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Journal of Chromatography B, 751 (2001) 341–348

JOURNAL OF CHROMATOGRAPHY B

www.elsevier.com/locate/chromb

Interaction between phosphofructokinase and aldolase from *Saccharomyces cerevisiae* studied by aqueous two-phase partitioning

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Received 10 July 2000; received in revised form 1 September 2000; accepted 1 September 2000

Abstract

Phosphofructokinase (EC 2.7.1.11) and aldolase (EC 4.1.2.13) have been highly purified from *Saccharomyces cerevisiae* by improved protocols. Partitioning of the enzymes in aqueous polymer two-phase systems was used to detect complex formation. The partition of each enzyme was found to be affected by the presence of the other enzyme. AMP affected the partition of the individual enzymes as well as the mixture of the two. The activities of the respective enzymes were stimulated in the putative complex in an AMP-dependent manner. Two strictly conserved residues belonging to an acidic surface loop of class II aldolases, are a potential site for electrostatic interaction with the positively charged regions close to the active site in phosphofructokinase.

Keywords: *Saccharomyces cerevisiae*; Enzyme–enzyme interaction; Partitioning; Phosphofructokinase; Aldolase; Enzymes

1. Introduction

In the living cell enzymes are part of multienzyme systems responsible for running biochemical pathways. These enzyme complexes are characterized by a high degree of organization and regulation to make their function optimized [1]. One very important and highly regulated process in the cytosol is the glycolysis, and in yeast cells, glycolytic enzymes constitute almost half of the total soluble protein [2]. High metabolic activity may require that the active sites of two sequential enzymes are close to each other and these proteins interact to channel intermediates. An enzyme–enzyme complex formation would have several biological advantages for the living cell such as regulation of metabolism, direct transfer of metabolites between enzymes and shielding metabolites from the aqueous cytosol. It is also possible that enzyme activities are controlled by association–dissociation and regulated through induced conformational changes. Evidences for the sequential enzyme–enzyme interactions with clearly advantageous overall effects on metabolism have been previously

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0378-4347/01/$ – see front matter
PII: S0378-4347(00)00492-8

documented [3–7] using phase partitioning, fluorescent anisotropy and isoelectric-focusing experiments.

Phase partitioning in aqueous two-phase systems can be used to detect minute changes in surface properties, such as hydrophobicity and charge, as the partition is determined by the surface properties of the partitioned substance. Preliminary attempts to purify enzymes from commercially available Bakers' yeast (*Saccharomyces cerevisiae*) provided us with an indication of mutual influences of several sequential glycolytic enzymes on each other's behaviour in aqueous two-phase systems. We found that the partitioning of aldolase and phosphofructokinase changed with increasing degree of enzyme purification, and hence decreasing degree of contaminating enzymes. In the present study we have found an influence of highly purified phosphofructokinase (EC 2.7.1.11) and highly purified aldolase (EC 4.1.2.13) isolated from *Saccharomyces cerevisiae* (strain CBS 8066), on each other's activity and partitioning in aqueous polymer two-phase systems. Multiple sequence alignment of Class II aldolases along with studies of enzyme 3D structure provided informations about two conserved residues, Glu 182 and Asp 183 (in the sequence *E. coli*; the Swissprotein Database number P11604) on the surface exposed loop. Their involvement in the interaction with phosphofructokinase is suggested.

2. Experimental

2.1. Microorganism

Saccharomyces cerevisiae, strain CBS 8066 (kindful gift from Professor Bärbel Hahn-Hägerdahl, Department of Applied Microbiology, Lund University, Lund, Sweden) was cultivated on Petri dishes on a medium with the following composition (per liter): 20 g glucose, 10 g yeast extract, 20 g proteous peptone and 20 g bacto agar at 30°C for 4 days. Cells were then transferred to 200 ml medium (similar composition as above, but without agar) in 1-l buffled Erlenmeyer flasks and grown on a rotary shaker (100 rpm) for one day at 30°C. Initial pH was 5.5. The cells were harvested in the late exponential phase. Prior to enzyme extractions the cells were washed twice with distilled water.

