REGULATION OF THE ACTIVITY OF PHOSPHOENOLYPYRUVATE CARBOXYLASE BY FRUCTOSE DIPHOSPHATE[1]

B. D. Sanwal and Peter Maeba[2]

Department of Microbiology, University of Manitoba, Winnipeg, Canada

Received December 17, 1965

In attempts to find whether precursor activation - formally the reverse of endproduct inhibition (Hilz et al., 1963; Sanwal et al., 1963; Sanwal and Stachow, 1965) - of allosteric enzymes was not more common than hitherto suspected, we tested the effect of various intermediates of the Glycolytic cycle prior to phosphoenolpyruvate (PEP) on the activity of PEP carboxylase of Salmonella typhimurium and found that the enzyme is markedly activated by fructose 1,6-diphosphate (FDP). The following report summarizes the specificity and some salient kinetic features of this activation.

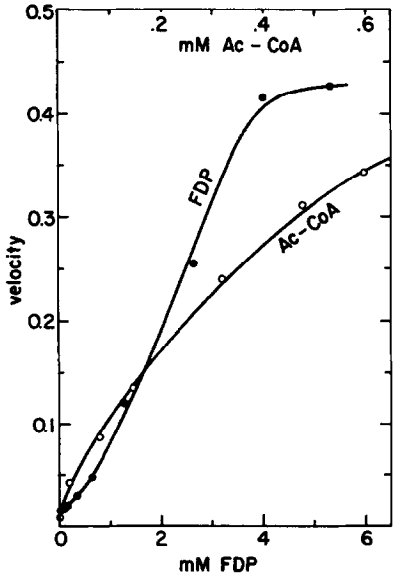
Materials and Methods- Approximately 60-fold purified PEP carboxylase was obtained from glucose grown S. typhimurium cells. The details of the method will be published elsewhere. The preparation was free of the following enzymes tested:

---

[1]Supported by NRC, Canada.
[2]Predoctoral Fellow.

DPNH oxidase, malate dehydrogenase, ATP-linked oxalacetate decarboxylase, pyruvate kinase, aldolase, malic enzyme and citrate synthase. The assay mixture, unless indicated otherwise, consisted of, 0.2 M Tris-HCl, pH 9.0; 0.133 mM DPNH; 3 μg pig heart malate dehydrogenase (Worthington); 10 mM $NaHCO_3$; 16 mM $MgCl_2$; 1.66 mM PEP; enzyme, other additives when required, and water to give a final volume of 3.0 ml. The oxidation of DPNH was measured at 340 mμ in a Gilford model 2000 spectrophotometer. Velocity is defined as the change in O.D./min. under our assay conditions.

Results and Discussion- It can be seen from Figs. 1 and 2



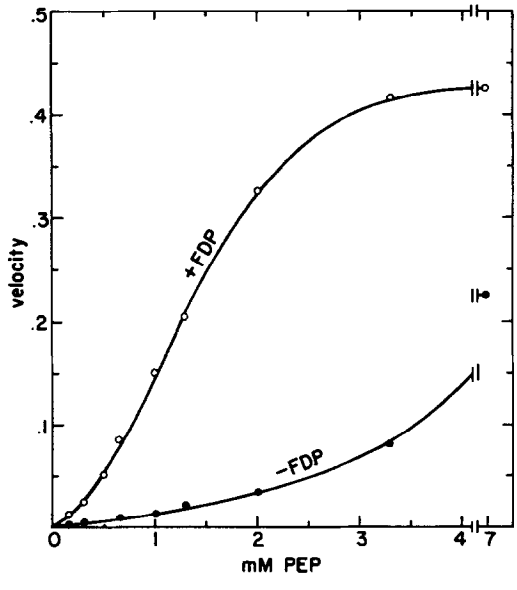


Fig. 1 (left). Activation of PEP carboxylase by FDP and acetylCoA. The fixed concentration of PEP was 1.66 mM. Enzyme concentration was 50 units for acetylCoA curve and 130 units for FDP curve.

Fig. 2 (right). The effect of FDP (7 mM, fixed) on the initial velocity of PEP carboxylase with PEP as the variable substrate.

that FDP activates the enzyme very markedly. In conformity with the results obtained by Canovas and Kornberg (1965), acetylCoA is also a powerful activator of the enzyme (Fig. 1). The stimulatory effect of FDP is quite specific as can be seen from data presented in Table I. Out of numerous glycolytic intermediates tested, glycerate and F 1-P stimulated the activity slightly, but this was nowhere comparable to the activation obtained with FDP. It may be mentioned that malate dehydrogenase, the second enzymic component of the assay mixture was not affected by acetylCoA, FDP or other compounds listed in Table I.

