REGULATION OF FDPase IN C. UTILIS*

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Specific fructose 1,6-diphosphatases (FDPases) which catalyze the dephosphorylation of fructose 1,6-diphosphate to fructose 6-phosphate and inorganic phosphate, have been described in a wide variety of living forms (Gormori, 1943; Racker and Schroeder, 1958; Pogell, 1962; Smillie, 1964; Fossitt and Bernstein, 1963). Where these enzymes have been well characterized, they have been found to exhibit alkaline pH optima, requirements for Mg^{++} or Mn^{++}, high degrees of substrate affinity and stimulation by EDTA. It has been suggested that specific FDPase may control gluconeogenesis and the formation of pentoses from 2- and 3-carbon precursors (McGilvery, 1964). On the other hand, phosphofructokinase (PFK) has been proposed as a rate limiting enzyme in glycolysis (Mansour, 1963; Passonneau and Lowry, 1962). Thus the net effect of the antagonistic activities of these two enzymes may determine whether carbohydrate metabolism proceeds in the direction of glycolysis or gluconeogenesis.

We have now purified FDPase from Candida utilis 1,000 fold and found it to exhibit the general properties attributed to the enzyme in other organisms[1]. The purpose of this communication is to report the control

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of enzyme levels by growth of C. utilis on glucose or glycerol and of enzyme activity by EDTA. It was anticipated that extracts from C. utilis grown on glycerol would exhibit higher FDPase activity than extracts from cells grown on glucose, since in the latter case, high FDPase activity would not be required for 5- and 6-carbon sugar synthesis and might indeed be wasteful of high energy intermediates.

Methods: C. utilis Strain ATCC 9950 was grown either in a minimal medium (Ramachandran and Walker, 1957) or in proteose peptone-beef extract (Difco). Glucose or glycerol (2%) was added and the cells grown with vigorous shaking at 35°. At appropriate intervals the cells were harvested, washed by centrifugation, ground with alumina and extracted with 0.05 M buffer[2], pH 7.5. FDPase was measured spectrophotometrically at room temperature. The reaction mixture contained 0.04 M glycine buffer,pH 9.5, 0.5 mM TPN, 0.2 mM FDP, 1.0 mM $MgCl_2$ and excess glucose-6-phosphate dehydrogenase and phosphohexose isomerase in a final volume of 1.0 ml. PFK (Wu and Racker, 1959), aldolase (Racker, 1947) and xylitol dehydrogenase (Chakravorty et al., 1962) were measured spectrophotometrically at room temperature. Protein was determined by the method of Lowry (Lowry et al., 1951).

Results: Extracts of C. utilis grown on glycerol showed levels of FDPase activity 4-5 fold greater than comparable extracts from cells grown on glucose (Table I). The changes in levels of aldolase and PFK were inversely related to those observed with FDPase, but these changes were smaller, and occurred only in peptone, and not in minimal medium.

FDPase activity in crude cell extracts had properties similar to that of the purified enzyme (Table II). In each case, the enzyme showed optimal activity at an alkaline pH, was dependent upon Mg^{++}, and showed no appreciable activity in the range from pH 7.5 to pH 8.5 unless EDTA

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[2] A variety of buffers including TRIS, triethanolamine, glycine and phosphate were found to give identical results.

TABLE I

Specific Activities of Enzymes in C. utilis

Enzyme	Growth on Glucose		Growth on Glycerol	
	Minimal Medium	Proteose Peptone	Minimal Medium	Proteose Peptone
	mμmoles/min/mg protein			
FDPase	13*	28	197	109
	26	28	132	107
	40	30	90	117
PFK	405	810	338	247
	215	410	227	242
	318	460	222	286
Aldolase	245	656	274	226
	-	600	-	295
	-	470	-	386
Xylitol Dehydrogenase	-	12	-	14

*Each line represents the results obtained with a series of cultures grown under the conditions specified. Cells were harvested during the logarithmic growth phase and extracts prepared as indicated in Methods. Aliquots containing 10-50 μg of protein (2-10 μl) were assayed.

was present. These requirements were not observed for the non-specific phosphatase of crude cell extracts, as measured by the hydrolysis of p-nitrophenylphosphate (PNPP) (Table II). The FDPase activity in extracts from C. utilis grown on glucose exhibited the same properties as the activity from cells grown on glycerol. Neither was inhibited by the presence of high concentrations of inorganic phosphate. When extracts from C. utilis grown on glucose or glycerol were combined, the resultant enzymatic activity was strictly additive, indicating that the low activity in glucose-grown cells was not due to the presence of an inhibitor in the extracts.

