The stimulation of phosphofructokinase from human erythrocytes by fructose 2,6-bisphosphate

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1. INTRODUCTION

Erythrocytes have an anaerobic life and rely entirely on glycolysis for their supply of ATP. In erythrocytes like in other tissues, the main rate-limiting enzyme of glycolysis at physiological pH appears to be PFK [1]. PFK from human erythrocytes has been purified and its properties investigated [2]. Its general properties are known to be similar to those of PFKs from other tissues.

Because glycolysis in erythrocytes is not known to be subjected to the control by hormones or by the availability of glucose it was of interest to investigate if, nevertheless, erythrocyte PFK would be sensitive to stimulation by Fru-2,6-P_2.

2. MATERIALS AND METHODS

PFK was purified from fresh human erythrocytes as in [2] up to the first ammonium sulfate precipitation step (inclusive). Several enzymatic preparations, with slightly different specific activities were used. Before the assays, the enzyme was filtered on Sephadex G-25 equilibrated with 100 mM KF, 15 mM EGTA, 50 mM Hepes (pH 7.1), 0.1 mM Fru-6-P and 0.35 mM Glc-6-P. The preparation was virtually free of adenylate kinase and aldolase. PFK was assayed as in [3], except that Glc-6-P was present at a 3.5-fold greater level than Fru-6-P. Unless otherwise indicated, the assay mixture contained 50 mM Hepes, 5 mM P_i, 0.1 mM AMP, 100 mM KCl, 0.15 mM NADH, 1 mM NH_4Cl and $MgCl_2$ in a 5 mM excess over ATP (pH 7.1). Fructose 2,6-bisphosphate was prepared as in [4].

Abbreviations: PFK, phosphofructokinase; Hepes, *N*-2, hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid

3. RESULTS

The effect of Fru-2,6-P_2 on the saturation curve of erythrocyte PFK for its two substrates, Fru-6-P and ATP, is shown in fig.1A,B. It appears that

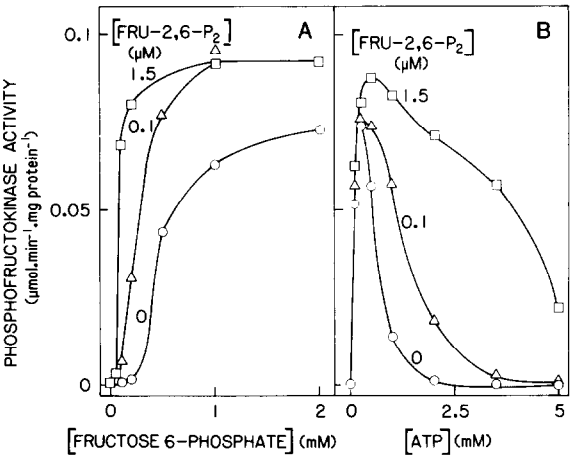

Fig.1. Effect of Fru-2,6-P_2 on (A) the affinity of PFK for Fru-6-P and (B) the inhibition of the enzyme by ATP: (A) ATP 1.5 mM; (B) Fru-6-P 0.25 mM. The activity measured at 5 mM Fru-6-P amounted to 0.084 $\mu mol \cdot min^{-1} \cdot mg\ protein^{-1}$ in the absence of Fru-2,6-P_2 and to 0.091 in its presence.

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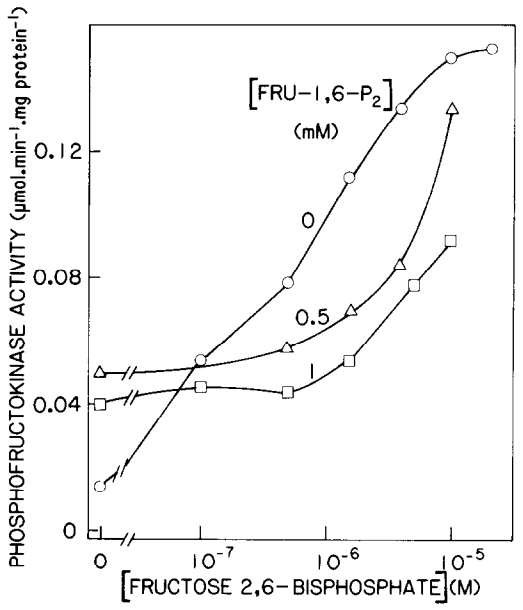

