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FRUCTOSE-2,6-BISPHOSPHATE INCREASES THE BINDING AFFINITY OF YEAST PHOSPHOFRUCTOKINASE TO AMP

Renate Kessler, Karl Nissler, Wolfgang Schellenberger and Eberhard Hofmann

Institute of Physiological Chemistry, Karl-Marx-University, Leipzig, German Democratic Republic

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SUMMARY: At saturating concentrations of AMP four molecules of this ligand are bound per octamer of yeast phosphofructokinase. Fructose-2,6-bisphosphate increases the binding affinity of the enzyme to AMP. This indicates synergistic cooperation of the two allosteric activators in the binding process. The stoichiometry of binding is not altered by fructose-2,6-bisphosphate.

INTRODUCTION: Phosphofructokinase (EC 2.7.1.11) is of significance to the regulation of glycolysis (1). The yeast enzyme is octameric, has a molecular weight of 835 000 and is composed of two different types of subunits (2). The enzyme exhibits cooperative kinetics with respect to fructose-6-phosphate, while the second substrate ATP acts also as an inhibitor. AMP is an efficient activator of yeast phosphofructokinase causing a relief of its inhibition by ATP.

Recently, fructose-2,6-bisphosphate has been identified as a powerful activator of phosphofructokinase from mammalian tissues (3 - 5). Yeast phosphofructokinase was found to be activated also by fructose-2,6-bisphosphate (5,6). This metabolite could be detected in yeast cells (7).

For understanding the regulatory role of phosphofructokinase interest is focused on AMP and fructose-2,6-bisphosphate, the effects of which being attributed to the homeostasis of the cellular ATP level as well as to the coordination of glycolysis and gluconeogenesis, respectively (8,3).

Binding experiments of yeast phosphofructokinase demonstrated that the sigmoidicity of the fructose-6-phosphate velocity curve is the result of its cooperative binding to the enzyme (9, 10). The fructose-6-phosphate binding is not altered

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by AMP (11). Two different types of ATP binding sites have been identified and were coordinated to the catalytic and regulatory sites (12, 13). AMP was shown capable of replacing ATP from only one of these ATP binding sites (13).
In this work the interaction of the two activators of yeast phosphofructokinase was studied by investigating the effect of fructose-2,6-bisphosphate on the binding of AMP to the enzyme.

MATERIALS AND METHODS: Phosphofructokinase was isolated from baker's yeast (Saccharomyces cerevisiae) (14). Fructose-2,6-bisphosphate was prepared, purified and determined by the methods of Van Schaftingen and Hers (15). [^{32}P]AMP was enzymatically synthesized with adenosine kinase using [γ-^{32}P]ATP and purified (16). Adenosine kinase was prepared by a modification of the procedure described in (17). The binding experiments were performed at 25 °C in 50 mM imidazole buffer, pH 6.8, containing 5 mM $MgCl_2$, 2 mM mercaptoethanol and 25 mM K_2HPO_4. The AMP binding experiments were carried out in presence and absence of fructose-2,6-bisphosphate applying the ultrafiltration technique (9). The analysis of the experimental data was performed by a regression procedure as well as by a nonparametric method (18). Protein was determined by the microbiuret method (19).

RESULTS AND DISCUSSION: Binding of AMP to yeast phosphofructokinase was measured in a concentration range of 1 - 280 µM at pH 6.8 in the presence of 25 mM phosphate. The experiments were performed under conditions where the enzyme is known to be most sensitive toward the activating influence of AMP.

