as those found in muscle cells be used, or that the ionic strength be decreased (Clarke & Masters, 1975, 1976).

Several structural analyses have provided evidence for a spatial separation between the catalytic site and the binding site for contractile proteins in the case of aldolase and GPDH (Humphreys *et al.*, 1986*a*,*b*). The topology of the actin-binding sites on the surfaces of aldolase and GPDH has been characterized. These studies show the involvement of some charged amino acid residues based on the electrostatic nature of the interactions (Humphreys *et al.*, 1986*a*,*b*). In contrast, nothing is yet known about the topology of the corresponding sites on the actin filaments.

In the present study, we investigated the location of the interaction sites for these glycolytic enzymes (PFK, GPDH, aldolase) by measuring their competition with anti-actin antibody populations directed against known

Abbreviations used: anti-(1-7) antibodies, anti-[actin-(1–7)-sequence] antibodies; anti-(18–28) antibodies, anti-[actin-(18–28)-sequence] antibodies; anti-(40–113) antibodies, anti-[actin-(40–113)-sequence] antibodies; anti-(285–375) antibodies, anti-[actin-(285–375)-sequence] antibodies; GPDH, glyceraldehyde-3-phosphate dehydrogenase; PFK, phosphofructokinase; F-actin, filamentous actin; S-1, myosin subfragment-1.

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limited portions of the actin sequence. Two phosphagen kinases, creatine kinase and arginine kinase, known to interact with myofibrils, were also used in the studies. This immunochemical approach has recently been used to investigate the interactions of some actin-binding proteins with actin, e.g., DNAase-I, myosin subfragment-1 (S-1), tropomyosin and gelsolin (Boyer *et al.*, 1985, 1987; Méjean *et al.*, 1986, 1987) or with ionic effectors (cations, polyamines, etc.) (Méjean *et al.*, 1988).

MATERIALS AND METHODS

Proteins

Rabbit skeletal muscle and *Pecten maximus* adductor muscle actins were purified (Spudich & Watt, 1971) from acetone powder. Rabbit muscle glycolytic enzymes aldolase (fructose-bisphosphate aldolase; EC 4.1.2.13), GPDH (EC 1.2.1.12) and PFK (EC 2.7.1.11) were provided by Boehringer, and creatine kinase (EC 2.7.3.2) was from Sigma. Arginine kinase (EC 2.7.3.3) from *Homarus vulgaris* tail muscle was purified as previously described (Der Terrossian *et al.*, 1966). The enzymes were freed of ammonium sulphate by extensive dialysis against 10 mM-imidazole/10 mM-β-mercaptoethanol/1 mM-EDTA (pH 6.8). In the case of PFK, 0.1 mM-ATP and 1.0 mM-$MgCl_2$ were added.

Antibodies

Anti-(1–7) antibodies, anti-(18–28) antibodies, anti-(40–113) antibodies and anti-(285–375) antibodies were purified by affinity chromatography as previously described (Benyamin *et al.*, 1986; Méjean *et al.*, 1988). The specificity of anti-(1–7) and anti-(18–28) antibodies has previously been determined (Roustan *et al.*, 1986). Anti-(40–113) antibodies have been shown to be specific for only one epitope located near residue 103 (Méjean *et al.*, 1988; Benyamin *et al.*, 1988), and the anti-(285–375) antibodies are specific for three epitopes in skeletal muscle actin, near residues 299, 325 and 355 (Boyer *et al.*, 1987; Benyamin *et al.*, 1988). The antibody subpopulation directed to the 299 region appears to be specific for skeletal muscle actin, and does not react with *Pecten maximus* actin (Boyer *et al.*, 1987). Anti-IgG antibodies labelled with alkaline phosphatase were from Biosys (Compiègne, France).

Analytical methods

Protein concentrations were determined from their absorbance (Roustan *et al.*, 1971; Benyamin *et al.*, 1986; Humphreys *et al.*, 1986*a*,*b*). SDS/polyacrylamide slab-gel electrophoresis was run according to Laemmli (1970). Co-sedimentation experiments were carried out using a Beckman Airfuge. F-actins (1.4 mg/ml) were incubated for 15 min in the presence of enzyme (0.3 mg/ml) in 10 mM-β-mercaptoethanol/2 mM-$MgCl_2$/10 mM-imidazo buffer, pH 6.8. After ultracentrifugation (30 min at 100000 ***g***), the pellets were analysed by slab-gel electrophoresis. The amount of each protein was determined by densitometry using a Shimadzu C.R. 3A densitometer.

The direct e.l.i.s.a. technique was used to monitor interactions between actin and antibody populations in the presence of enzymes. The assay has been described in detail (Méjean *et al.*, 1987).

