YEAST PHOSPHOFRUCTOKINASE
IV. REVERSIBILITY OF THE ATP-DESENSITIZATION

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Received 18 December 1972

1. Introduction

Yeast phosphofructokinase (PFK) exists in two forms. An ATP-sensitive form (PFKs) is inhibited by ATP concentrations higher than 0.1 mM and by Mg-citrate, while PFKd – the ATP-desensitized form – is not inhibited by these effectors [1]. Vinuela et al. [1] postulated a chemical interconversion of the two forms by a phosphorylation/dephosphorylation mechanism catalyzed by a "desensitizing protein". In the first two papers of this series [2, 3] it has been shown that no "desensitizing protein" is necessary for conversion of PFKs to PFKd. PFKs can be desensitized by incubation with ADP, Fru-6-P, Mg^{2+}, NH_4^+ and F^-. Since MgF^+ is the effective complex of desensitization [3] and since the ATP desensitized form behaves like an enzyme fixed in the allosteric R-state, as defined by Monod et al. [4], ATP-desensitized PFK is called PFKd (MgF^+) [5]. In the present paper it is shown that the desensitization of PFKs to PFKd (MgF^+) is completely reversible. For desensitization all effectors are necessary. Once in the desensitized form, PFKd (MgF^+) could be stabilized only by Mg^{2+} and F^-. Complexing of Mg^{2+} by EDTA or citrate as well as removal of F^- or addition of ATP favours resensitization of PFKd (MgF^+) to PFKs. This resensitization is 2–3 times slower than desensitization.

* Part of a Ph.D. thesis to be submitted to the Faculty of Biology at the University of Freiburg.

2. Materials and methods

PFK was purified from baker's yeast according to Atzpodien and Bode [6]. The activity of PFK and the rate of de- and resensitization was determined as described elsewhere [2]. By definition,

$$R_{ATP} = \frac{\text{activity at 0.05 mM ATP}}{\text{activity at 1.0 mM ATP}} \leq 1$$

is characteristic for PFKd (MgF^+) and $R_{ATP} > ?$ for PFKs. The desensitization was performed at 25° in a standard desensitization mixture containing 50 mM imidazole/HCl buffer, pH 6.8, 0.1 mM ADP, 0.1 mM Fru-6-P, 1 mM $MgCl_2$, 10 mM NH_4Cl, 10 mM NaF and 40–80 μg PFKs in a total volume of 1.0 ml [3]. For resensitization either ATP, citrate or EDTA was added to the complete desensitization mixture or to an aliquot of it. In some experiments the desensitized enzyme was separated from effectors by filtration on a Sephadex G-25 column (1 × 15 cm). The speed of elution was adjusted so that the enzyme left the column 4 min after application to it. Nucleotides and PFK assay enzymes were purchased from Boehringer (Mannheim). All other chemicals used were analytical grade reagents obtained from commercial sources.

3. Results and discussion

As shown in table 1 PFKd (MgF^+) is stable to dialysis against the complete desensitization mixture for at least 5 hr, while removal of F^- from the dialyzing mixture results in a resensitization. A similar effect is achieved by adding EDTA to the dialysis

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Table 1
Resensitization of PFKd (MgF^+).

Dialysis of PFKd (MgF^+) against	R_{ATP}
Complete "desensitization mixture"	0.9
Complete "desensitization mixture" $-F^-$	5.6
Complete "desensitization mixture" $-Mg^{2+}$, +40 mM EDTA	3.0
1 mM $MgCl_2$ and 10 mM NaF in 50 mM K-PO_4 buffer pH 6.8	1.0

For preparation of PFKd (MgF^+) see Materials and methods. 40 µg PFKd (MgF^+) in 0.5 ml of the "desensitization mixture" [3] were dialyzed for 5 hr at 0° against 250 ml of the desensitization mixture containing 0.1 mM ADP, 0.1 mM Fru 6-P, 10 mM NH_4, 1 mM Mg^{2+} and 10 mM F^- in 50 mM K-PO_4 buffer pH 6.8. R_{ATP} is defined as: activity 0.05 mM ATP/activity at 1.0 mM ATP.

