The Hydrolysis of Phosphoenol Pyruvate Catalyzed by Rabbit Liver Fructose-1,6- diphosphatase+

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ABSTRACT

Fructose-1,6-diphosphatase isolated from rabbit liver has been found to catalyze the hydrolysis of phosphoenol pyruvate (PEP). Hydrolysis of either fructose diphosphate (FDP) or PEP is dependent on the presence of Mn^{++} or Mg^{++} and is inhibited by AMP. The hydrolysis of PEP is inhibited by FDP. The K_m for PEP is 1.1×10^{-3} M and the V_{max} is 2-4% of that observed with FDP. Some implications of this activity of fructose diphosphatase are mentioned.

The enzyme fructose-1,6-diphosphatase* (FDPase)** from rabbit liver has been shown to catalyze the hydrolysis of the 1-phosphate from fructose diphosphate or sedoheptulose diphosphate but not from a number of other sugar phosphates (Bonsignore et al, 1963; Horecker et al, 1966). In addition, there is a preliminary report that the FDPase from beef liver catalyzes the hydrolysis of β-glycerophosphate (Byrne et al, 1965) although this activity has not yet been confirmed with rabbit liver FDPase. In this communication we report that rabbit liver FDPase catalyzes the hydrolysis of phosphoenol pyruvate (PEP).

Rabbit liver FDPase was purified by a procedure based on that of Pontremoli et al, (1965) which includes homogenization, ammonium sulfate fractionation, chromatography on CM-cellulose, and crystallization. The steps of the original procedure which include the use of organic solvents, extremes of pH and heating were not used. The overall yield and specific activity of the pure enzyme were comparable with those reported by Pontremoli et al. In all the experiments reported below the amount of enzyme used is reported in terms of the international

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* IUB #3.1.3.11, D-Fructose-1,6-diphosphate 1-phosphohydrolase.

** Abbreviations used in this paper: FDPase, fructose-1,6-diphosphatase; PEP, phosphoenol pyruvate; CM-cellulose, carboxymethyl cellulose; "PEPase", phosphoenol pyruvate phosphohydrolase; TCA, Trichloroacetic acid.

units of FDPase activity which that enzyme would have had with FDP as substrate at pH 9.3 at 25° (Pontremoli et al, 1965).

Table 1

Release of Phosphate from PEP in Presence of FDPase

FDPase	pH	mμmoles P_i/ml			
		0	15 min.	30 min.	60 min.
+	7.2	0	13.8	30.0	64.3
-	7.2	0	1.7	-	4.3
+	9.2	0	0	0.8	2.5
-	9.2	0	0	0	0

Each incubation mixture contained 1.0 mM PEP, 0.5 mM $MnCl_2$ and either 10 mM Tris, pH 7.4 or 40 mM glycine, pH 9.4 in a total volume of 2.0 ml at 25°. The enzymatic reactions were begun by addition of 0.1 ml FDPase (0.55 units FDPase). At the indicated times 0.4 ml aliquots were removed and the reaction was stopped with 30% TCA. Phosphate was measured by the procedure of Chen, Toribara and Warner (Chen et al, 1956).

The hydrolysis of PEP as measured by the release of inorganic phosphate is shown in Table 1. At pH 7.2 in the presence of rabbit liver FDPase the formation of inorganic phosphate is linear with time for at least one hour. The non-enzymatic hydrolysis of PEP under these conditions is very slow. At pH 9.2 there is very little PEP hydrolysis in the absence or presence of the enzyme. In several preparations of FDPase the maximal rate of PEP hydrolysis has been 2 - 4% of that for FDP at pH 7.2.

The initial rate of phosphate release as a function of PEP concentration is shown in Figure 1. The K_m for PEP is 9.1×10^{-4} M at pH 7.2. Under the same conditions the K_m for FDP is 3×10^{-6} M. Both PEP and FDP give normal hyperbolic kinetic curves and linear double reciprocal plots and, consequently, show no evidence of cooperative binding.

One of the characteristic properties of the FDPase-catalyzed hydrolysis of FDP is allosteric inhibition by AMP (Taketa and Pogell, 1965). As shown in Table 2 the enzymatic hydrolysis of PEP is also strongly inhibited by AMP.

The hydrolysis of PEP should produce inorganic phosphate and pyruvate. The formation of pyruvate was assayed by coupling the reaction with lactate dehydrogenase and following the PEP-dependent oxidation of NADH. This

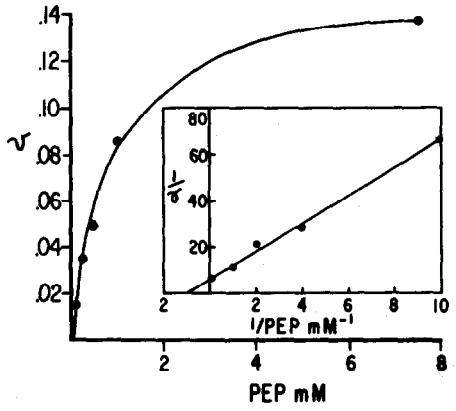

Figure 1. Initial rate of hydrolysis of PEP as a function of PEP concentration.