Each well of the microtitre plate was coated with 50 μg of F-actin. The coated plates were washed and incubated with 5% protamine solution to saturate all of the free protein-binding sites. After washing, the plate was incubated for 1 h with a selected dilution of enzyme in 3% (w/w) gelatin hydrolysate/0.5% (w/w) gelatin/2 mM-$MgCl_2$/10 mM-imidazole buffer, pH 6.8, and supplemented with the specific anti-actin antibody. The assay was then carried out as described in Méjean *et al.* (1987). Staining was monitored at 405 nm with a Titertek Multiscan Plus instrument (Flow Laboratories). Each sample was assayed in triplicate and the mean values were calculated. Non-specific absorption was determined for each sample using a deactivated uncoated well treated under the same conditions.

RESULTS

Association of PFK, GPDH and aldolase with F-actins

The interactions of the three selected glycolytic enzymes (PFK, GPDH and aldolase) with filamentous actins were allowed to proceed in 2 mM-$MgCl_2$/10 mM-imidazole buffer, pH 6.8, under conditions similar to those described by Clarke & Masters (1976). As shown in Table 1, these enzymes co-sedimented well with skeletal muscle actin filaments under our experimental conditions. Experiments carried out with adductor muscle actin from *Pecten maximus* instead of skeletal muscle actin gave similar results (Table 1). Thus these associations do not appear to depend on the origin (isoform) of the actin.

Under the same experimental conditions, the selected phosphagen kinases, arginine kinase and creatine kinase, did not co-sediment with skeletal muscle actin. An

Table 1. Co-sedimentation of enzyme with F-actin followed by ultracentrifugation

n.d., Not determined.

	Binding (%) to F-actin from:	
	Rabbit skeletal muscle	*Pecten maximus* adductor muscle
PFK	100	100
Aldolase	100	100
GDPH	92	90
Arginine kinase	0	n.d.
Creatine kinase	0	n.d.

Table 2. Apparent dissociation constants of the various specific anti-actin antibody populations for skeletal-muscle F-actin

Values are means ± S.D. of at least three determinations.

Antibody population	$10^8 \times$ Apparent K_D (M)
Anti-(1–7)	2.0 ± 0.5
Anti-(18–28)	2.0 ± 0.5
Anti-(40–113)	1.0 ± 0.3
Anti-(285–375)	0.7 ± 0.2

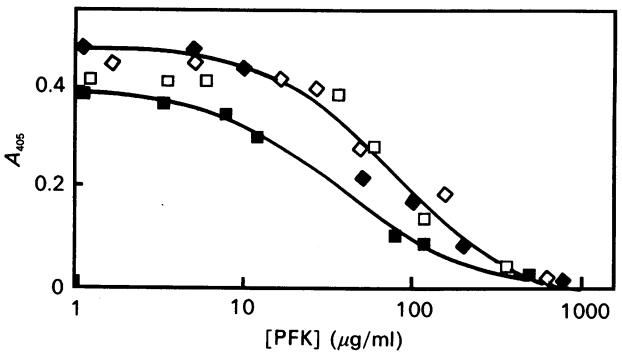

Fig. 1. Reactivity of anti-actin antibodies in the presence of PFK monitored by e.l.i.s.a.

The binding of antibodies was monitored at 405 nm. Specific antibodies were reacted with skeletal muscle F-actin in the presence of increasing PFK concentrations: □, anti-(1–7) antibodies (6 μg/ml); ■, anti-(18–28) antibodies (3 μg/ml); ◆, anti-(40–113) antibodies (2.5 μg/ml); ◇, anti-(285–375) antibodies (6 μg/ml).

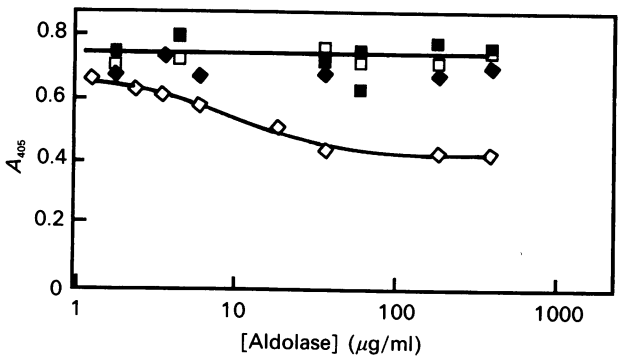

Fig. 2. Reactivity of anti-actin antibodies in the presence of aldolase monitored by e.l.i.s.a.

