STUDIES ON THE FRUCTOSE 1-PHOSPHATASE ACTIVITY OF PIG KIDNEY FRUCTOSE 1,6-DIPHOSPHATASE

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1. Introduction

Mammalian liver and kidney fructose 1,6-diphosphatase (EC 3.1.3.11) (FDPase) has been shown to catalyze the hydrolysis of the 1-phosphate from fructose 1,6-diphosphate and the next higher homolog, sedoheptulose 1,7-diphosphate [1]. During the last few years it has also been shown that FDPase catalyzes the hydrolysis of other structurally unrelated substrates, such as β-glycerophosphate [2], phosphoenol pyruvate [3], and *p*-nitrophenyl phosphate [4]. However, little attention has been given to earlier indications pointing out that rabbit liver [5] and pig kidney FDPase [6] show some activity with fructose-1-P. Since fructose-1-P is formed by fructokinase in those tissues (e.g. liver and kidney) metabolizing fructose [7, 8], it was deemed important to study some parameters of the hydrolysis of fructose-1-P by FDPase, because the concurrent function of both fructokinase (Fructose + ATP → Fructose-1-P + ADP) and of FDPase (Fructose-1-P → Fructose + P_i) within a single cell compartment could lead to a futile cycle of carbon and a net hydrolysis of ATP.

2. Methods

FDPase activity was measured by the rate of formation of inorganic phosphate either from fructose 1,6-diphosphate or fructose-1-P at 30°C, as previously described [9]. Unless otherwise stated, the incubation mixture of 1 ml contained 50 mM Tris–HCl (pH 7.5), 0.1 mM EDTA, 5 mM $MgSO_4$, 0.10 ml of a solution containing FDPase, and the substrate as indicated in the figure legends. Enzyme dilutions were always made in 20 mM Tris–HCl (pH 7.5), containing 2 mM $MgSO_4$ and 0.1 mM EDTA. A unit of FDPase activity is defined as that amount of enzyme which catalyzes the formation of 1 μmole of inorganic phosphate per min under the described conditions. Specific activity is expressed in terms of units per mg of protein. FDPase concentration was determined by its absorbance at 280 nm using a value of 0.755 for the absorbance per mg per ml [10].

Pig Kidney FDPase with maximal activity at neutral pH was prepared as previously described [11]. Its specific activity, measured as described above, using 0.5 mM fructose 1,6-diphosphate as substrate and 75 mM K_2SO_4, was 30.2. When K^+ was omitted from the assay system, the specific activity diminished to 10.1.

Modification of FDPase with pyridoxal-P and $NaBH_4$ reduction was carried out as previously described under preparation of pyridoxyl-P FDPase (see ref. [12]) except that the modification was carried out at 4°C and 2.5 mM AMP was added to the incubation system.

3. Results

Purified pig kidney FDPase hydrolyzed fructose-1-P. The formation of inorganic phosphate was linear with time up to a consumption of 50% of the substrate, and was proportional to enzyme concentration. Clearly contrasting with our previous findings showing that K^+ is an activator of FDPase when fructose 1,6-diphosphate is the substrate of the reaction [11, 13], the activity with fructose-1-P was significantly lower in the presence of 150 mM K^+ than in its

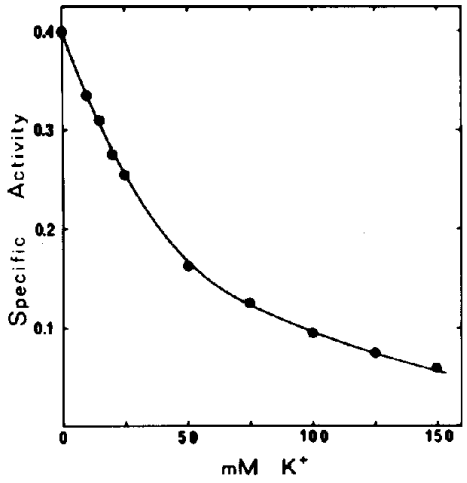

Fig. 1. Effect of K^+ on the activity of FDPase with fructose-1-P as the substrate. Except for the variable concentration of potassium ions (added as K_2SO_4), the assays were performed at 1.34 mM fructose-1-P as described under Methods.

absence. The inhibitory effect of K^+ on hydrolysis of fructose-1-P is shown in fig. 1.

