The stimulation of yeast phosphofructokinase by fructose 2,6-bisphosphate

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1. INTRODUCTION

Fructose 2,6-bisphosphate was discovered as a stimulator of rat liver PFK [1–3]. Its effect on liver and muscle PFKs is to increase the affinity of the enzyme for Fru-6-P and to relieve the inhibition by ATP with no effect on V_{max} [4,5]. It is the most potent positive effector of this enzyme known at the present time. It exerts its effect at concentrations which are 1000-fold smaller than those of Fru-1,6-P_2, a classical positive effector of PFK, required for the same effect [4]. Fru-2,6-P_2 is present in glucose-grown *Saccharomyces cerevisiae* [6]; it stimulates *S. cerevisiae* PFK [4,7] and, in [4] this effect was described to be due to a change in both K_m and V_{max}. We now report these data in detail. We also show that the positive effect of Fru-2,6-P_2 is synergistic with that of AMP and can be suppressed by Fru-1,6-P_2. Furthermore, PFK from yeasts of the genus *'Rhodotorula'* was greatly stimulated by Fru-2,6-P_2. These yeasts were first reported to lack PFK [8–10] but were found, more recently, to contain an enzyme highly cooperative for Fru-6-P [11,12].

2. MATERIALS AND METHODS

PFK, purified to homogeneity from baker's yeast [13,14] was kindly provided by Dr E. Hofmann (Leipzig). This preparation was used in experiments shown in fig.1–4. *S. cerevisiae*, *Rh. glutinis* and *Rh. gracilis* were grown at 29°C in a rotary shaker in a minimum ammonium–glucose (3%, w/v) medium and harvested by filtration in the exponential phase of growth. The pellets were resuspended (10% wet wt/vol.) in 0.1 M Tris–HCl (pH 7.2) and extracted in a French pressure cell. Cell debris were removed by centrifugation at 22 000 × *g* for 20 min. The extracts were stored at −20°C and used within 2 days for the enzymatic assay.

PFK was assayed spectrophotometrically [4] by the production of ADP (fig.3) or of Fru-1,6-P_2 (other figures). The incubation mixture contained 100 mM KCl, 2.5 mM dithiothreitol, 0.15 mM NADH and buffer, substrates and effectors as indicated in the figure legends. Fructose 2,6-bisphosphate was prepared as in [18].

Abbreviations: PFK, phosphofructokinase; MES, 2-[morpholino]ethanesulfonic acid

3. RESULTS

The saturation curve of purified *S. cerevisiae* PFK for Fru-6-P measured at pH 6.4 in the presence of various concentrations of Fru-2,6-P_2 is shown in fig.1. The effect of Fru-2,6-P_2 was to increase the affinity of the enzyme for Fru-6-P and also to increase V_{max}. At 2.5 mM ATP and 0.5 mM Fru-6-P, a half-maximal stimulation was obtained with 2 μM Fru-2,6-P_2. Similar effects on K_m and V_{max} were observed at pH 7.6 both with the pure enzyme and a crude extract of *S. cerevisiae* (not shown). The inhibition of PFK by ATP was measured at various concentrations of Fru-6-P in the mM range or of Fru-2,6-P_2 in the μM range (fig.2).

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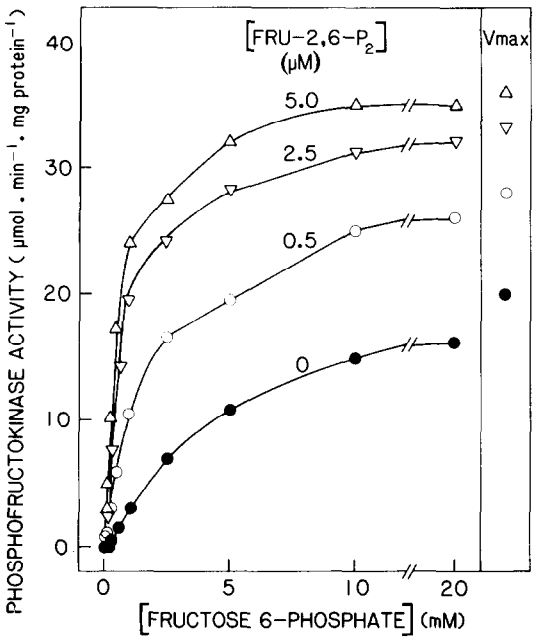

Fig.1. Effect of Fru-2,6-P_2 on the saturation curve of yeast PFK for Fru-6-P. The incubation mixture contained 50 mM MES (pH 6.4), 10 mM NH_4Cl, 2.5 mM ATP, 5 mM $MgCl_2$ and Fru-6-P and Fru-2,6-P_2 as indicated. The Hill coefficient was 1.32 in the absence of Fru-2,6-P_2 and 1.18, 1.12 and 1.04 in the presence of increasing concentrations of the stimulator.