Table 2

Inhibition by AMP of the Hydrolysis of PEP

Addition	mμmoles P_i/minute
None	1.55
0.1 mM AMP	0.25
1.0 mM AMP	0

Each incubation mixture contained 1.0 mM PEP, 0.5 mM $MnCl_2$, 10 mM Tris and AMP as indicated in a total volume of 2.0 ml with final pH of 7.2. Reaction was initiated by addition of 50 μm FDPase (0.32 units FDPase) and the progress of the reaction was followed as described in Table 1.

assay is linear with time through at least 50% hydrolysis of the added PEP and the activity is dependent on the presence of FDPase and the cation activator.

The hydrolysis of both FDP and PEP has an absolute requirement for divalent cations. As shown in Table 3 the activity with Mn^{++} is higher than that with Mg^{++} for both substrates. In other experiments 0.5 mM $MnCl_2$ has been found to be optimum for the hydrolysis of both substrates.

When the coupled assay for PEP hydrolysis is allowed to go to completion the amount of NADH oxidized is equal to the total amount of added PEP. Because the coupled assay measures only pyruvate it can be used to determine the effect of FDP on the rate of hydrolysis of PEP by

Table 3

Effect of Manganese and Magnesium on Hydrolysis of FDP or PEP by FDPase

Activator	FDP Hydrolysis		PEP Hydrolysis	
	mμmoles/min	% of activity with 0.5 mM $MnCl_2$	mμmoles/min	% of activity with 0.5 mM $MnCl_2$
None	0	-	0	-
0.5 mM $MnCl_2$	0.87	100	3.41	100
0.5 mM $MgCl_2$	0.24	28	1.35	38
5.0 mM $MgCl_2$	0.29	33	1.44	41

FDP hydrolysis was measured by the spectrophotometric assay of Pontremoli et al (1965). Each cuvet contained 0.1 mM FDP, 0.5 mM NA DP, 10 mM Tris, 2 units phosphoglucose isomerase, 0.3 units glucose 6-phosphate dehydrogenase and the additions indicated in a total volume of 1.0 ml with a final pH of 7.2. Reaction was initiated by addition of 10 μl FDPase (8.6 munits FDPase) and the rate of increase at 340 mμ was measured.

PEP hydrolysis was measured by coupling the reaction with lactate dehydrogenase. Each cuvet contained 1.0 mM PEP, 0.25 mM NADH, 10 mM Tris, 0.16 units lactate dehydrogenase and the additions indicated in a total volume of 1.0 ml with a final pH of 7.2. Reaction was initiated by addition of 20 μl FDPase (0.172 units FDPase) and the rate of decrease at 340 mμ was measured.

Table 4

Inhibition of PEP Hydrolysis by FDP

Addition	PEP Hydrolysis mμmoles/minute
None	1.45
0.0. mM FDP	0.96
0.1 mM FDP	0.42

Each cuvet contained 0.4 mM PEP, 0.5 mM $MnCl_2$, 10 mM NADH, 0.16 units lactate dehydrogenase and FDP as indicated in a total volume of 1.0 ml with a final pH of 7.2. Reaction was initiated by addition of 40 μl FDPase (0.67 units FDPase) and the rate of reaction was followed as described in Table 3.

FDPase. The data in Table 4 show that FDP inhibits the hydrolysis of PEP as is expected if both compounds are substrates for the same enzyme.

These results show that pure preparations of rabbit liver FDPase

catalyze the hydrolysis not only of FDP and sedoheptulose diphosphate but also of phosphoenol pyruvate. The evidence which suggests that the "PEPase" activity is due to FDPase and not to a contaminating enzyme includes the following:

1. several pure FDPase preparations have been tested and found to contain "PEPase" activity;
2. both FDPase and "PEPase" activities require Mn^{++} or Mg^{++} and show higher activity in the presence of Mn^{++};
3. both activities are inhibited by AMP;
4. "PEPase" activity is inhibited by FDP.

While the FDPase-catalyzed hydrolysis of FDP shows two pH optima, 9.3 and 7.3, the hydrolysis of PEP occurs only at the lower optimum. Although the significance of the biphasic pH-activity profile for FDP hydrolysis is not yet clear, there are several reports that the activity at pH 7.3 can be modified independently of that at pH 9.3 (Pontremoli et al, 1965, Pontremoli et al, 1967). It seems reasonable to expect that the physiological activity of this enzyme is related to its function at pH 7.3 rather than at the higher pH.

Because of the low V_{max} and high K_m of the "PEPase" activity of FDPase it is not clear that this activity has any physiological significance. However, the fact that PEP is a substrate shows that there is a specific interaction between this compound and the enzyme and it is possible that other conditions exist in which the "PEPase" activity is accentuated or in which PEP modifies the FDPase activity of the enzyme. It is interesting to note that this is not the first example of an effect of both FDP and PEP on a common enzyme. Recent studies have shown that pyruvate kinase, for which PEP is a substrate, is specifically activated by FDP (Tanaka et al, 1967; Maeba and Sanwal, 1968). This effect has been cited as a possible example of "feed-forward activation" in which FDP, as an early precursor of PEP, serves to stimulate PEP utilization through activation of the enzyme. Regardless of whether PEP has any function with FDPase other than as a weak substrate it will be of interest to compare the mutual interactions of FDP and PEP with pyruvate kinase and with FDPase in order to determine if two such apparently different enzymes have common mechanisms of interaction with these compounds.

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