FEEDBACK INHIBITION OF PHOSPHOENOLPYRUVATE CARBOXYLASE OF SALMONELLA[1]

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Considerable amount of evidence exists that out of the three enzymes (phosphoenolpyruvate carboxylase, "malic enzyme" and ATP-linked oxalacetate decarboxylase) that can synthesize C_4-acids from C_3-compounds in Enterobacteriaceae, only phosphoenolpyruvate (PEP) carboxylase serves the 'anaplerotic' function (Kornberg, 1965), i.e., it seems to be the only enzyme of any physiological importance in replenishing the supply of oxalacetate drained away for the synthesis of proteins from the tricarboxylic acid cycle. The evidence rests primarily in the demonstration that mutants of Escherichia coli (Amarsingham, 1959; Ashworth and Kornberg, 1963) and S. typhimurium (Theodore and Englesberg, 1964) which lack PEP carboxylase fail to grow on pyruvate or its precursors unless tricarboxylic acid cycle intermediates are also added to the growth medium. Since oxalacetate is removed from the tricarboxylic acid cycle for the synthesis of aspartate via

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aspartate-glutamate transaminase, PEP carboxylase could formally be considered as the first enzyme of the pathway leading from PEP to the end-product, aspartate (or glutamate). In analogy with the regulation of other biosynthetic sequences in microorganisms (Umbarger, 1964), it was, therefore, expected that aspartate (or glutamate) would control the activity of PEP carboxylase by allosteric inhibition. This communication substantiates the veracity of this assumption.

Methods - Salmonella typhimurium, strain LT2 was grown in shake cultures in a glycerol-salts medium and the cells were harvested in the late log phase of growth. Partially purified PEP carboxylase was prepared essentially in the same way as outlined by Canovas and Kornberg (1965).

Two assay procedures were used. Assay 1 consisted of the measurement of incorporation of radioisotope from $NaHC^{14}O_3$ in malate. The assay mixture contained 0.2 M Tris-HCl (pH 8.0), 10.0 mM $NaHC^{14}O_3$ (2 μC), 5 mM $MgCl_2$, 0.1 mM acetyl-coA, 0.83 mM PEP, 0.70 mM DPNH, 3 μg pig heart malic dehydrogenase (Worthington) and suitably diluted PEP carboxylase preparation. The final volume of the assay mixture was 1 ml. The reaction was carried out at 24-25° for 10 minutes and was stopped by the addition of perchloric acid (5% final concentration). After aeration for 1 minute, the protein precipitate was centrifuged down and radioactivity was measured in suitable aliquots with a thin-window Geiger tube. Assay 2 utilized the measurement of decrease in absorbance at 340 mμ in assay mixtures. The concentration of various components was the

same as in Assay 1, except that cold $NaHCO_3$ was used and the final volume of the reaction mixture was 3 ml. The oxidation of DPNH was measured in Silica cuvettes of 1 cm light path in a Gilford-2000 recording spectrophotometer. In both kinds of assays linearity with regard to time and enzyme concentration was established.

Results and Discussion - As already demonstrated by Canovas and Kornberg (1965) in Escherichia coli, PEP carboxylase from Salmonella is powerfully activated by acetyl-coA. Thus, at a fixed concentration of 2.5 mM PEP (Fig. 1a) the absorbance change was 0.007, but increased to 0.378 in the presence of 0.35 mM acetyl-coA. The initial velocity data with PEP as the variable substrate at a fixed concentration of 0.1 mM acetyl-coA gave a marked S-shaped curve (Fig. 1a). This kind of curve is characteristic of many allosteric enzymes (Monod et al., 1965), but no conclusions can be drawn as to the reasons for this departure from a normal hyperbolic curve, until a more purified preparation is available. It is possible, however, that PEP binds at two or more sites on the enzyme surface, although a random addition of substrates is also likely to lead to departures from normality (Sanwal et al., 1965). The initial velocity data obtained with radioactive assay also yields a plot similar to that shown in Fig. 1a.