The binding of antibodies was monitored at 405 nm. Specific antibodies were reacted with skeletal muscle F-actin in the presence of increasing aldolase concentrations: □, anti-(1–7) antibodies (6 μg/ml); ■, anti-(18–28) antibodies (3 μg/ml); ◆, anti-(40–113) antibodies (2.5 μg/ml); ◇, anti-(285–375) antibodies (6 μg/ml).

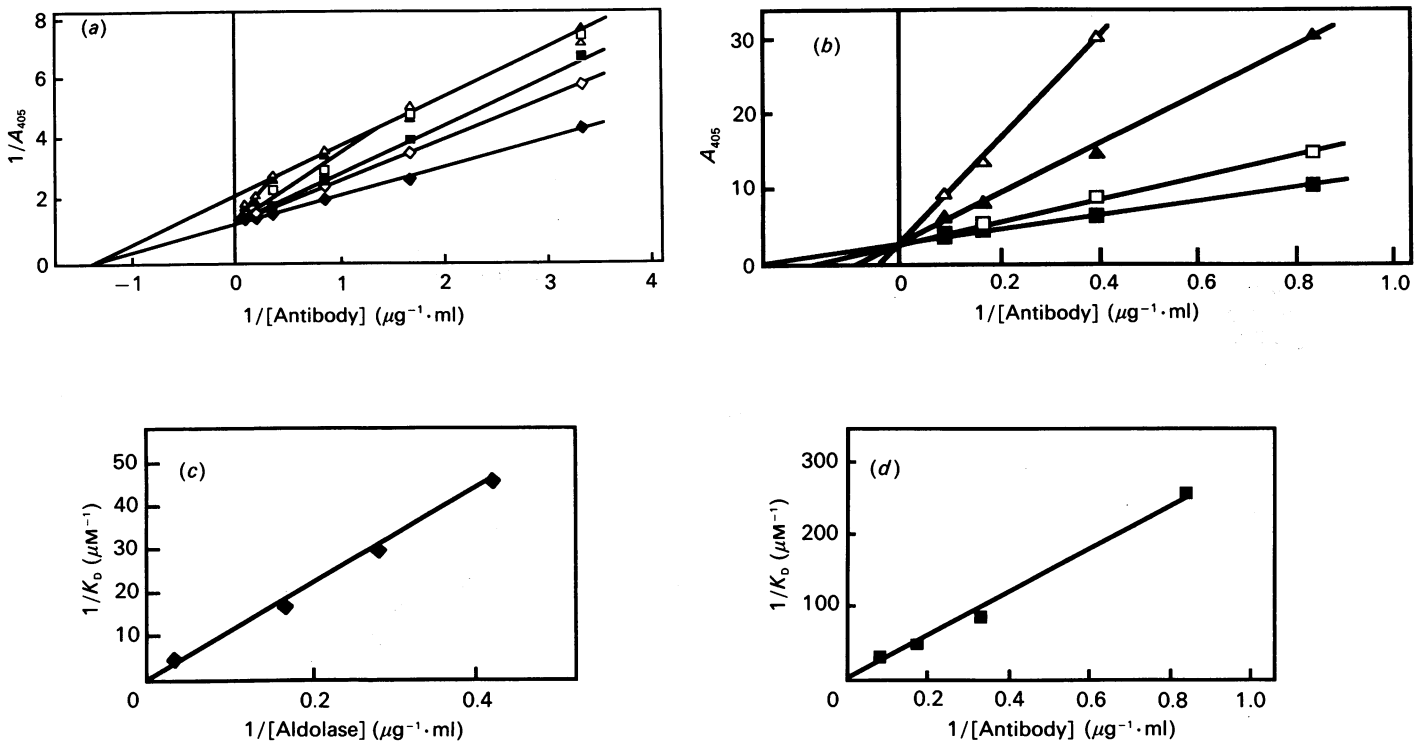

Fig. 3. Analysis of the effect of aldolase on anti-(285–375) antibody reactivity

E.l.i.s.a. was carried out as described in the Materials and methods section. (*a*) Specific antibodies at various concentrations (0.3–12 μg/ml) were reacted with coated skeletal muscle F-actin in the presence of several aldolase concentrations. A plot of $1/A_{405}$ versus 1/[antibody] was made using the following aldolase concentrations: ◆, 0 μg/ml; ◇, 0.6 μg/ml; ■, 2.4 μg/ml; □, 3.6 μg/ml; ▲, 6 μg/ml; △, 36 μg/ml. (*b*) The interaction of the antibody subpopulation affected by aldolase binding was analysed by plotting the corresponding $1/A_{405}$ values versus 1/[antibody] at several aldolase concentrations: ■, 2.4 μg/ml; □, 3.6 μg/ml; ▲, 6 μg/ml; △, 36 μg/ml. (*c*) Plot of apparent $1/K_D$ for antibodies versus 1/[aldolase]. (*d*) Replot of apparent $1/K_D$ for aldolase versus 1/[antibody].

interaction between filamentous actin and enzymes of the phosphagen kinase group thus appears improbable.