2.2. Chemicals

All auxiliary enzymes, enzyme substrates and coenzymes were from Sigma Chemical Co (St. Louis, MO). Dextran 500 ($M_r = 500\,000$) was from Pharmacia (Uppsala, Sweden) and polyethylene glycol 8000 ($M_r = 8000$) was from Union Carbide (New York, NY). Sepharose and Sephadex gels were from Pharmacia (Uppsala, Sweden). DEAE-cellulose (DE 52) was from Whatman (Heidelberg, Germany). 1,6-diaminohexane was from Aldrich (Stenheim, Germany). All other chemicals were of reagent grade.

2.3. Enzyme assays

The phosphofructokinase activity was assayed according to Hofmann and Kopperschläger [8] while aldolase was assayed according to Rutter [9]. Both enzymes were assayed at 340 nm at room temperature by using a Hitachi 100-60 spectrophotometer. One unit of phosphofructokinase is defined as the amount of enzyme that catalyzes the conversion of 1 μmol of fructose 6-phosphate/min at 25°C. One unit of aldolase activity is defined as the amount of enzyme that catalyzes the aldol cleavage of 1 μmol of fructose 1,6-bisphosphate/min at 25°C.

2.4. Protein determination

Protein content was determined according to Bradford [10] at 595 nm using bovine serum albumin as a standard. Concentrations of phosphofructokinase and aldolase were determined photometrically according to Vértessy [11] using molar absorption coefficients at 280 nm of 1.66×10^5 M^{-1} cm^{-1} for phosphofructokinase and 1.18×10^5 M^{-1} cm^{-1} for aldolase.

2.5. Purification of phosphofructokinase

Phosphofructokinase was purified using a modified version of Hofmann and Kopperschläger [8], as described below. Unless otherwise stated, all steps were performed on ice.

2.5.1. Step 1. Disruption of the yeast cells and extraction of phosphofructokinase

Ten g of yeast (wet weight) was suspended in 84 ml of buffer A (10 m*M* sodium phosphate, 1 m*M* EDTA, pH 7.2, 5 m*M* 2-mercaptoethanol, 0.5 m*M*

phenylmethylsulphonyl fluoride). Cell disruption was achieved by sonication (Sonifier® Cell Disruptor B-30 of Branson Sonic Power Co., Danbury, CT). The yeast suspension, cooled with ice-water, was sonified for 12 min (with 1 min break after each minute) at 50% effect and output 4. Solid polyethylene glycol 8000 (final concentration 5% (w/w)) was added over a period of 15 min under vigorous stirring. After additional 15 min of stirring, the homogenate was centrifuged at 5000 *g* for 20 min, to remove insoluble components.

Fifty millilitres of the resulting supernatant was used for the purification of phosphofructokinase (step 2–7) and the rest (about 35 ml) was stored at 4°C for 3 days and then used for the purification of aldolase (see 2.6).

2.5.2. Step 2. 12% (w/w) Polyethylene glycol precipitation

Precipitation of phosphofructokinase from the supernatant was achieved by addition of polyethylene glycol 8000 as above, to give a concentration of 12%. The turbid solution was allowed to stand for another 15 min before centrifugation (as described above). The pellet containing phosphofructokinase was suspended in buffer A to give a final volume of 5 ml.

2.5.3. Step 3. Chromatography on immobilized Cibacron Blue F3G–A

Affinity matrix was synthetized according to Hofmann and Kopperschläger [8], with 1/4 of the recommended Cibacron Blue F3G-A amount. The gel was packed in the column (1.6×8 cm) and equilibrated with buffer B (10 m*M* sodium phosphate, 1 m*M* EDTA, pH 7.2, and 5 m*M* 2-mercaptoethanol). The enzyme solution obtained at the end of step 2 was diluted 5-fold with buffer B (final volume 25 ml) and applied on the column. Prior to enzyme elution, the gel was washed with 100 ml of buffer B. Enzyme elution was performed with 50 m*M* buffer A containing 2 *M* ammonium sulfate. The eluate was collected in 3 ml fractions. The active fractions were pooled (18 ml).

2.5.4. Step 4. Ammonium sulfate precipitation

From the pooled fractions obtained in step 3, the phosphofructokinase was precipitated by addition of solid ammonium sulfate to give a concentration of 2.53 *M*. The solution was stirred for 30 min and then centrifuged at 25 000 *g* for 30 min. The pellet was dissolved in 1 ml of buffer C (50 m*M* sodium phosphate, 1 m*M* EDTA, pH 7.2, 5 m*M* 2-mercaptoethanol, 0.5 m*M* PMSF, 1 m*M* fructose 6-phosphate).