buffer at a final conc. of 40 mM, for after 5 hr dialysis the R_{ATP} will have reached a value of 3.0. PFKd (MgF^+) however, remains in the desensitized form upon dialysis against 50 mM phosphate buffer, pH 6.8, containing 1 mM $MgCl_2$ and 10 mM NaF. These results show that Mg^{2+} and F^- are essential to the stabilization of PFKd (MgF^+), while all the other effectors necessary for desensitization of PFKs [3] can be dispensed with after desensitization has taken place.

Resensitization is slower than desensitization. As shown in fig. 1 PFKs is desensitized in the complete incubation mixture within 10 min. If EDTA is now added in excess to Mg^{2+} resensitization takes place within about 30 min. Subsequent addition of $MgCl_2$ in concentrations exceeding EDTA results again in a desensitization, which is at a higher rate than the resensitization. The de- and re-sensitization cycle can be repeated several times. In these experiments neither AMP (5 mM) nor ATP (2 mM) have any effect on the velocity of resensitization. Removal of F^- from PFKd (MgF^+) by chromatography on Sephadex G-25, equilibrated with a normal desensitization mixture $-F^-$ gave comparable results.

ATP and citrate also resensitize PFKd (MgF^+). As depicted in fig. 2, addition of 1 mM ATP to the desensitizing mixture leads to a slow resensitization of PFKd (MgF^+) to PFKs. If 0.05 mM ATP is added to the desensitization mixture, no resensitization occurs.

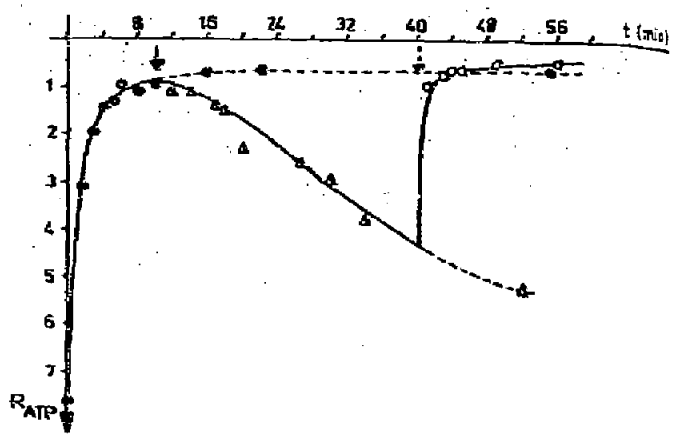

Fig. 1. Desensitization and resensitization of PFK. The desensitization was carried out in the standard desensitization mixture (●). After 10 min incubation 2 mM EDTA were added to an aliquot of the desensitization mixture (↓, ▲). After another 30 min the desensitization was again started by addition of 5 mM $MgCl_2$ (↓, ○). – – –.= control.

Because PFKs shows strong inhibition by 1.0 mM ATP, whereas at 0.05 mM there is none [2, 5], this means that only concentrations of ATP inhibitory for PFKs favour resensitization of PFKd (MgF^+). Further increases in the ATP concentration up to 5 mM do not further enhance the velocity of resensitization. Resensitization occurs at the same velocity