These experimental conditions (ionic strength, pH, etc.) were then used in the following e.l.i.s.a. experiments in which the enzymes were allowed to interact with filamentous actins coated on microtitre plates, in competition with immunological probes. We first studied the interaction of the specific antibodies under the experimental conditions required for enzyme–actin association by co-sedimentation experiments (Table 2). In the case of anti-(1–7), anti-(18–28) and anti-(40–113) antibodies, only one antigenic sequence (Benyamin *et al.*, 1988), specific for each population, was recognized. In contrast, the antibodies directed against the 280–375 segment, contained several subpopulations which were specific for three related epitopes, and the corresponding apparent K_D thus reflects their mean affinity.

PFK–F-actin interface

The antibody populations described above were used in direct e.l.i.s.a. to monitor the antigenic reactivity of rabbit skeletal muscle F-actin in the presence of various concentrations of the enzyme PFK. As shown in Fig. 1, all antibody populations used [anti-(1–7), anti-(18–28), anti-(40–113) and anti-(285–375) antibodies] were completely released at enzyme concentrations above 0.5 mg/ml. The apparent K_D for the PFK interaction in the presence of each specific antibody can be deduced from the curves shown in Fig. 1, giving values of 2.7×10^{-7} M in the presence of anti-(1–7) antibodies, 1.1×10^{-7} M in the presence of anti-(18–28) antibodies and 2.1×10^{-7} M in the presence of anti-(40–113) or anti-(285–375) antibodies.

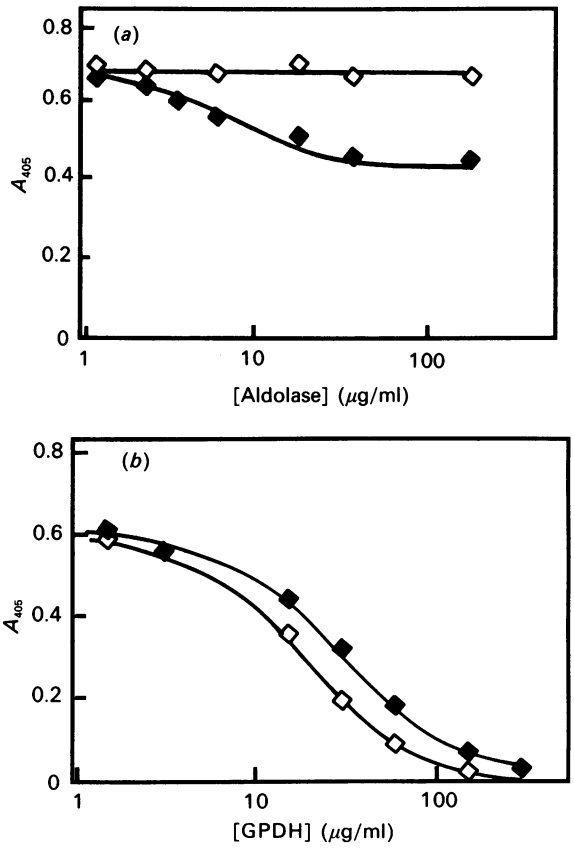

Fig. 4. Reactivity of anti-(285–375) antibodies for two actin isoforms in the presence of aldolase (*a*) or GPDH (*b*)

The actins were from rabbit skeletal muscle (◆) and *Pecten maximus* adductor muscle (◇). The binding of anti-(285–375) antibodies (6 μg/ml) was monitored at 405 nm in the presence of increasing enzyme concentrations.

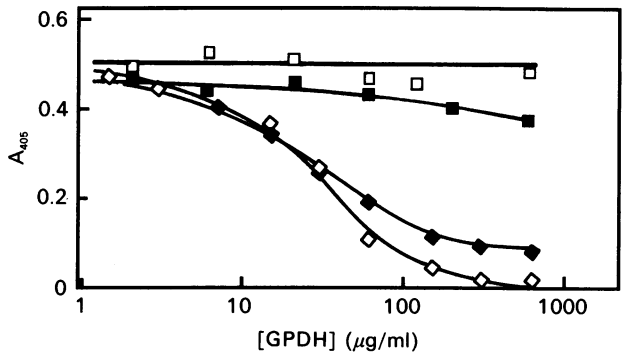

Fig. 5. Reactivity of anti-actin antibodies in the presence of GPDH monitored by e.l.i.s.a.