2.5.5. Step 5. Gel filtration

The enzyme solution obtained at the end of step 4 was applied on a Sephadex G-200 column (2.7×18 cm) equilibrated with buffer C. Enzyme elution was performed with the same buffer at the flow-rate 66 ml/h. The eluate was collected in 3-ml fractions. The active fractions were pooled (17.5 ml).

2.5.6. Step 6. Ion-exchange chromatography

The pooled fraction in step 5 was applied on a DEAE–cellulose column (5.5×2 cm) equilibrated with buffer C. Phosphofructokinase was eluted by means of a linear sodium chloride gradient (0.05 to 0.3 *M*) prepared in 200 ml of buffer C. The active fractions were pooled (31 ml).

2.5.7. Step 7. Polyethylene glycol precipitation

Solid polyethylene glycol 8000 was added over a period of 30 min with vigorous stirring to give a final concentration of 15% (w/w). The solution was stirred for an additional 60 min and then centrifuged at 30 000 *g* for 60 min. The pellet was suspended in 1.5 ml of buffer C. The enzyme suspension was stored at 4°C.

2.6. Purification of aldolase

Aldolase was purified as described by Lubini et al. [12] except for the first step, cell disruption, which was done as described for phosphofructokinase. The affinity chromatography step was slightly modified. Sepharose 4B was activated with divinylsulfone then aminated with 1,6-diaminhexane according to Porath [13]. Fructose 1,6-bisphosphate was then coupled and immobilized by sodium borohydride reduction of the Schiff base formed with the aminated Sepharose according to [12].

2.7. Two-phase partitioning

After initial screening, a phase system with general composition (final concentrations after addition of

enzyme): 7.80% (w/w) Dextran 500; 4.29% (w/w) polyethylene glycol 8000; 100 m*M* Li_2SO_4; 10 m*M* sodium phosphate buffer, pH 7.2; 0.1 m*M* $ZnSO_4$; 0.1 m*M* $(NH_4)_2SO_4$; 0.1 m*M* $MgSO_4$; 0.33 m*M* fructose 6-phosphate, 0.1 m*M* ATP and ±0.25 m*M* AMP was chosen. Li_2SO_4 controls interfacial potential in the system. The additional components were added in order to ensure optimal catalytic activities of studied enzymes. The systems had total mass of 4 g and the volume ratio of top and bottom phase was 1. After enzyme addition, the phase systems were thoroughly mixed by shaking for 30 min at 4°C (shaking table). Phase separation was speeded up by low speed centrifugation (2000 *g* for 5 min) using a fixed-angle rotor.

2.8. Affinity partitioning of partly purified aldolase

A partly purified fraction containing both aldolase and phosphofructokinase was obtained by fractional precipitation of yeast extract (see Section 2.5, step 1 for yeast extract preparation), and the material precipitated between 12–18% polyethylene glycol was collected. The fraction was partitioned in two-phase systems with following composition: 7.80% (w/w) Dextran 500; 4.29% (w/w) polyethylene glycol 8000 (with and without 0.25% Cibacron blue–polyethylene glycol); 100 m*M* $LiSO_4$ and 10 m*M* sodium phosphate buffer, pH 7.2.

2.9. Calculations

For the determination of the partition coefficients (K) of phosphofructokinase, aldolase and proteins, suitable volumes of each phase (interface mixed with bottom phase) were withdrawn and analyzed for either enzyme activities or protein concentration. The partition coefficient of phosphofructokinase and aldolase is defined as the ratio of their respective activities (u/ml) in top and bottom phase. The partition coefficient of protein is defined as the ratio of protein concentration (mg/ml) in top and bottom phase. Each analysis was done in duplicate.

2.10. Structural studies

Amino acid sequences were obtained from Protein Sequence Data Base (SWISS-PROT; http://www.expasy.ch/sprot/). Multiple sequence alignment was done on the European Bioinformatics Institute server (EBI; http://www2.ebi.ac.uk), by using analysis tool ClustalW. Secondary structure elements were obtained from the Protein Data Bank (PDB; http://www.pdb.bnl.gov). 3-D macromolecular structure data were found in the Protein Data Bank and analysed by means of following programs: Swiss PDB viewer, RasMol and MAGE.

