# INHIBITION OF YEAST PYRUVATE CARBOXYLASE BY L-ASPARTATE AND OXALOACETATE

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Received July 18, 1966

Pyruvate carboxylase catalyzes the direct carboxylation of pyruvate to oxaloacetate in the presence of ATP and magnesium ions. Several pyruvate carboxylases from different biological sources have been described, but their activities are not affected in the same way by acetyl-CoA. The avian liver enzyme requires absolutely acetyl--CoA (Utter and Keech, 1963); the enzymes from photosynthetic bacteria (Fuller et al., 1961), rat liver (Henning and Seubert, 1964), and baker's yeast (Ruíz-Amil et al., 1965; Cooper and Benedict, 1966) are activated by acetyl-CoA; the enzymes from Aspergillus niger (Bloom and Johnson, 1962) and Pseudomonas citronellolis (Seubert and Remberger, 1961) are not affected at all by acetyl-CoA.

Phosphoenolpyruvate carboxylase from Salmonella typhimurium (Theodore and Englesberg, 1964) and Escherichia coli (Amarasingham, 1959; Ashworth et al., 1965) have a function equivalent to that of yeast pyruvate carboxylase in the synthesis of oxaloacetate and are also activated by acetyl-CoA (Cánovas and Kornberg, 1965; Maeba and Sanwal, 1965). The inhibition of phosphoenolpyruvate carboxylase from Salmonella by L-aspartate has been recently described (Maeba and Sanwal, 1965).

This communication shows that yeast pyruvate carboxylase is inhibited by L-aspartate, a fact that increases the similarities between the two carboxylating enzymes. Oxaloacetate, the reaction

product of pyruvate carboxylation, has also been found to inhibit yeast pyruvate carboxylase activity.

METHODS - The baker's yeast pyruvate carboxylase preparation used in this work was the ammonium sulfate fraction previously described (Ruíz-Amil et al., 1965). Protein was determined by the method of Warburg and Christian (1941). Enzyme assays were carried out by either the spectrophotometric or the radioactive method (Ruíz-Amil et al., 1965). To study the effect of oxaloacetate on the $CO_2$-fixing reaction, a modification of the radioactive method was employed, since, under the usual conditions, the oxaloacetate added would be immediately trapped by the auxiliary NADH-malate dehydrogenase system. In order to avoid this difficulty, the pyruvate carboxylase reaction was allowed to proceed in the absence of NADH, oxalate being then added, together with the coenzyme, to inhibit the carboxylation of pyruvate without affecting the malate dehydrogenase reaction (Losada et al., 1964; Ruíz-Amil et al., 1965). The ammonium sulfate fraction contained enough malate dehydrogenase to trap in 10 min, under the conditions of the assay, the maximal possible amount of oxaloacetate present.

RESULTS AND DISCUSSION - As shown in Fig. 1, both L-aspartate and oxaloacetate inhibited pyruvate carboxylase activity. D-aspartate, L-glutamate, citrate, L-malate, fumarate and succinate at a concentration of 2.5 mM did not have practically any effect on the activity of the enzyme. The inhibition by L-aspartate was noncompetitive with respect to pyruvate. Appropriate controls excluded the possibility that L-aspartate would inhibit the coupled malate dehydrogenase reaction involved in the assay. It is apparent from the data presented in Table I that the inhibitory effect of L-aspartate was also independent of the concentration of ATP, $Mg^{++}$ and bicarbonate, and thus corresponded to the noncompetitive type. On the other hand, oxaloacetate is a competitive inhibitor with respect to pyruvate (Fig. 1, right). From the Lineweaver and Burk plots

(Fig. 1), Ki values of 1.9 mM and 0.22 mM were deduced for L-aspartate and for oxaloacetate, respectively. Fig. 2 shows the

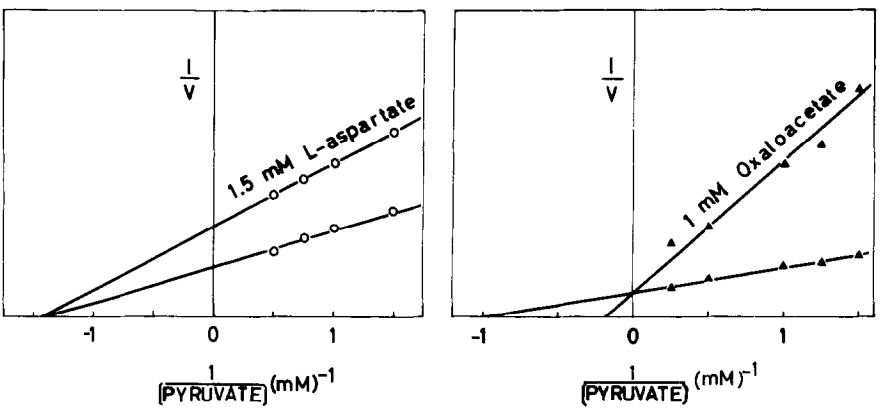


Figure 1, left. Noncompetitive inhibition of pyruvate carboxylase by L-aspartate. The assays were carried out spectrophotometrically at room temperature by measuring optical density changes at 340 mμ. Each cuvette contained in a final volume of 3 ml: 300 μmoles of Tris--HCl (pH 7.6); 30 μmoles of $MgCl_2$; 15 μmoles of ATP; 45 μmoles of $KHCO_3$; 0.4 μmoles of NADH; 0.36 mg of protein (ammonium sulfate fraction); sodium pyruvate and L-aspartate as indicated.