Specific antibodies were reacted with coated skeletal muscle F-actin in the presence of increasing GPDH concentrations: □, anti-(1–7) antibodies (6 μg/ml); ■, anti-(18–28) antibodies (3 μg/ml); ◆, anti-(40–113) antibodies (2.5 μg/ml); ◇, anti-(285–375) antibodies (3 μg/ml).

Aldolase–F-actin interface

The second enzyme studied, aldolase, was one of the glycolytic enzymes that interacted most strongly with actin. In contrast with PFK, only the anti-(285–375) antibody population was affected when aldolase associated with F-actin. As shown in Fig. 2, anti-(1–7), anti-(18–28) and anti-(40–113) antibodies showed unmodified activity in the presence of up to 0.4 mg of aldolase/ml. The effect induced by aldolase on anti-(285–375) antibody reactivity was then studied in detail. We observed only a partial decrease (Fig. 2) in antibody reactivity at high enzyme concentrations (above 36 μg/ml); the related effect corresponded to about 30%. This result suggests that only part of the antibody population is involved in the enzyme interaction. This observation was quantified by varying the enzyme and antibody concentrations. The data were then analysed by plotting $1/A_{405}$ versus 1/[antibody] at several enzyme concentrations (Fig. 3*a*). The double-reciprocal plot did not yield linear curves. However, it should be noted that at enzyme concentrations above 3.6 μg/ml, the linear parts of the curves overlap. By extrapolating the linear part of the curve, the apparent K_D and maximum A_{405} can be determined in the absence ($K_D = 0.5 \times 10^{-8}$ M; $A_{405,max.} = 0.740$) or in the presence ($K_D = 0.5 \times 10^{-8}$ M; $A_{405,max.} = 0.425$) of enzyme used at saturating concentrations. From these parameters, it is evident that the amount of antibodies not affected by the enzyme is $\frac{2}{3}$ of the total antibodies bound in the absence of enzyme, and that this fraction showed the same apparent K_D.

The behaviour of the antibody population affected by aldolase is analysed in Fig. 3(*b*). It can be seen that the variation in the binding of the population as a function of the aldolase concentration shows a competitive pat-

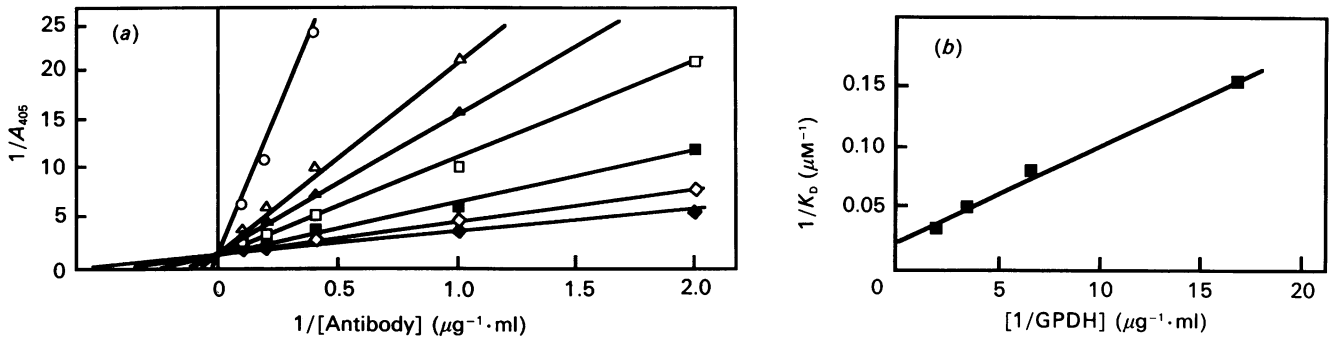

Fig. 6. Analysis of the reactivity of anti-(40–113) antibodies in the presence of GPDH by using e.l.i.s.a.

(*a*) Anti-(40–113) antibodies at various concentrations (0.5–10 μg/ml) were reacted with skeletal muscle F-actin in the presence of several GPDH concentrations. A plot of $1/A_{405}$ versus 1/[antibody] was drawn using the following GPDH concentrations: ◆, 0 μg/ml; ◇, 15 μg/ml; ■, 30 μg/ml; □, 60 μg/ml; ▲, 150 μg/ml; △, 300 μg/ml; ○, 600 μg/ml. (*b*) Plot of apparent $1/K_D$ for antibodies versus 1/[GPDH].

tern. The corresponding apparent K_D for the antibodies was very high at saturating enzyme concentrations (Fig. 3*c*).