3. Results

The modified protocol for yeast phosphofructokinase purification gave purification factor and yield of 190 and 11%, respectively (Table 1), which is comparable with results reported earlier [10]. Much time was saved by the precipitation with polyethylene glycol 8000 (Table 1, steps 2 and 6) instead of two precipitation steps with ammonium sulfate, since, the dialysis prior to the ion-exchange chromatography (step 5) could be omitted.

The modified protocol for yeast aldolase purification resulted in 71% yield (Table 2) compared to the reported value of 10% [12]. The higher yield was probably due to the following: Amination of di-

Table 1
Purification of phosphofructokinase

Purification step	Volume (ml)	Total activity (u)	Total protein (mg)	Spec activity (u/mg)	Purification (fold)	Yield (%)
1. Enzyme extraction	50.0	83.0	138	0.60	1	100
2. 12% PEG 8000 precipitation	5.0	58.2	51.6	1.13	1.9	70
3. Affinity chromatography	18.0	37.1	3.50	10.6	18	45
4. Gel filtration	17.5	28.5	0.31	91.9	153	34
5. Ion-exchange chromatography	31.0	20.6	0.18	114	190	25
6. 15% PEG 8000 precipitation	1.5	9.10	0.08	114	190	11

Table 2
Purification of aldolase

Purification step	Volume (ml)	Total activity (u)	Total protein (mg)	Spec activity (u/mg)	Purification (fold)	Yield (%)
Enzyme extraction	34	98	77.7	1.26	1	100
Heated supernatant after dialysis	35	94.5	14.8	6.37	5	96
Ion-exchange chromatography	60	78	6.40	12.2	9.7	80
Affinity chromatography	36	69.4	0.81	85.7	68	71

vinylsulfone activated Sepharose 4B with 1,6-diaminohexane resulted in attachment of 13 carbon long spacer arms on the gel matrix in contrast to the 10 carbon long spacer arms attached to the gel matrix of the commercially available AH–Sepharose 4B [12]. The attachment of fructose-1,6-bisphosphate proceeded by reduction of the Schiff base formed between aminated gel matrix and the carbonyl group of the fructose-1,6-bisphosphate according to [12]. By this procedure, Schiff base was converted into a stable secondary amine. The much higher yield was obtained without any apparent loss in purification. Although our ion-exchange step was less efficient than earlier reported with a purification factor of 9.7 (Table 2) compared to 33 [12], the overall purification was the same (68-fold).

The first indication that phase partitioning could be used to identify interactions between phosphofructokinase and aldolase came from the partitioning of partly purified aldolase in a phase system with or without Cibacron Blue–polyethylene glycol. This partly purified aldolase preparation (protein precipitated between 12–18% polyethylene glycol), contained some phosphofructokinase (and also minor extents of triose phosphate isomerase and glyceraldehyde 3-phosphate dehydrogenase activities). Using a system without Cibacron Blue–polyethylene glycol, aldolase activity partitioned predominantly to the lower phase, giving a partition coefficient, K(aldolase), of 0.9 (Table 3). By replacing 0.25% polyethylene glycol with Cibacron Blue–polyethylene glycol but otherwise keeping conditions the same, K(aldolase) increased to 1.4. This indicated that the addition of Cibacron Blue–polyethylene glycol, which was enriched in the upper phase together with unmodified polyethylene glycol, affected the partitioning of aldolase. It has been reported that the partitioning of aldolase alone is not affected by Cibacron Blue–polyethylene glycol in contrast to phosphofructokinase which is strongly affected [14]. Therefore, the higher partitioning of aldolase obtained in the presence of Cibacron Blue–polyethylene glycol (Table 3) indicated an interaction between aldolase and phosphofructokinase bound to Cibacron Blue–polyethylene glycol. If so, the results also indicate that the binding sites for aldolase and Cibacron Blue–polyethylene glycol on phosphofructokinase are separated from each other. To investigate the possible interaction between aldolase and phosphofructokinase, the partition behaviour of the pure enzymes were studied.