Fig. 1 presents the dependence of the amount of AMP bound to phosphofructokinase on the concentration of free AMP measured in the presence and the absence of 10 µM fructose-2,6-bisphosphate. Yeast phosphofructokinase was reported to be maximally stimulated at this fructose-2,6-bisphosphate concentration (6). The binding data of AMP in presence and absence of fructose-2,6-bisphosphate can be described by a hyperbolic binding function. There is no experimental evidence for cooperativity in the binding process of AMP (Fig. 1B).
The binding curve demonstrates that at saturation the enzyme binds 0.5 molecules of AMP per subunit of phosphofructokinase. This indicates a stoichiometry of four AMP binding sites per octamer. Fructose-2,6-bisphosphate does not influence the stoichiometry of AMP binding, however it is capable of shifting significantly the binding curve of AMP to the left (Fig. 1A).

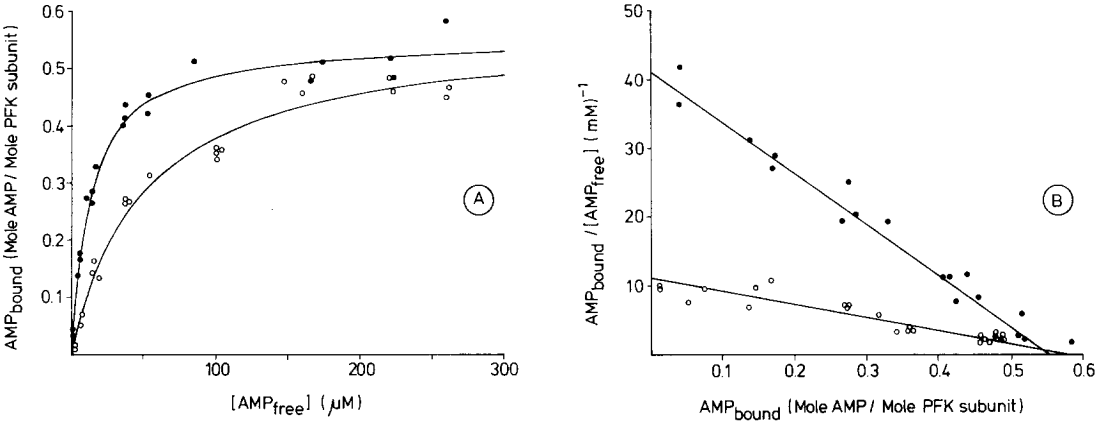

Fig. 1: Influence of fructose-2,6-bisphosphate on the binding of AMP to yeast phosphofructokinase (PFK)

A: The amount of AMP per subunit of phosphofructokinase (molecular weight 100 000) is plotted versus the free concentration of AMP.

Binding of AMP to yeast phosphofructokinase (1 - 4 mg/ml) was measured in 50 mM imidazole buffer, pH 6.8, containing 5 mM $MgCl_2$, 2 mM mercaptoethanol, 25 mM K_2HPO_4 in absence of fructose-2,6-bisphosphate (o) and in presence of 10 µM fructose-2,6-bisphosphate (●) using the ultrafiltration technique (10).

B: Scatchard-plot of the experimental data shown in Fig. 1A.

The calculated dissociation constants indicate a three-fold increase of the affinity of phosphofructokinase to AMP by fructose-2,6-bisphosphate (Table 1).

The binding stoichiometry (0.5 moles of AMP per subunit) is consistent with the results of Laurent et al. (13). In compa-

Table 1: Calculated constants of AMP binding to yeast phosphofructokinase (PFK) in absence and in presence of fructose-2,6-bisphosphate

	Regression analysis (equally weighted data)		Nonparametric method (18)	
Concentration of fructose-2,6-bisphosphate [µM]	0	10	0	10
K [µM]	51.8	13.4	53.3	13.3
C_B^{MAX} $\left[\frac{\text{Mole AMP}}{\text{Mole PFK subunit}}\right]$	0.577	0.555	0.575	0.549

The constants were obtained by fitting the data to the hyperbolic function $C_B = C_B^{MAX} \cdot [AMP] / (K + [AMP])$

rison, one mole of fructose-6-phosphate is bound per phosphofructokinase subunit (9). The difference in the binding stoichiometry of AMP and fructose-6-phosphate may possibly be related to the different activating mechanisms underlying the actions of the two ligands (20).