Similarly, the apparent K_D for aldolase was very high at saturating antibody concentrations (Fig. 3*d*). The corresponding apparent K_D for aldolase in the absence of antibodies can be calculated by extrapolation to be about 1.0×10^{-9} M.

In conclusion, the analysis demonstrates that an antibody subpopulation (about $\frac{1}{3}$ of total antibodies) competed with aldolase for F-actin and did not form a ternary complex. Furthermore, a similar study using *Pecten maximus* actin gave a completely different pattern (Fig. 4*a*). The reactivity of the anti-(285–375) antibodies was not at all affected in this case. Since aldolase was brought down by *Pecten maximus* actin in sedimentation experiments (Table 1), it appears that the antibody subpopulation involved in the aldolase–actin interaction is related to a variable part of the 285–375 sequence and does not react with *Pecten maximus* actin.

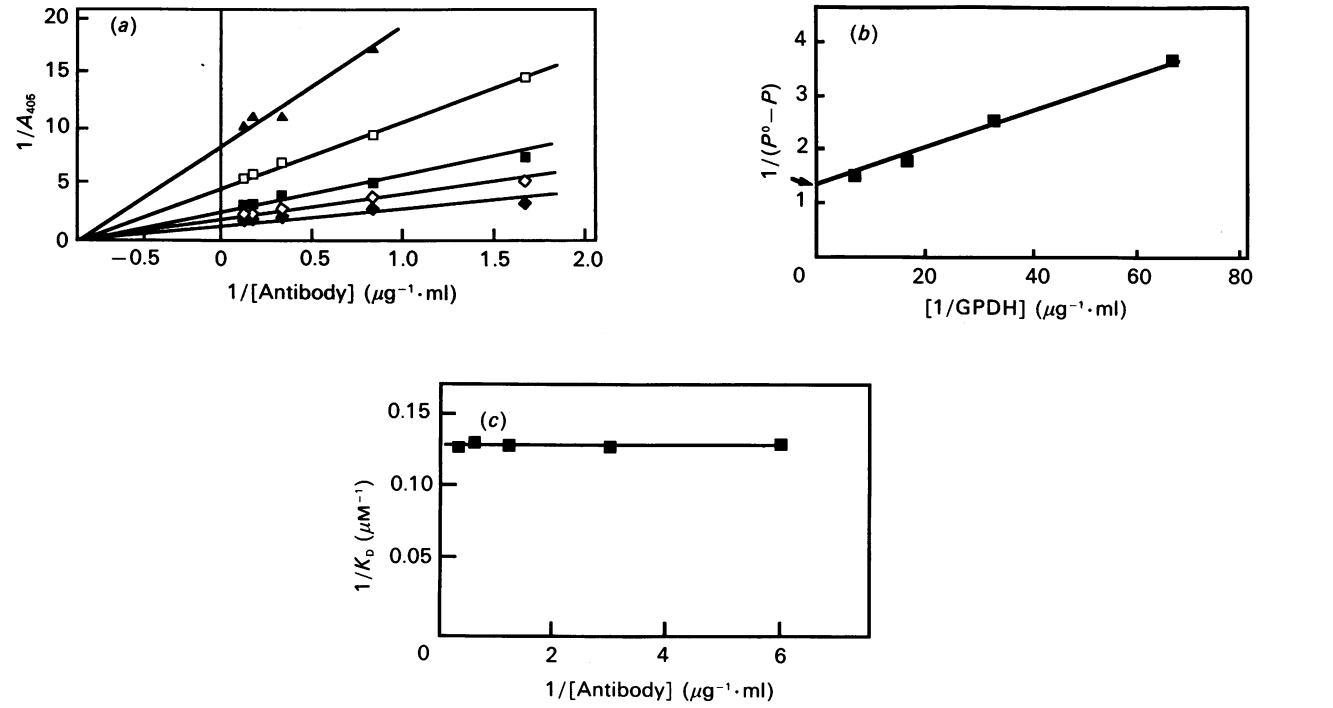

Fig. 7. Analysis of the reactivity of anti-(285–375) antibodies in the presence of GPDH by using e.l.i.s.a.

(*a*) Anti-(285–375) antibodies at various concentrations (0.6–8 μg/ml) were reacted with skeletal muscle F-actin in the presence of several GPDH concentrations. A plot of $1/A_{405}$ versus 1/[antibody] was drawn at the following GPDH concentrations: ◆, 0 μg/ml; ◇, 15 μg/ml; ■, 30 μg/ml; □, 60 μg/ml; ▲, 150 μg/ml. (*b*) Replot of $1/(P^0 - P)$ versus 1/[GPDH] where P^0 corresponds to maximum A_{405} without enzyme and P to that at the considered enzyme concentration. At infinite enzyme concentration, $1/(P^0 - P) = 1/P^0$ and then $P = 0$. (*c*) Replot of apparent $1/K_D$ for enzyme versus 1/[antibody].