The regulatory nature of PEP carboxylase is reflected

Table I

Effect of various intermediates of the glycolytic cycle on the activity of PEP carboxylase

Additions	Concentration (mM)	Velocity*
None	-	0.036
Glycerol, Glucose, 2-PGA, 3-PGa, DHAP, F-6-P, G-1-P	5	0.036-.040
G-6-P	3.3	0.044
DL-Glycerate	1.66	0.053
F-1-P	5.0	0.090
FDP	1.3	0.288

PGA = Phosphoglycerate, DHAP = dihydroxyacetone phosphate
*Measured at a fixed concentration of 1.66 mM PEP.

in the sigmoid rate-concentration data obtained with PEP as the variable substrate (Fig. 2). Unlike some other allosteric

enzymes (Monod et al., 1965), however, the activator (FDP) at nearly saturating concentrations is unable to convert the initial velocity plot to a hyperbola (Fig. 2). When the data presented in Fig. 1 for acetylCoA and FDP are plotted in the form of $1/v-v_o$ versus 1/conc. of activator (v and v_o being velocities in the presence and absence of activator, respectively), the plot is linear (not shown) for acetylCoA (yielding a $K_{activation}$ value of 0.5 mM), but curved for FDP. This suggests that acetylCoA binds only once in the reaction sequence while binding of more than one molecule of FDP may contribute to the activation process. That the binding sites for FDP and acetylCoA are possibly different from one another is suggested by an experiment where at a fixed level of 1.66 mM PEP in the presence of 9 mM FDP and 1.0 mM acetylCoA (nearly saturating values) separately, the velocities were .28 and .40 respectively, but changed to .53 when both activators were present together. In the absence of detailed kinetic studies these interpretations, however, may only be considered tentative.

It is pertinent to enquire here into the possible physiological importance of enzyme activation by FDP. Since the functioning of PEP carboxylase is absolutely necessary when the organism is growing on glucose (Kornberg, 1965) it is tempting to consider FDP activation to be a mechanism ensuring uninterrupted supply of oxalacetate. However, the extreme specificity of FDP activation coupled with the observation (Utter and Keech, 1963; Kornberg, 1965) that

adequate supply of oxalacetate is already ensured by acetylCoA activation of the enzyme suggest that this simple interpretation is not likely to be correct. Although entirely speculative, it seems more reasonable to consider FDP activation to be related to the energy metabolism of the cell (Atkinson, 1965). From the calculations of Krebs (1964) it is apparent that the ATP/AMP ratio rather than the absolute concentration of ATP or ATP/ADP in a cell is a controlling factor for large number of enzymes involved in carbohydrate metabolism. When the ATP/AMP ratio is high the level of FDP is expected to drop (owing to the inhibition of phosphofructokinase) and that of citrate is expected to increase (see, Atkinson, 1965) with a resulting removal of acetylCoA for fatty acid synthesis (due to an activation by citrate of acetylCoA carboxylase). Under such conditions there is neither a necessity (because of already augmented levels of citrate) nor a capability (owing to decreased levels of acetylCoA and FDP) for increased production of oxalacetate. However, when the ATP/AMP ratio is low FDP concentration will tend to increase and that of citrate will tend to decrease (thus increasing the levels of acetylCoA by slowing down fatty acid synthesis); adequate supplies of oxalacetate would consequently be generated by a concerted activation of PEP carboxylase by FDP and acetylCoA to augment the levels of citrate.

REFERENCES

Atkinson, D. E., Science, 150, 851 (1965).

Canovas, J. L., and Kornberg, H. L., Biochim. Biophys. Acta, 96, 169 (1965).
Hilz, H., Tarnowski, W., and Arend, P., Biochem. Biophys. Res. Commun., 10, 492 (1963).
Kornberg, H. L., Angew. Chem., 4, 558 (1965).
Krebs, H. A., Proc. Roy. Soc. London, Ser. B, 159, 545 (1964).
Monod, J., Wyman, J., and Changeux, J. P., J. Mol. Biol., 12, 88 (1965).
Sanwal, B. D., Zink, M. W., and Stachow, C. S., Biochem. Biophys. Res. Commun., 12, 510 (1963).
Sanwal, B. D., and Stachow, C. S., Biochim. Biophys. Acta, 96, 28 (1965).
Utter, M. F., and Keech, D. B., J. Biol. Chem., 238, 2603 (1963).