Kinetic studies with fructose-1-P as substrate for FDPase demonstrated that the enzyme displays Michaelis–Menten kinetics either in the presence of 150 mM K^+ or in its absence, with no indication of

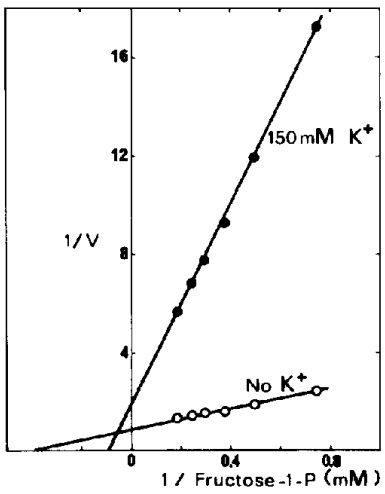

Fig. 2. Double reciprocal plots for the hydrolysis of fructose-1-P by FDPase, in the presence (●—●—●) and absence (o—o—o) of 150 mM K^+. V = μmoles of inorganic phosphate formed per min per mg of enzyme.

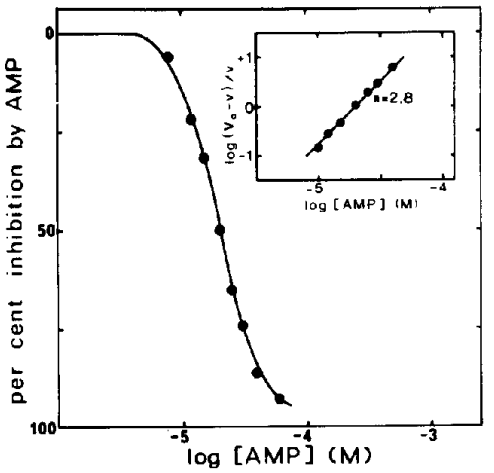

Fig. 3. Inhibition by AMP of FDPase activity with fructose-1-P as the substrate. The assays were performed at 1.34 mM fructose-1-P in the absence of K^+, as described under Methods, except that AMP was added as indicated. The *inset* shows the Hill plots according to Taketa and Pogell [14] of data from this figure. n Represents the slope of the straight line.

inhibition by excess substrate. Lineweaver–Burk plots are shown in fig. 2. The extrapolated Michaelis constants (K_M) for fructose-1-P are 2.3 mM in the absence of K^+, and 11.1 mM in the presence of 150 mM K^+. V_{max} decreased 2.2-fold due to the presence of 150 mM K^+.

The characteristic allosteric AMP inhibition of FDPase [14] was also observed when fructose-1-P was the substrate of the reaction (fig. 3). The K_i and n values, 0.02 mM and 2.8, respectively, obtained from Hill plots (*inset* fig. 3) were in good agreement with those reported for fructose 1,6-diphosphate as the substrate of the reaction [11].

Although the structural similarity of the substrates, fructose-1-P and fructose 1,6-diphosphate, as well as the similar effect of AMP on enzyme activity with both substrates, indicate that both are probably hydrolyzed at the same active site, chemical modification of the enzyme was also carried out to provide additional evidence in favour of the above hypothesis. The results shown in fig. 4 indicate that modification of the enzyme with pyridoxal-P, under conditions which appear to be selective for modification of FDPase at the active site region [15], results in a similar pattern of loss of both activities.

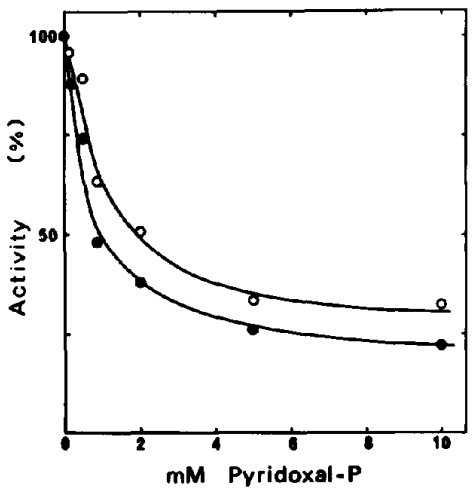

Fig. 4. Inactivation of FDPase activities by modification with pyridoxal-P. After modification with the indicated concentrations of pyridoxal-P (see Methods), enzyme activity was measured with 0.5 mM fructose 1,6-diphosphate and 150 mM K^+ (o—o—o), as well as with 1.34 mM fructose-1-P (•—•—•), as described under Methods.