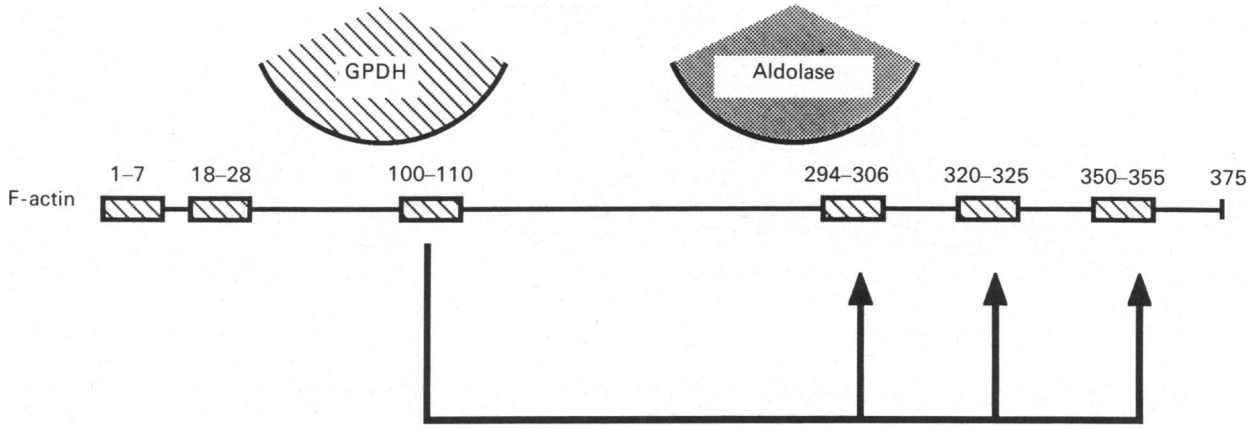

Fig. 8. Location of the potential interaction sites for GPDH and aldolase in the actin sequence

▨, Antigenic epitopes; ⌐, long-range conformational effects.

GPDH–F-actin interface

The interaction of GPDH with F-actin also induced perturbation of antibody reactivity. The anti-(1–7) antibodies were not at all perturbed by GPDH association even at high concentrations (Fig. 5). Similarly, the anti-(18–28) antibody population showed only an insignificant effect under the same conditions (Fig. 5). In contrast, a strong decrease in the anti-(40–113) antibody reactivity occurred when enzyme concentrations were increased. However, low residual binding was always observed (Fig. 5). We studied the interaction of antibodies at various GPDH and anti-(40–113) antibody concentrations (Fig. 6*a*). A plot of $1/A_{405}$ versus 1/[antibody] shows that the maximum amount of antibodies able to react with skeletal-muscle F-actin was not GPDH-dependent. Furthermore, the apparent K_D for anti-(40–113) antibodies (Table 2) differs by a factor of 25 from the apparent K_D extrapolated in the presence of an infinite GPDH concentration (Fig. 6*b*). Similarly, a variation of about 6-fold was estimated for the apparent K_D values of GPDH extrapolated in the presence ($K_D = 7 \times 10^{-7}$ M) or in the absence ($K_D = 1.2 \times 10^{-7}$ M) of antibodies (results not shown).

The effect of GPDH on anti-(285–375) antibody binding is more complex. The antibody populations appeared to be almost completely excluded by GPDH (Fig. 5). Although the amount of interacting antibodies appeared to be strongly affected by GPDH, the apparent K_D did not change (Figs. 7*a* and 7*b*). Similarly, the apparent K_D of GPDH (19 μg/ml) was not affected by the presence of antibodies (Fig. 7*c*).

These results suggest that the binding of GPDH near the epitope specific for anti-(40–113) antibodies induces long-range conformational changes in the *C*-terminal domain of actin, burying the corresponding antigenic sequences. The effects observed in the *C*-terminal domain of actin did not depend on the origin of the actin; a similar pattern (Fig. 4*b*) was observed with *Pecten maximus* actin which showed an apparent K_D for GPDH of about 1.1×10^{-7} M.