Table 3
The effect of Cibacron Blue on the partitioning of partly purified aldolase in polyethylene glycol–Dextran two-phase systems[a]

	No Cibacron Blue	With Cibacron Blue
K(aldolase)	0.9	1.4

[a] The partly purified aldolase fraction contained also phosphofructokinase as well as traces of triose phosphate isomerase and glyceraldehyde 3-phosphate dehydrogenase.

Purified aldolase (Table 2), was subjected to partitioning in the two-phase system with the same polymer concentration as described above, but without Cibacron Blue–polyethylene glycol. Upon addition of phosphofructokinase the K-value for aldolase increased from 0.7 to 1.2 by (Table 4), again indicating an interaction between phosphofructokinase and aldolase. In the presence of AMP, which is a strong signal forward for glycolysis and hence expected to promote enzyme–enzyme interaction, the corresponding shift in K(aldolase) by phosphofructokinase addition was from 0.5 to 0.8 (Table 4). Thus, the addition of phosphofructokinase raised K(aldolase), again pointing to an interaction between the two. We noted that AMP affected K(aldolase), also in absence of phosphofructokinase. Evidence for NADH binding leading to conformational changes were reported from the class I aldolase of rabbit

Table 4
The effect of AMP and phosphofructokinase on the partitioning of purified aldolase in polyethylene glycol/Dextran two-phase systems[a]

	No phosphofructokinase	With phosphofructokinase
K(aldolase) (no AMP)	0.7	1.2
K(aldolase) (with AMP)	0.5	0.8

[a] Phosphofructokinase concentration was 0.012 μ*M* and its partition coefficient 0.021 (with AMP) and 0.042 (no AMP), respectively.

muscle [15]. The AMP effect found in our study suggests to that there may be a nucleotide binding site also on class II aldolases such as yeast aldolase. The yeast nucleotide binding site must show some differences to that of phosphofructokinase, since Cibacron Blue–polyethylene glycol, which has affinity to nucleotide binding sites [16] does not bind aldolase, but phosphofructokinase with high affinity.

To study the effect of aldolase on phosphofructokinase, phase systems were made with constant concentration of phosphofructokinase and increasing concentrations of aldolase (Fig. 1). In the absence of aldolase and no AMP, the K for phosphofructokinase was 0.04. Increasing the concentration of aldolase resulted in an increase in K(phosphofructokinase) to 0.17 at maximum which corresponded to an aldolase concentration of 0.025 μ*M*. At higher aldolase concentrations, K(phosphofructokinase) decreased to a level of around 0.1, that is a higher value than without aldolase. The same results were obtained in the presence of AMP, but with overall lower partition ratios.

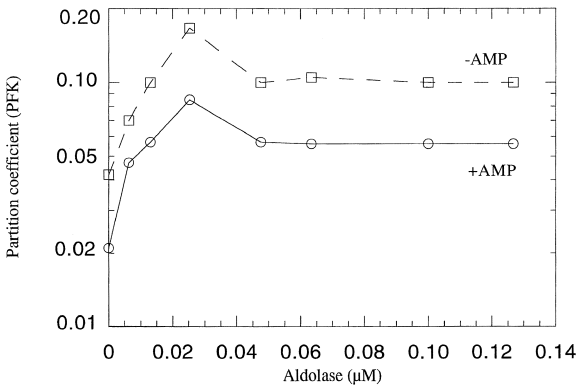

Fig. 1. Effect of aldolase on the partitioning of phosphofructokinase (phosphofructokinase) in the aqueous two-phase system. ————, in the presence of AMP, ------, in the absence of AMP. The partition coefficient is defined as the ratio of enzyme activities (u/ml) in top and bottom phase. Phosphofructokinase = 0.012 μ*M*, *AMP* = 0.25 m*M*. Error of the partition coefficient determination was 3% ($n = 2$).