Both sugar phosphates had a similar effect to counteract the inhibition by ATP.

Yeast PFK, contrary to the mammalian enzyme,

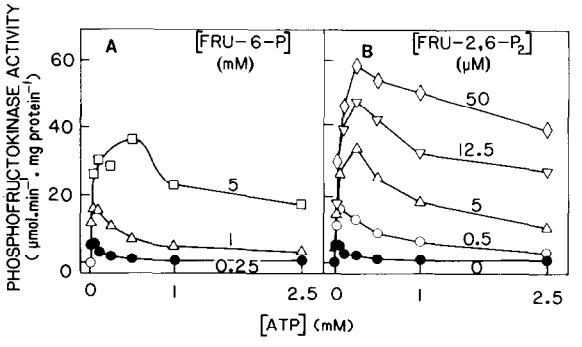

Fig.2. Effect of Fru-6-P (A) and Fru-2,6-P_2 (B) on the inhibition by ATP. The incubation mixture contained 50 mM imidazole (pH 7.6), 1 mM NH_4Cl, ATP, Fru-6-P and Fru-2,6-P_2 as indicated, and $MgCl_2$ in a 5 mM excess over ATP; in (B) Fru-6-P was 0.25 mM.

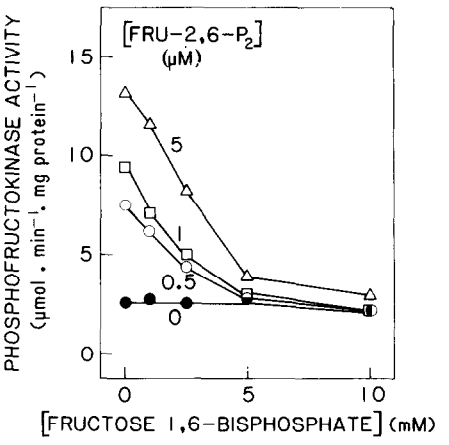

Fig.3. Effect of Fru-1,6-P_2 on the enzymic activity measured at various concentrations of Fru-2,6-P_2. The incubation mixture contained 50 mM MES (pH 6.4), 10 mM NH_4Cl, 1 mM ATP, 0.25 mM Fru-6-P, 3.5 mM $MgCl_2$ and 5 mM P_i. The ADP-coupled assay was used.

is known not to be stimulated by Fru-1,6-P_2 [15]. We show in fig.3 that Fru-1,6-P_2 counteracted the positive effect of Fru-2,6-P_2. AMP is another positive effector the action of which is synergistic with that of Fru-2,6-P_2 on liver and muscle PFKs [4,5]. We show in fig.4 that the stimulation of the yeast

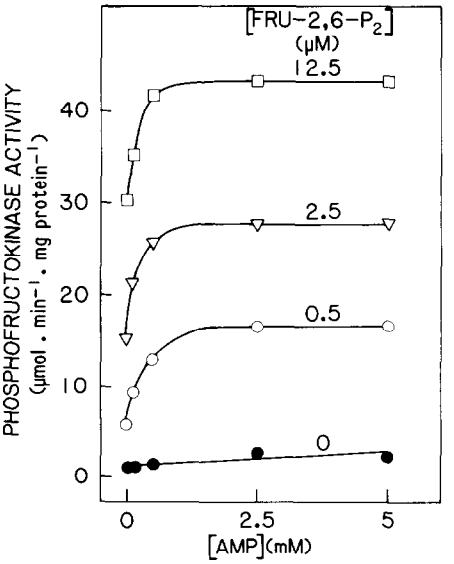

Fig.4. Effect of Fru-2,6-P_2 on the stimulation of the enzymic activity by AMP. Fru-6-P was 1 mM and ATP was 1 mM; other assay conditions, as in fig. 2.

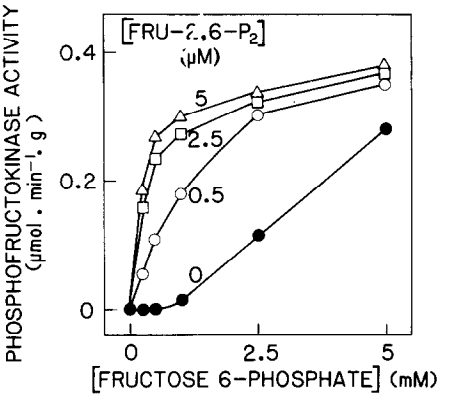

Fig.5. Effect of Fru-2,6-P_2 on the saturation curve of *Rh. glutinis* phosphofructokinase. The incubation mixture contained 50 mM imidazole (pH 7.0), 10 mM NH_4Cl, 1 mM ATP, 3.5 mM $MgCl_2$ and Fru-6-P and Fru-2,6-P_2 as indicated.

enzyme by AMP was only ~ 2-fold both in the presence and in the absence of Fru-2,6-P_2. The K_a for Fru-2,6-P_2 was close to 1.4 μM in the presence of 2.5 mM AMP and to 3 μM in its absence. It is remarkable, however, that the presence of Fru-2,6-P_2 did increase the affinity of the enzyme for AMP.