DISCUSSION

Previous studies (Boyer *et al.*, 1985, 1987; Méjean *et al.*, 1987) have shown that insolubilization or coating of actin does not alter its binding properties towards, e.g., DNAase-I, gelsolin or the myosin head. Moreover, in the case of the myosin head (S-1), some results obtained in the heterogeneous phase by direct e.l.i.s.a. (Méjean *et al.*, 1986, 1987) have been confirmed by other studies in solution (Miller *et al.*, 1987) and are in accordance with data obtained by physical methods such as H^1 n.m.r. (Moir *et al.*, 1987). Consequently, an approach using sequential antibodies as probes was chosen for locating the interfaces between actin and selected glycolytic enzymes.

Our results show that filamentous actin interacts specifically with the three glycolytic enzymes, PFK, aldolase and GPDH, at different interfaces. The electrostatic nature of these interactions, which has been inferred (Humphreys *et al.*, 1986*a*) on the basis of ionic strength effects, does not involve the *N*-terminal-negative charged cluster of actin in the case of aldolase and GPDH.

The present results suggest that the interface between PFK and F-actin is large and that boundaries are ill-defined, given that all antibodies tested are similarly affected, in contrast with what is observed with the two other enzymes. As the antibodies tested interact with independent antigenic domains (Benyamin *et al.*, 1988), the most probable explanation is related to the large size of this polymeric molecule (Luther *et al.*, 1985) in equilibrium with up to 16-mers (1520 kDa) which may impede antibody binding. In addition, the presence of two actin-binding sites per enzyme monomer (Humphreys *et al.*, 1986*b*) (eight sites per functional entity) supports such a conclusion. However, the possibility of large conformational changes modifying the reactivity of all antibody populations cannot be completely excluded.

The other two enzymes showed more precise interfaces at the surface of actin, and detailed analysis of their interactions reveals their locations.

We found that only the *C*-terminal region of actin was involved with aldolase association. More precisely, a subpopulation of the anti-(285–375) antibodies was in direct and complete competition with the enzyme for actin. Three continuous epitopes have been characterized in the *C*-terminal region. Two of them, including Met-325 and Met-355, correspond to conserved sequences in various actins, while the third (Met-299) corresponds to a variable region and is characteristic of skeletal-muscle

actin (Boyer *et al.*, 1987). The related antibody subpopulation showed no reactivity towards other actins (e.g. *Pecten maximus* actin). Consequently, the unchanged antibody reactivity in the presence of aldolase when *Pecten maximus* actin was used instead of skeletal muscle actin implies the participation of a region near or at residue 299 of actin in the interface (Fig. 8).

The location of the actin-binding site for GPDH was determined by a similar analysis. Our results show that two antibodies [anti-(40–113) and anti-(285–375)] were perturbed by the association. However, only the anti-(40–113) antibodies were in strong though partial competition with the enzyme. Apparent dissociation constants for enzyme and antibodies were decreased in the ternary complex. This result is in keeping with a similar study showing interaction of S-1 with the same region in the actin sequence. The analysis of S-1–anti-(40–113) antibody competition was confirmed by structural determination (Roustan *et al.*, 1988). Bearing in mind the specificity of our antibody (in the region around residue 103), it can be assumed that the GPDH–actin interface is located in this actin region (Fig. 8). Since the GPDH–actin interaction is known to be an electrostatic interaction, the two glutamic residues at positions 99 and 100 are potentially interesting. They, like the *N*-terminal acidic residues, are very reactive with carbodi-imide and therefore relatively exposed (Elzinga, 1987). Such residues could account for the interaction, the counterpart in GPDH probably consisting of sequences around Lys-191 and/or Lys-212 (Humphreys *et al.*, 1986*a*).

The other antibody population affected was directed to the *C*-terminal of actin (285–375 sequence). The results show that only antibody binding was lost and the interaction of the enzyme was not affected. This behaviour suggests that antigenic regions in the 285–375 sequence become buried upon interaction with GPDH. Similar conformational changes in this region have previously been reported for cation or spermine interactions (Méjean *et al.*, 1988). We also found that the binding of S-1 induces long-range conformational changes affecting the accessibility of Cys-374 (Méjean *et al.*, 1987). The plasticity of the actin molecule and of the microfilament may play an important role in the physiological behaviour of actin during polymerization or S-1 activation. Thus the mutual influences of actin and associated enzymes may modify the kinetics of the enzyme pathway and therefore the energy transduction processes.

In contrast with glycolytic enzymes, phosphagen kinases did not interact with F-actin. The interactions of creatine kinase and arginine kinase with myosin as well as the preferential localization of the binding sites of these two enzymes (Mani & Kay, 1976; Y. Benjamin & C. Roustan, unpublished work) on the rod part of myosin constitute a strong argument in favour of a selective compartmentalization of the enzymes involved in energetic metabolism on different components of the myofibrils.

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