Conclusion: The identification of a specific FDPase in C. utilis adds to the growing list of organisms known to possess this enzyme and

TABLE II

Properties of Crude and Purified FDPase from C. utilis

Assay Conditions	Crude Extract	Purified Enzyme
	ΔOD 340/10 minutes	
FDPase, pH 9.5		
complete*	0.600	0.500
-EDTA	0.400	0.360
-FDP	0.0	0.0
-Mg	0.0	0.0
$+P_i$	0.600	0.500
FDPase, pH 8.0[4]		
complete**	0.620	0.510
-EDTA	0.030	0.0
-FDP	0.0	0.0
-Mg	0.0	0.0
$+P_i$	0.610	0.520
PNPPase		
complete***	0.340	-
$+Mg^{++}$	0.420	-
$+Mg^{++}$, + EDTA	0.400	-

Aliquots of either crude cell extract (50 ug) or purified enzyme (0.1 μg) were assayed at room temperature. The Δ OD (340 mμ) for a ten minute period is recorded.

*The complete mixture contained 0.04 M glycine buffer, pH 9.5, 0.5 mM TPN, 0.2 mM FDP, 1.0 mM $MgCl_2$, 0.5 mM EDTA, and excess glucose-6-phosphate dehydrogenase and phosphoglucose isomerase in a final volume of 1.0 ml. Where indicated inorganic phosphate (0.01 M) was present.

**The complete mixture was identical to the pH 9.5 reaction mixture except that glycine was replaced by 0.04 M triethanolamine buffer, pH 8.0.

***The reaction mixture contained 1 μg p-nitrophenylphosphate and 0.5 M triethanolamine buffer, pH 7.5, in a final volume of 1.0 ml. Where indicated 1.0 mM $MgCl_2$ and/or 0.5 mM EDTA were present. PNPPase activity was measured spectrophotometrically at 420 mμ.

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supports the suggestion that it is involved in the control of carbohydrate metabolism. The activity of such an enzyme must, therefore, be

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[4] Maximal stimulation by EDTA occurred between pH 7.5 and pH 8.5. There was some variability from one extract to another in the pH at which the effect was maximal.

critically regulated in vivo. Increased synthesis, activation and/or changes in cellular localization may be involved to various extents in different organisms.

The cellular slime mold, Polysphondylium pallidum, has a specific FDPase[3]. This organism undergoes a morphogenetic sequence which is paralleled by a marked increase in gluconeogenesis and polysaccharide biosynthesis. Extracts of cells obtained at various times during differentiation showed no changes in specific activity of FDPase. The FDPase levels of E. coli (Fraenkel and Horecker, 1965) were also not observed to change during growth on various carbon sources. On the other hand, extracts of Saccharomyces cerevisiae (Grancedo et al., 1965) grown on glucose contained essentially no FDPase activity, while comparable extracts from cells grown on 2- and 3-carbon intermediates showed significant levels. These levels were similar to those observed in glucose-grown C. utilis. The relatively high levels of FDPase in glucose-grown C. utilis may be attributed to the fact that this strain, unlike S. cerevisiae, is normally an aerobic organism and possesses an extraordinarily active pentose phosphate pathway perhaps related to a requirement for high levels of TPNH (Horecker, 1963). It is possible that under ordinary aerobic conditions this organism requires resynthesis of hexose from C_3 products because of the formation of excess pentose phosphate.

The strict requirement of the FDPase activity for EDTA between pH 7.5 and pH 8.5 suggests that the enzyme may be under metabolic control and it will be of considerable interest to determine whether EDTA can be replaced by more physiologic agents.

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[3] Rosen, O. M. Unpublished.

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