The mutual influence of aldolase and phosphofructokinase in two-phase systems was reflected also in the activities of both enzymes. In these experiments, the enzymes were partitioned ±AMP, after which samples were withdrawn to measure enzyme activity (assay with AMP). In the presence of AMP during phase partitioning, the phosphofructokinase activity was 0.02 u/ml in the absence of aldolase, but this activity increased to 0.032 u/ml when aldolase was present at concentrations of 0.04 μ*M* and above (Table 3). In the absence of AMP during phase partitioning these stimulations were not seen, but instead a small decrease of the phosphofructokinase activity was obtained by adding aldolase. The activity of aldolase was also stimulated by the presence of phosphofructokinase at concentrations above 0.04 μ*M* aldolase (Fig. 2B), but only if AMP had been present during partitioning. This indicates that the interaction between aldolase and phosphofructokinase per se is not dependent on the presence of AMP, since they affected each other's partitioning also in it absence of AMP (Table 4, Fig. 1). However, only when the enzyme complex had been formed in the presence of AMP, were stimulated enzyme activities obtained (Fig. 2). This means that the complex remained once it had been formed, at least as long as the activity assay was performed (since AMP was added here). Also inserted in Fig. 2B. are the activities obtained in the absence of phosphofructokinase. This shows that addition of 0.012 μ*M* phosphofructokinase to a phase system with 0.065 μ*M* aldolase, doubled the aldolase activity (no AMP) or increased its activity by about 50% (+AMP).

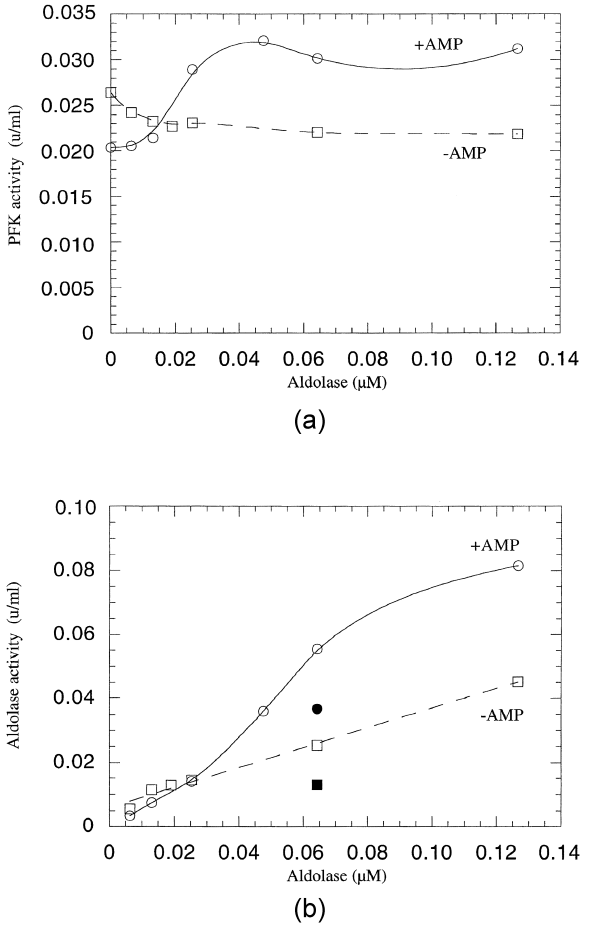

Fig. 2. Effect of increasing concentration of aldolase on the activity of phosphofructokinase (A) and aldolase (B). ————, in the presence of AMP, ------, in the absence of AMP. Phosphofructokinase = 0.012 μ*M*, *AMP* = 0.25 m*M*. Closed symbols represent aldolase activity at concentration 0.064 μ*M* in the absence of phosphofructokinase. Error of the activity determination was 5% ($n = 2$).

4. Discussion

We here show that phosphofructokinase and aldolase interact to form a complex with different partitioning in Dextran–polyethylene glycol two-phase systems, compared to that of either enzyme. Complex formation took place both in the presence and absence of AMP, but presence of AMP was obligatory to get enhanced activities of the individual enzymes. The concentration of aldolase needed to get maximum change in K(phosphofructokinase) was around 0.025 μ*M*, whereas that needed to get stimulation of activity was about the double (cf Figs. 1 and 2). A simple explanation to this discrepancy is that at 0.025 μ*M* aldolase, a 'supra'complex with very specific partitioning (induced by size increase as well as conformational changes), was formed where the phosphofructokinase molecules somehow share aldolase molecules (provided these have two binding sites for phosphofructokinase, e.g. are dimers). With increasing concentration of aldolase, K(phosphofructokinase) decreased probably reflecting a dissociation of the 'supra'complex into smaller complexes of phosphofructokinase saturated with aldolase, complexes that allow stimulations of activity since each phosphofructokinase is provided with its 'own' aldolase. At around 0.05 μ*M* aldolase, a new K(phosphofructokinase) was reached that remained also at higher aldolase concentrations (Fig. 1). This K(phosphofructokinase) probably reflected the active complex that gave increased enzyme activities, since the aldolase concentration needed to reach it was the same (compare Figs. 1 and 2). This notion is supported by the observed K(aldolase) that at high aldolase concentration K(aldolase) is substantially the same as when the enzyme is partitioned alone (results not shown).