Fig.2. Effect of Fru-2,6-P_2 on the activity of PFK in the presence of various concentrations of Fru-1,6-P_2. The assays were performed in the presence of 0.2 mM Fru-6-P and 3 mM ATP.

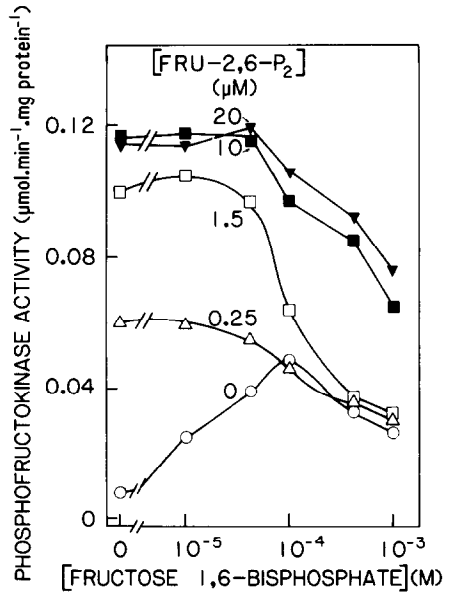

Fig.3. Effect of Fru-1,6-P_2 on the activity of PFK, in the presence of various concentrations of Fru-2,6-P_2. The assays were performed in the presence of 0.05 mM P_i, 0.1 mM Fru-6-P and 1.5 mM ATP.

Fru-2,6-P_2, at μM levels increased the affinity for Fru-6-P and released the inhibition by ATP. V_{max} was not affected. The dose–response curve to Fru-2,6-P_2 is shown in fig.2. Under the experimental conditions chosen, a half-maximal effect was obtained at close to μM levels of Fru-2,6-P_2. The same figure shows that 0.5 mM Fru-1,6-P_2 had some stimulatory action on the enzyme when the activity was measured in the absence of Fru-2,6-P_2. However, in the presence of Fru-2,6-P_2, Fru-1,6-P_2 counteracted the positive action of its isomer. Glc-1,6-P_2 had effects similar to that of Fru-1,6-P_2 but at 5-fold greater concentrations (not shown). The interaction between the two fructose bisphosphates is further illustrated in fig.3. It appears again that Fru-1,6-P_2 by itself had a slight positive effect at $\geq 10^{-5}$ M but that in the presence of Fru-2,6-P_2 it became inhibitory at 5×10^{-5} M.

The effect of pH on the activity of erythrocytes PFK in the absence or in the presence of 1.5 μM Fru-2,6-P_2 is shown in fig.4. It is apparent that Fru-2,6-P_2 allowed enzyme activity over pH 6.6–7.4, at which no activity could be detected in its absence. Maximal stimulation was observed at pH 7.4.

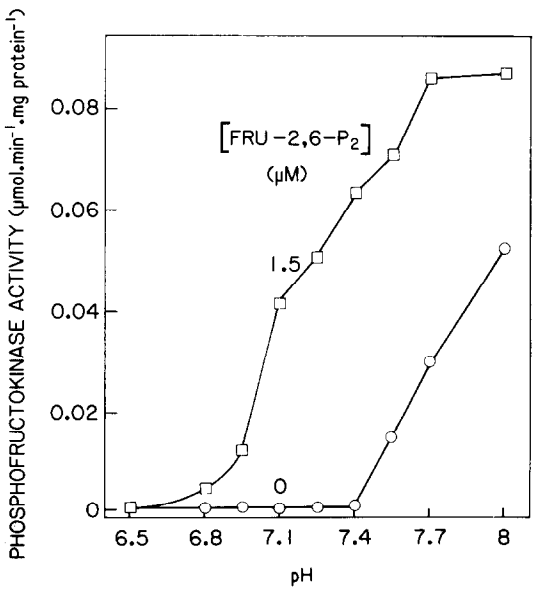

Fig.4. Effect of pH on the activity of PFK in the presence or in the absence of Fru-2,6-P_2. The assays were performed in the presence of 0.2 mM Fru-6-P and 1.5 mM ATP.