In kinetic experiments a synergistic action of AMP and fructose-2,6-bisphosphate on liver phosphofructokinase was found. The $K_{0.5}$ value of phosphofructokinase from liver for AMP is significantly decreased by fructose-2,6-bisphosphate (5, 21). In respect to the activation of the yeast enzyme a synergism in the effects of AMP and fructose-2,6-bisphosphate was made likely (6).

This contribution demonstrates that the kinetic interaction of the two positive effectors with yeast phosphofructokinase is due to their synergistic cooperation in the binding process. This synergism may be of regulatory significance to the coordination of glycolysis and gluconeogenesis by the phosphofructokinase/fructose-1,6-bisphosphatase cycle.

REFERENCES

1. Uyeda, K. (1979) Adv. Enzymol. Relat. Areas Mol. Biol. 48, 193-244.
2. Kopperschläger, G., Bär, J., Nissler, K., and Hofmann, E. (1977) Eur. J. Biochem. 81, 317-325.
3. Van Schaftingen, E., Hue, L., and Hers, H.-G. (1980) Biochem. J. 192, 887-901.
4. Furuya, E. and Uyeda, K. (1980) Proc. Natl. Acad. Sci. USA 77, 5861-5864.
5. Van Schaftingen, E., Jett, M.-F., Hue, L.,and Hers, H.-G. (1981) Proc. Natl. Acad. Sci. USA 78, 3483-3486.
6. Avigad, G. (1981) Biochem. Biophys. Res. Commun. 102, 985-991.
7. Lederer, B., Vissers, S., Van Schaftingen, E.,and Hers, H.-G. (1981) Biochem. Biophys. Res. Commun. 103, 1281-1287.
8. Schellenberger, W., Eschrich, K.,and Hofmann, E. (1981) Eur. J. Biochem. 118, 309-314.
9. Nissler, K., Kessler, R., Schellenberger, W., and Hofmann, E. (1977) Biochem. Biophys. Res. Commun. 79, 973-978.
10. Laurent, M., Seydoux, F.J., and Dessen, Ph. (1979) J. Biol. Chem. 254, 7515-7520.
11. Nissler, K., Kessler, R., Schellenberger, W., and Hofmann, E. (1979) Biochem. Biophys. Res. Commun. 91, 1462-1467.
12. Nissler, K., Schellenberger, W., and Hofmann, E. (1977) Acta biol. med. germ. 36, 1027-1033.
13. Laurent, M., Chaffotte, A.F., Tenu, J.-P., Roucous, C., and Seydoux, F.J. (1978) Biochem. Biophys. Res. Commun. 80, 646-652.
14. Diezel, W., Böhme, H.-J., Nissler, K., Freyer, R., Heilmann, W., Kopperschläger, G., and Hofmann, E. (1973) Eur. J. Biochem. 38, 479-488.

15. Van Schaftingen, E., and Hers, H.-G. (1981) Eur. J. Biochem. 117, 319-323.
16. Khym, J.K., and Cohn, W.E. (1953) J. Amer. Chem. Soc. 75, 1153-1156.
17. Leibach, T.K., Spiess, G.I., Neudecker, T.J., Peschke, G.J., Puchwein, G., and Hartmann, G.R. (1971) Hoppe-Seyler's Z. Physiol. Chem. 352, 328-344.
18. Cornish-Bowden, A., and Eisenthal, R. (1978) Biochim. Biophys. Acta 523, 268-272.
19. Janatova, J., Fuller, J.K., and Hunter, M.J. (1968) J. Biol. Chem. 243, 3612-3622.
20. Reuter, R., Eschrich, K., Schellenberger, W., and Hofmann, E. (1979) Acta biol. med. germ. 38, 1067-1079.
21. Uyeda, K., Furuya, E., and Luby, L.J. (1981) J. Biol. Chem. 256, 8394-8399.