The situation may be more complex, however, since different oligomers of phosphofructokinase exist, and they may have different activities and affinities for aldolase. In studies with mammalian enzymes Vértessey et al. [11] found that aldolase, by binding to phosphofructokinase, prevented the dissociation-induced inactivation of the tetrameric form of phosphofructokinase. In this complex, also aldolase was more active [11]. In yeast, the octameric form, rather than a tetrameric form, is the most active form of phosphofructokinase [17], but otherwise the situation may be the same, i.e. that binding of aldolase to phosphofructokinase prevents it's deactivation.

From the data in Figs. 1 and 2, a rough estimate about the stochiometry of the activated complex could be made. The molar ratio was ca. 0.05 μ*M* aldolase to 0.012 μ*M* phosphofructokinase, which is about four aldolase molecules per phosphofructokinase. Regarding the fact that yeast phosphofructokinase is an octamer and aldolase is a dimer this could mean that each aldolase molecule interacts

with two phosphofructokinase subunits at the same time. The effector binding site in phosphofructokinase on the entrance to the active site [18] is located on the interface between two subunits and is well characterised. Binding of the effector regulates the width of the active site cleft [18]. If aldolase binds to phosphofructokinase on the entrance to the active site, the conformational changes on the effector site could influence enzyme–enzyme interaction. This speculation is in agreement with the observed effects of AMP on partition coefficients and activities of both enzymes. The aldolase concentration needed to saturate phosphofructokinase was the same regardless of whether AMP was present or not (Fig. 1), even if the presence of AMP resulted in an overall lower partitioning.

Investigations were also made to see if the known three-dimensional structures could give information on possible interactions between aldolase and phosphofructokinase. Amino acid sequence alignment of Class II aldolases (Fig. 3), revealed two strictly conserved residues, Glu 182 and Asp 183 (numbering system of *Esherichia coli*, P11604). These two residues belong to the extended, mainly acidic surface loop and they have not so far been implicated to be involved in neither catalysis nor the stabilisation of 3D structure. Studies of the known phosphofructokinase structure, especially on the entrance to the active sites, revealed positively charged regions meaning that the potential for the electrostatic interaction exists. We therefore suggest that these phosphofructokinase regions interact with the conserved residues of the acidic loop of aldolase and that the formed complex may function in metabolic channeling. The latter is suggested since complex formation led to enhanced activities of both enzymes but only in the presence of the effector AMP.

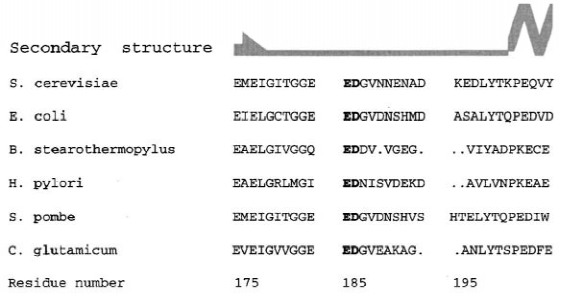

Fig. 3. Amino–acid similarity in Class II aldolases. The Swissprotein Database numbers are *Saccharomyces cerevisiae*, P14540; *Escherichia coli*, P11604; *Bacillus stearothermophylus*, P94453; *Helicobacter pylori*, P56109; *Schizosaccharomyces pombe*, P36580 and *Corynebacterium glutamicum*, P19537. Residue numbers correspond to *Escherichia coli*, P11604 residues. The strictly conserved residues are highlighted in bold.

Acknowledgements

We thank Professor Bärbel Hahn–Hägerdahl, Department Applied Microbiology, Lund University for providing us with the yeast strain.

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