STIMULATION OF THE PROTEIN-DEPENDENT INTERCONVERSION OF TWO FORMS OF YEAST PHOSPHOFRUCTOKINASE BY A HEAT-STABLE FRACTION FROM YEAST

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1. Introduction

The conversion of yeast phosphofructokinase (ATP:D-fructose-6-phosphate 1-phosphotransferase (EC 2.7.1.11)) sensitive to inhibition by high ATP concentrations (PFK*s*) into a desensitized form (PFK*d*) not inhibited by excess ATP, has been described by Viñuela et al. [1]. The conversion, catalyzed by a yeast fraction, was reported to require ATP-Mg, NaF and cyclic 3′,5′-AMP.

Recently the reaction has been reinvestigated, using purified yeast phosphofructokinase [2]. A heat-labile yeast fraction with catalytic activity has been partially purified and characterized as a protein [3]. It has been confirmed that the desensitization reaction requires also ATP-Mg and NaF, but no effect of cyclic 3′,5′-AMP has been observed.

In the present paper it is shown that when the studies are done with a partially purified desensitizing protein the conversion of PFK*s* to PFK*d* depends on the addition of a heated and ultrafiltrated yeast extract. The heat-stable fraction can be replaced by a mixture of 5′-AMP and fructose-6-phosphate.

2. Methods

Purified yeast phosphofructokinase was used [2]. Enzyme activity was determined as described elsewhere [2] testing at 0.25 mM Fru-6-P and 0.05 and 1 mM ATP, respectively.

Incubations for conversion (total volume: 0.1 ml) were performed at 25° for 20 min using a 25- to 50-fold purified desensitizing protein fraction from yeast [3]. Other details are described elsewhere [3].

Definition of "% conversion": the ATP-sensitive phosphofructokinase is inhibited by 1 mM ATP. The extent of the conversion to the desensitized form is expressed in % conversion taking the maximal phosphofructokinase activity (with 1 mM ATP) as 100% and the activity without addition of the desensitizing protein to the conversion mixture as 0% conversion [3].

Preparation of the heated and ultrafiltrated yeast extract: crude yeast extract [3] was heated for 5 min at 90°. After centrifugation, the supernatant was ultrafiltrated (device and filters SM 12136 from Sartorius-Membranfilter GmbH, Göttingen). The

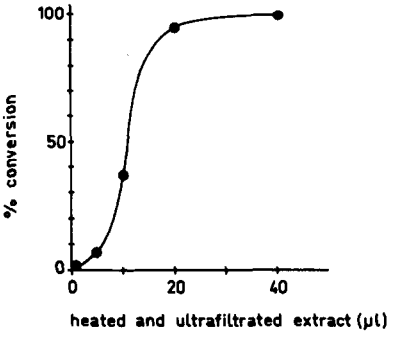

Fig. 1. Addition of heated and ultrafiltrated yeast extract to the conversion mixture. For details see "Methods" and "Results and discussion".

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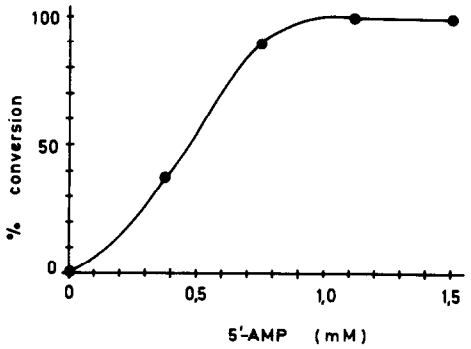

Fig. 2. Dependence of the conversion of PFK*s* to PFK*d* on the 5′-AMP concentration. Fru-6-P: 0.07 mM. Other details of the incubation for the conversion see "Methods" and "Results and discussion".

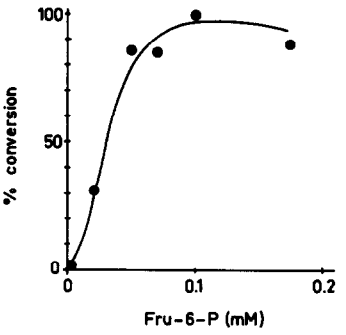

Fig. 3. Influence of Fru-6-P concentration on the conversion of PFK*s* to PFK*d*. 5′-AMP: 1.0 mM. Other details of the conversion see "Methods" and "Results and discussion".

clear filtrate was stored at −15° without loss of activity.

5′-AMP and Fru-6-P were determined enzymatically [4, 5].

3. Results and discussion

The partially purified protein fraction from yeast described elsewhere [3], catalyzes the conversion of PFK*s* to PFK*d* in the presence of 0.1 mM ATP, 1 mM Mg^{++}, and 10 mM sodium fluoride in 20 mM potassium phosphate buffer pH 6.5, if a heated and ultrafiltrated yeast extract is added. Fig. 1 shows that 20 µl of such an extract are sufficient for a maximal conversion in an incubation mixture of 100 µl. The following substances, added separately, are not able to replace the heated and ultrafiltrated extract: 3 mM NH_4Cl, 1.0 mM 5′-AMP, 0.5 mM ADP, 0.5 mM Fru-6-P, 0.2 mM ADP + 0.07 mM Fru-6-P, 0.5 mM Fru-1,6-P_2, 0.5 mM P-enolpyruvate, 0.5 mM citrate, 1 mM acetyl-CoA, and 0.2 mM 3′,5′-AMP. Though 5′-AMP and Fru-6-P, separately added, do not stimulate, a mixture of both can replace the heated and ultrafiltrated extract completely. Fig. 2 illustrates that in the presence of 0.07 mM Fru-6-P about 1 mM 5′-AMP is necessary for a maximal stimulation of the conversion. Fig. 3 shows that 0.05 to 0.1 mM Fru-6-P are needed for maximal stimulation of the conversion in the presence of 1.0 mM 5′-AMP.

The amounts of Fru-6-P and 5′-AMP in the heated and ultrafiltrated extract gave a final concentration in the conversion mixture ranging from 0.025 to 0.04 mM Fru-6-P and 0.02 to 0.05 mM 5′-AMP.

It appears therefore that the amounts of Fru-6-P and 5′-AMP in the heated and ultrafiltrated extract do not alone account for the stimulating effect observed. Preliminary experiments indicate that the stimulating compound(s) present in the heated and ultrafiltrated extract is adsorbed by anion-exchange resins (Amberlite IRA-400) and eluted by 0.5 M NaCl.

4. Summary

Besides ATP-Mg, fluoride and a desensitizing protein fraction, the conversion of yeast phosphofructokinase, sensitive to inhibition by excess ATP, into a desensitized form requires a heated and ultrafiltrable fraction from yeast extract. This heat-stable fraction can be replaced by a mixture of Fru-6-P and 5′-AMP. The concentrations of Fru-6-P and 5′-AMP in the heated extract are too low to explain the stimulation completely. The stimulating material present in the heated and ultrafiltrable extract, can be adsorbed to Amberlite IRA-400.

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