4. Discussion

The results presented in this paper show that fructose-1-P is a substrate for pig kidney FDPase, and suggest that both fructose 1,6-diphosphate and fructose-1-P are hydrolyzed at the same active site. Since the kinetics of the reaction are simple, with no additional complications such as the excess substrate inhibition and the very low K_M values (approx. 0.002 mM) of the reaction with fructose 1,6-diphosphate as the substrate, fructose-1-P would make a particularly useful substrate in future studies on the mechanism of the reaction. It may also be a useful tool for following the results of chemical modification studies at the active site of FDPase (i.e., to differentiate a 6-P binding site from a 1-P binding site).

The activation of fructose 1,6-diphosphate hydrolytic activity by K^+ on one side, and the inhibitory effect of K^+ on fructose-1-P hydrolysis on the other, appeared at first sight rather puzzling. However, two explanations which may have a physiological basis can be given: i) the antagonistic effects of K^+ on both activities represent a considerable gain in FDPase specificity for its normal substrate (fructose 1,6-diphosphate), and reduces activity for an analog substrate (fructose-1-P) which is a normal intermediate of another metabolic pathway. For instance, when using the FDPase activity values at 0.5 mM fructose 1,6-diphosphate and 1.34 mM fructose-1-P, the fructose 1,6-diphosphate/fructose-1-P activity ratio increases from 25 to 500 due to the antagonistic effect of K^+ on both activities; ii) the inhibitory effect of K^+ on fructose-1-P hydrolytic activity of FDPase also establishes an antagonistic effect on the first reaction of fructose metabolism, since fructokinase (EC 2.7.1.3) is activated by K^+ [16, 17]. Thus, the possibility of an 'in vivo' futile cycle (see Introduction) is greatly diminished.

Acknowledgements

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References

[1] Pontremoli, S. and Horecker, B.L. (1971) in: The Enzymes (Boyer, P.D., ed), Vol. 4, p. 611, Academic Press, New York.
[2] Cohen, H.J., Harris, T.M., Geller, A.M. Byrne, W.L. (1971) Biochem. Biophys. 146, 144.
[3] Kirtley, M.E. and Dix, J.C. (1969) Biochem. Biophys. Res. Commun. 37, 634.
[4] Chou, T. and Kirtley, M.E. (1971) Biochem. Biophys. Res. Commun. 45, 98.
[5] Mokrasch, L.C. and McGilvery, R.W. (1956) J. Biol. Chem. 221, 909.
[6] Mendicino, J. and Vasarhely, F. (1963) J. Biol. Chem. 238, 3528.
[7] Sillero, M.A.G., Sillero, A. and Sols, A. (1969) Eur. J. Biochem. 10, 345.
[8] Kranhold, J.F., Loh, D. and Morris, R.C. (1969) Science 165, 402.
[9] Marcus, F. (1967) Arch. Biochem. Biophys. 122, 393.
[10] Marcus, F. and Hubert, E. (1968) J. Biol. Chem. 243, 4923.
[11] Colombo, G. and Marcus, F. (1973) J. Biol. Chem. 248, 2743.
[12] Colombo, G., Hubert, E. and Marcus, F. (1972) Biochemistry 11, 1798.

[13] Hubert, E., Villanueva, J., González, A.M. and Marcus, F. (1970) Arch. Biochem. Biophys. 138, 590.
[14] Taketa, K. and Pogell, B.M. (1965) J. Biol. Chem. 240, 651.
[15] Colombo, G. and Marcus, F., in preparation.
[16] Parks, R.E., Ben-Gershom, E. and Lardy, H.A. (1957) J. Biol. Chem. 227, 231.
[17] Sánchez, J.J., González, N.S. and Pontis, H.G. (1971) Biochim. Biophys. Acta 227, 79.