L-aspartate serves as a powerful inhibitor of the reaction (Fig. 1b). Thus at a concentration of 2.5 mM PEP and 0.1 mM acetyl-coA 50% inhibition is obtained at a concentration of 8.2×10^{-5} M aspartate. The inhibition by

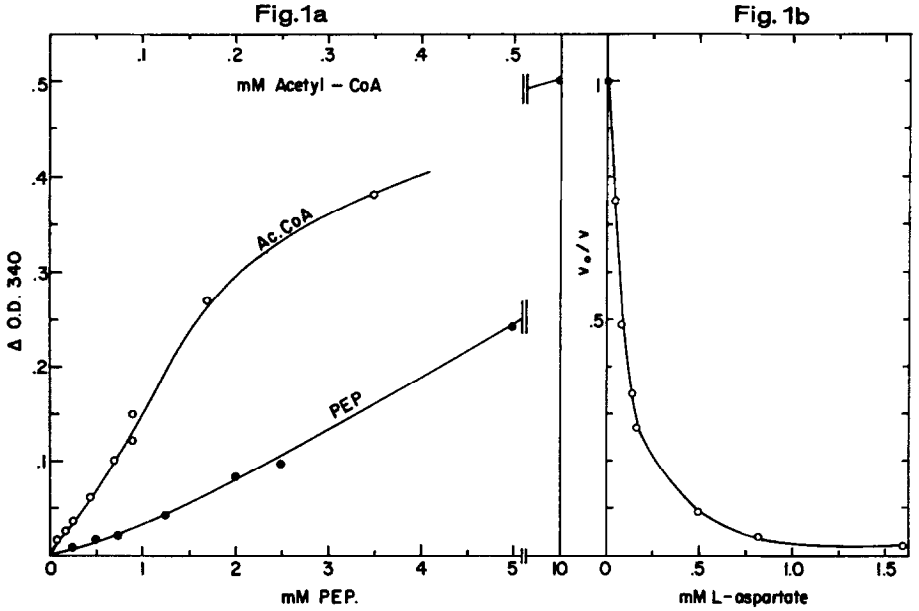

Fig. 1a (left). Saturation curves of PEP carboxylase with acetyl-coA and PEP. Fixed concentration of PEP for the upper curve was 2.5 mM and for the lower curve acetyl-coA concentration was 0.1 mM.

Fig. 1b (right). The inhibition of PEP carboxylase by L-aspartate. Acetyl-coA and PEP concentrations were 0.1 mM and 2.5 mM respectively. Assay 2 was used.

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aspartate seems to be quite specific. As can be seen from Table I, various other intermediates of the tricarboxylic acid cycle and amino acids derived from aspartate do not affect enzyme activity significantly. D-aspartate and DL-β-methylaspartate each at a concentration of 1.66 mM inhibited the enzyme activity 50% and 33% respectively. It may be mentioned that aspartate (5 mM) does not inhibit the activity of malate dehydrogenase which is the other enzymic component of our assay system.

It is of some interest to note that while the enzyme functionally equivalent to PEP carboxylase in mammalian cells, pyruvic carboxylase (Utter et al., 1964), is also

TABLE I

Effect of various compounds on the activity of PEP carboxylase

Components of reaction mixture	$C^{14}O_2$ incorporated (counts/min)
Complete*	10130
Complete minus PEP	170
Complete + citrate	9040
Complete + L-alanine	8790
Complete + L-glutamate	8890
Complete + succinate	8300
Complete + L-threonine	8300
Complete + DL-homoserine	10010
Complete + L-aspartate	560

*The assay mixture is described in the text. PEP was used at a concentration of 1.25 mM. The final concentration of all compounds tested was 5 mM.

activated by acetyl-coA, the inhibition characteristics of the two enzymes are completely different. As shown above, the PEP carboxylase is specifically inhibited by aspartate, but the mammalian pyruvic carboxylase is non-specifically inhibited by citrate, fumarate, succinate, malate and α-ketoglutarate (Freedman and Kohn, 1964).

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