The effect of Fru-2,6-P_2 on the affinity of PFK present in a crude extract of *Rh. glutinis* is shown in fig.5. At 0.05 mM Fru-6-P, the activity reached a nearly maximal value in the presence of 5 μM Fru-2,6-P_2, whereas it was barely detectable in its absence. Similar results were obtained on the activity of PFK present in a crude extract of *Rh. gracilis*.

4. DISCUSSION

Fru-2,6-P_2 has been identified in *S. cerevisiae* grown on glucose although not in the same organism grown on pyruvate [6]. One could therefore assume that Fru-2,6-P_2 plays an important role in the initiation of glycolysis and in the inhibition of gluconeogenesis by glucose. It not only stimulates PFK but also inhibits yeast fructose-1,6-bisphosphatase [16] at the levels found in glucose-grown yeast. Its effect on yeast PFK is similar although not identical to that on the liver enzymes, the main difference being the change in the V_{max} and the weak synergism with AMP. Thus, AMP decreased the K_a for Fru-2,6-P_2 < 2-fold in the case of the yeast enzyme and > 50-fold in the case of the liver [4] and of the erythrocyte [19] enzyme. Not all our kinetic data agree with those in [7] with a 9-year-old preparation of commercial yeast PFK; indeed no effect of Fru-2,6-P_2 at pH < 7 and little or no change in V_{max} was observed [7].

It also appears that in the presence of Fru-2,6-P_2, PFK from *Rhodotorula* yeast has an affinity for Fru-6-P comparable to that of the *Saccharomyces* enzyme. A positive effect of Fru-1,6-P_2 on PFK has often been assumed to play a role in the control of its activity [17]. However, in agreement with [15], Fru-1,6-P_2 did not stimulate *S. cerevisiae* PFK, but acted as an inhibitor of the Fru-2,6-P_2-stimulated enzyme. This feedback inhibition of yeast PFK by the reaction product is likely to play an important role in adapting the rate of fructose 6-phosphate phosphorylation to the glycolytic capacity of the cell.

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REFERENCES

[1] Van Schaftingen, E., Hue, L. and Hers, H.G. (1980) Biochem. J. 192, 887–895.
[2] Van Schaftingen, E., Hue, L. and Hers, H.G. (1980) Biochem. J. 192, 897–901.
[3] Van Schaftingen, E. and Hers, H.G. (1980) Biochem. Biophys. Res. Commun. 96, 1524–1531.
[4] Van Schaftingen, E., Jett, M.-F., Hue, L. and Hers, H.G. (1981) Proc. Natl. Acad. Sci. USA 78, 3483–3486.
[5] Uyeda, K., Furuya, E. and Luby, L.J. (1981) J. Biol. Chem. 256, 8394–8399.
[6] Lederer, B., Vissers, S., Van Schaftingen, E. and Hers, H.G. (1981) Biochem. Biophys. Res. Commun. 103, 1281–1287.
[7] Avigad, G. (1981) Biochem. Biophys. Res. Commun. 102, 985–991.
[8] Brady, R.J. and Chambliss, G.H. (1967) Biochem. Biophys. Res. Commun. 29, 898–903.
[9] Höfer, M., Becker, J.U., Brand, K., Deckner, K. and Betz, A. (1969) FEBS Lett. 3, 322–324.

[10] Gancedo, J.M. and Gancedo, C. (1971) Arch. Microbiol. 76, 132–138.
[11] Mazon, M.J., Gancedo, J.M. and Gancedo, C. (1974) Biochem. Biophys. Res. Commun. 61, 1304–1309.
[12] Zeidan, H.M. (1981) Biochem. Biophys. Res. Commun. 100, 681–687.
[13] Diezel, W., Böhme, H.-J., Nissler, K., Freyer, R., Heilmann, W., Kopperschläger, G. and Hofmann, E. (1973) Eur. J. Biochem. 38, 479–488.
[14] Kopperschläger, G., Bär, J., Nissler, K. and Hofmann, E. (1977) Eur. J. Biochem. 81, 317–325.
[15] Viñuela, E., Salas, M.L. and Sols, A. (1963) Biochem. Biophys. Res. Commun. 12, 140–145.
[16] Van Schaftingen, E. and Hers, H.G. (1981) Proc. Natl. Acad. Sci. USA 78, 2861–2863.
[17] Boiteux, A., Hess, B. and Sel'kov, E.E. (1980) Curr. Top. Cell. Regul. 17, 171–203.
[18] Van Schaftingen, E. and Hers, H.G. (1981) Eur. J. Biochem. 117, 319–323.
[19] Heylen, A., Van Schaftingen, E. and Hers, H.G (1982) FEBS Lett. 143, 141–143.