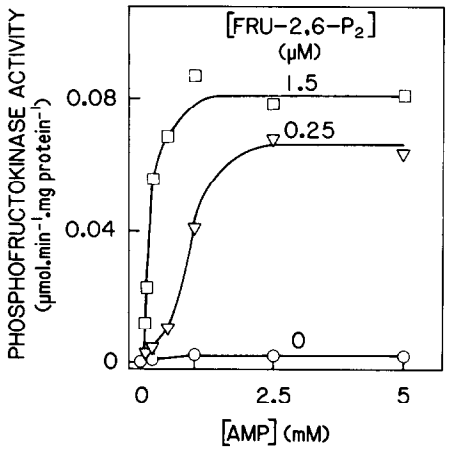

Fig.5. Effect of Fru-2,6-P_2 on the activity of PFK measured at various concentrations of AMP. The assays were performed in the presence of 50 μM Fru-6-P and 1.5 mM ATP.

The synergism between AMP and Fru-2,6-P_2, which was most striking in the case of liver PFK, is illustrated by fig.5. One can see that in the conditions chosen, almost no activity could be detected, in the presence of AMP ($\leq$ 5 mM) or of Fru-2,6-P_2 alone. In contrast, the enzyme became very active in the simultaneous presence of small concentrations of both stimulators.

4. DISCUSSION

The effects of Fru-2,6-P_2 on human erythrocytes PFK reported here are very similar to the effect of the same effectors on liver and muscle PFKs [3,5]. This is particularly apparent for the effect of Fru-2,6-P_2 to increase the affinity for Fru-6-P and to reduce the inhibition by ATP without affecting V_{max} and also to be highly synergistic with that of AMP.

Other effects had not been investigated with other mammalian PFKs. Fru-1,6-P_2 was a weak stimulator of the enzyme and became inhibitory in the presence of Fru-2,6-P_2. This inhibitory action is similar to that observed with yeast PFK [6] and suggests that the two fructose bisphosphates have affinity for a common site of fixation. The interrelationship between the stimulation by Fru-2,6-P_2 and pH had not been reported although it was forseeable. It is known that H^+ is a negative effector of PFKs in general and therefore that the effect of positive effectors would be the most apparent in the acid range of pH. The effect of Fru-2,6-P_2 in this respect is, therefore, not different from that of other positive effectors.

One can conclude from this work that the property to be stimulated by Fru-2,6-P_2 is a general property of many PFKs and up to now of all mammalian PFKs. This is despite the fact Fru-2,6-P_2 could not be detected in erythrocytes (unpublished) and that glycolysis in erythrocytes is little subjected to regulation.

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REFERENCES

[1] Rapoport, S. (1968) Essays Biochem. 4, 69–103.
[2] Tarui, S., Kono, N. and Uyeda, K. (1972) J. Biol. Chem. 247, 1138–1145.
[3] Van Schaftingen, E., Jett, M.-F., Hue, L. and Hers, H.G. (1981) Proc. Natl. Acad. Sci. USA 78, 3483–3486.
[4] Van Schaftingen, E. and Hers, H.G. (1981) Eur. J. Biochem. 117, 319–323.
[5] Uyeda, K., Furuya, E. and Luby, L.J. (1981) J. Biol. Chem. 256, 8394–8399.
[6] Bartrons, R., Van Schaftingen, E., Vissers, S. and Hers, H.G. (1982) FEBS Lett. 143, 137–140.