Figure 1, right. Competitive inhibition of pyruvate carboxylase by oxaloacetate. Enzyme assays were carried out by measuring $^{14}CO_2$ fixation. A total volume of 2.0 ml contained: 200 μmoles of Tris--HCl (pH 7.6); 20 μmoles of $MgCl_2$; 10 μmoles of ATP; 38 μmoles of $KH^{14}CO_3$ (50 μC); 0.24 mg of protein (ammonium sulfate fraction); sodium pyruvate and oxaloacetate as indicated. After the pyruvate carboxylase reaction proceeded for 15 min at 30°, aliquots of 0.5 ml were pipetted into tubes containing, in 0.2 ml, 1 μmole of NADH and 0.7 μmoles of oxalate (Losada et al., 1964; Ruíz-Amil et al., 1965). The malate dehydrogenase reaction was then allowed to proceed for 10 min at 30°, at which time 0.1 ml of 50% trichloroacetic acid was added. After centrifugation, the radioactivity was measured in the supernatant.

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ratios of pyruvate carboxylase activities with and without L-aspartate at different concentrations of this aminoacid.

Maeba and Sanwal (1965) have pointed out that the inhibition of phosphoenolpyruvate carboxylase from Salmonella by L--aspartate is a property that distinguishes this enzyme from the mammalian pyruvate carboxylase. Our results indicate that yeast

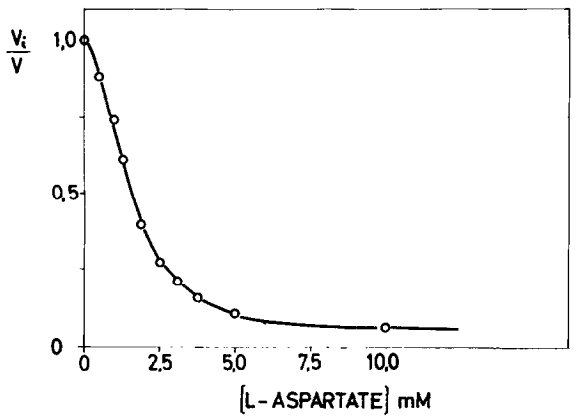


Figure 2. Inhibition of pyruvate carboxylase activity at different concentrations of L-aspartate. Enzyme assays were carried out by measuring $^{14}CO_2$ fixation. The reaction mixture contained in a final volume of 2.0 ml: 200 µmoles of Tris-HCl (pH 7.6); 20 µmoles of $MgCl_2$; 10 µmoles of ATP; 20 µmoles of sodium pyruvate; 30 µmoles of $KH^{14}CO_3$ (20 µC); 0.6 µmoles of NADH; 0.24 mg of protein (ammonium sulfate fraction); L-aspartate as indicated. After incubation for 30 min at 30°, 2.0 ml of 50% trichloroacetic acid was added and the radioactivity was measured in the supernatant after centrifugation.

pyruvate carboxylase, like phosphoenolpyruvate carboxylase from Salmonella, is inhibited by L-aspartate, although its Ki is greater.

Since in yeast the oxaloacetate formed by the carboxylation of pyruvate can be removed for the synthesis of aspartate, pyruvate carboxylase may be regarded as the first enzyme of the pathway leading from pyruvate to aspartate. Therefore, the inhibition of pyruvate carboxylase activity by aspartate may be considered as an allosteric inhibition by end-product. The oxaloacetate inhibition of pyruvate carboxylase activity is of the competitive type. The inhibitory effect by the reaction product may be of interest in the regulation of the oxaloacetate level within the cell.

Table I - INHIBITION BY L-ASPARTATE OF PYRUVATE CARBOXYLASE ACTIVITY AT DIFFERENT CONCENTRATIONS OF THE REACTION COMPONENTS

| Experiment | System | $^{14}CO_2$ fixed | | Inhibition |
|---|---|---|---|---|
| | | No L-aspartate | L-aspartate (3 mM) | |
| | | Counts x $10^{-3}$/ min | | % |
| 1 | Complete | 11.3 | 4.9 | 57 |
| | Complete but 0.25 mM ATP | 5.6 | 2.4 | 57 |
| | Complete but 3.5 mM $Mg^{++}$ | 14.3 | 7.6 | 47 |
| 2 | Complete | 10.2 | 4.0 | 61 |
| | Complete but 15 mM $KHCO_3$ | 15.6 | 6.1 | 61 |
| | Complete but 3 mM $KHCO_3$ | 31.2 | 9.7 | 69 |

Enzyme assays were carried out by measuring $^{14}CO_2$-fixation. The complete system contained in a final volume of 2.0 ml: 200 µmoles of Tris-HCl (pH 7.6); 20 µmoles of $MgCl_2$; 5 µmoles of ATP; 20 µmoles of sodium pyruvate; 66 µmoles of $KH^{14}CO_3$ (55 µC in experiment 1, and 5.5 µC in experiment 2); 1 µmole of NADH; 6 µmoles of L-aspartate; 0.36 mg of protein (ammonium sulfate fraction). After incubation for 10 min at 30°, 0.2 ml of 50 % trichloroacetic acid was added and the mixture was centrifugated. The radioactivity was measured in the supernatant with a thin window Geiger counter in experiment 1 and with a gas flow Geiger counter in experiment 2.

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