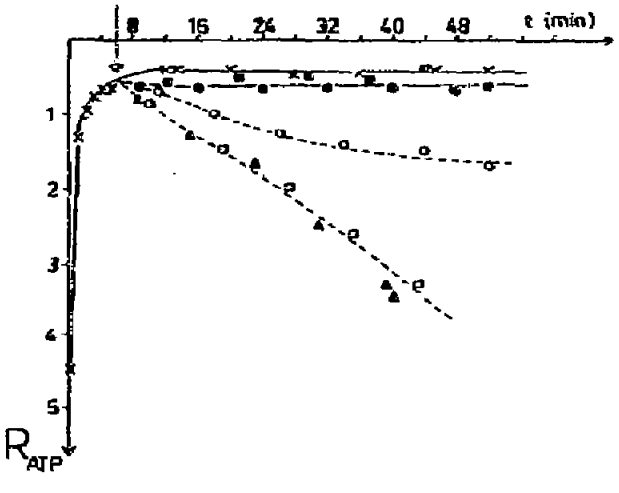

Fig. 2. Resensitization of PFKd (MgF^+) by ATP and citrate. The desensitization of PFKs was performed in the standard desensitization mixture. After 6 min the indicated effectors were added to aliquots of the desensitization mixture: × = control, ○ = 10 mM citrate, ◐ = 10 mM Mg-citrate, ■ = 0.05 mM ATP, □ = 1.0 mM ATP, ▲ = 5.0 mM ATP.

regardless of whether ATP of Mg-ATP (1:1) is added to the desensitization mixture. On the other hand, citrate is not effective as an Mg–citrate complex. Resensitization proceeds more slowly with 10 mM citrate than with 1 mM ATP.

These experiments show that the desensitization of PFKs to PFKd (MgF^+) is completely reversible.

$$\text{PFKs} \underset{-Mg^{2+} \text{ or } -F^- \text{ or } +\text{ATP}}{\overset{\text{ADP} + \text{Fru-6-P} + NH_4^+ + Mg^{2+} + F^-}{\rightleftharpoons}} \text{PFKd}\,(MgF^+)$$

As reported in the second paper of this series [3] ADP, Fru-6-P, NH_4^+, Mg^{2+} and F^- favour desensitization concertedly. Once desensitized the enzyme could be stabilized in that form by Mg^{2+} and F^- alone. If Mg^{2+}, F^- or both are removed from PFKd (MgF^+) the enzyme is reconverted to PFKs. ATP and citrate, which are inhibitors of the desensitization [3], cause a resensitization when added to the complete desensitization mixture.

ATP effects resensitization not by complexing Mg^{2+}, which is an obligatory effector of desensitization. Rather it apparently binds directly to PFKd (MgF^+) at a site distinct from that for MgF^+; at sufficiently high concentrations ATP elicts a slow resensitization. Citrate, on the contrary, permits resensitization in that it complexes with Mg^{2+}, as does EDTA. In the presence of 10 mM citrate the concentration of free Mg^{2+} in the reaction mixture is about 0.1 mM. Under these conditions the concentration of the MgF^+ complex is too small to fix PFKd as PFKd (MgF^+). An ATP-insensitive form of yeast PFK was obtained by Salas et al. [7] and Freyer et al. [8] after tryptic degradation of PFKs. The reversibility of the desensitization rules out any proteolytic mechanisms.

References

[1] E. Viñuela, M.L. Salas, M. Salas and A. Sols, Biochem. Biophys. Res. Commun. 15 (1964) 243.

[2] E.G. Afting, D. Ruppert and H. Holzer, Arch. Biochem. Biophys. 143 (1971) 587.

[3] E.G. Afting, D. Ruppert and H. Holzer, Arch. Biochem. Biophys. 152 (1972) 433.

[4] J. Monod, J. Wyman and J.R. Changeux, J. Mol. Biol. 12 (1965) 88.

[5] E.G. Afting, D. Ruppert and H. Holzer, submitted to Arch. Biochem. Biophys.

[6] W. Atzpodien and H. Bode, European J. Biochem. 12 (1970) 126.

[7] M.L. Salas, J. Salas and A. Sols, Biochem. Biophys. Res. Commun. 31 (1968) 461.

[8] R. Freyer, S. Lötze, G. Kopperschläger and E. Hofmann, European J. Biochem. 17 (1970) 386.