REGULATION OF PYRUVATE CARBOXYLASE OF PENICILLIUM CAMEMBERTI BY THE ADENYLATE SYSTEM

H.-J. Stan and J. Schormüller

Institut für Lebensmittelchemie
Technische Universität 1 Berlin 12
Deutschland

Received June 18, 1968

Since the discovery of the negative feedback by Umbarger (1956) and Yates and Pardee (1956), metabolic control of the key enzymes of the biosynthetic and degradative sequences has been recognized as an important factor in the regulation of metabolism. Generally the first step of a biosynthetic sequence is inhibited by the endproduct in a specific manner. Degradative sequences produce energy in the form of adenosine-5'-triphosphate (ATP) which is the endproduct of catabolic pathways. Key enzymes of degradative sequences are under the negative feedback control of ATP. A well-known example of this type of enzyme is phosphofructokinase (PFK) which is thought to be the pacemaker in the glycolysis. PFK of different sources is inhibited by ATP and activated by AMP. On the other hand conservation of the level of ATP is a major feature of metabolic regulation. Therefore, a control by the energy level of the cell must be superposed on the specific controls that regulate the rates of synthesis of individual products. Atkinson et al. (1967) proposed as a working hypothesis that all ATP-linked enzymes are controlled by a universal parameter, the energy charge of the adenylate system defined as (ATP + 1/2 ADP)/(AMP + ADP + ATP). The equilibrium between AMP, ADP, and ATP is catalyzed by adenylate kinase which is assumed to be in sufficient concentration in all living cells

for this equilibration. In the case of the anabolic citrate cleavage enzyme (EC 4.1.3.8) from rat liver and phosphoribosylpyrophosphate synthase (EC 2.7.6.1) from E. coli, it was shown that the enzymic activity was low with a low energy charge and high with a high energy charge. When the enzymic activity was plotted against a linear energy charge scale steep curves were obtained. An opposite behavior can be expected from phosphofructokinase. Nothing is known about anaplerotic enzymes in this connection. Pyruvate carboxylase (PC) is an anaplerotic enzyme which plays an important role in gluconeogenesis in mammalian liver and kidney (Keech and Utter, 1963) and replacement of intermediates of the Krebs cycle in microorganisms grown on glucose as the sole carbon source. PC (EC 6.4.1.1) was found in Penicillium camemberti, var. candidum to be an allosteric enzyme (Stan and Schormüller). The results reported in this paper demonstrate that PC is under control of the energy charge of the adenylate system and under specific feedback control of L-aspartate.

Methods: Pen. camemberti was grown in shake cultures in a glucose-salts medium and the mycelia were harvested in the late log phase of growth. Mycelia were suspended in 0.1 M triethanolamine buffer pH 7.8 containing 2 mM cysteine and 0.1 mM EDTA and disrupted by grinding with sea sand in a mortar. After centrifugation for 30 minutes at 20000 x g, the supernatant solution was fractionated with ammonium sulfate. The protein with the anticipated enzyme activity was precipitated between 40 and 50% of saturation with ammonium sulfate. This protein fraction, dissolved in 0.1 M Tri pH 8.0 was used for the experiments reported. Enzymic activity was measured by the incorporation of radioisotope from $Na_2{}^{14}CO_3$ in malate. The assay mixture initially contained in a total volume of 1.0ml: 100mM Tri-NaCl pH 8.0; 5mM $MgSO_4$; 10mM sodium pyruvate; 0.7 mM NADH; 5 µg malate dehydrogenase (pig heart, Boehringer); 3 µg adenylate kinase (rabbit muscle, Boehringer); 1 mM $Na_2{}^{14}CO_3$ (10 μC_i);

ATP was indicated on the abscissa; AMP, to bring the total adenylate concentration to 1, 2 or 3 mM. The added enzyme fraction contained 0.1 mg of protein. The reaction was carried out at 30°C for 15 minutes and was stopped by the addition of trichloracetic acid (5% final concentration). After aeration for 5 minutes, the protein precipitate was centrifuged down and radioactivity was measured in 0.2 ml of the supernatant solution with a liquid scintillation counter (Tri-Carb).

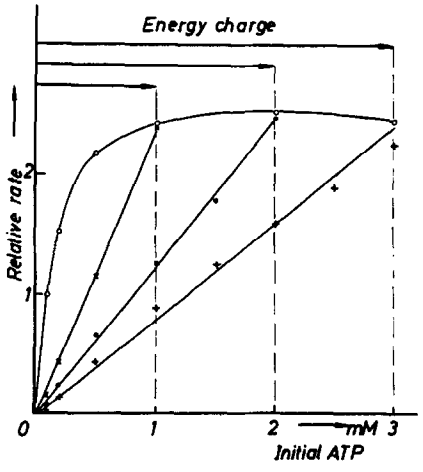

Fig. 1. Rate of the reaction catalyzed by pyruvate carboxylase as a function of the "energy charge" of the adenylate system. The assay mixture is described in the text. Open circles indicates the rate of the reaction as a function of the ATP concentration without adenylate kinase and AMP.

Results: Figure 1 shows the enzymic activity as a function of the concentration of ATP (circles) and of the energy charge of the adenylate system. In the absence of AMP the enzyme shows normal Michaelis-Menten kinetics. In the presence of AMP and adenylate kinase the total concentration was chosen 1, 2, and 3 mM as indicated. With each concentration a linear increase of enzyme activity was found when plotted against a linear energy charge scale. The rate of the enzymic reaction shows no linear function of the ATP concentration when plotted

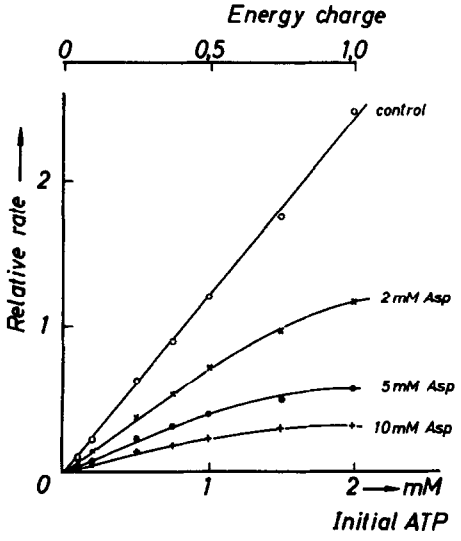

Fig.2. Rate of the reaction catalyzed by pyruvate carboxylase as a function of the "energy charge" of the adenylate system. Effect of L-aspartate. The assay mixture is described in the text. Aspartate is added as indicated.

against the actual concentration of ATP in the assay mixture, calculated from the equilibrium constant of the adenylate kinase reaction. AMP does not affect the enzymic activity, but ADP is an inhibitor of the reaction (Stan and Schormüller). Therefore, the unusual kinetic behavior may be the result of two effects: Decrease of ATP concentration and inhibition by ADP. Figure 2 demonstrates the inhibition of the enzymic activity as a function of the energy charge of the adenylate system by various concentrations of L-aspartate. It can be seen that the inhibition by aspartate is independent of the energy charge.

Discussion: PC occupies a position from which different pathways of metabolism branch off. As an anaplerotic enzyme it produces oxalacetate in order to replace the acids of the Krebs cycle which have been removed for biosyntheses. For this replacement process oxalacetate or another C_4-acid (malate or aspartate) must be transported from the cytoplasma to the mitochondrion by an active transport through the

membrane of the mitochondrion (Haslam and Krebs, 1968). In this way the PC reaction leads subsequently to the production of ATP by way of the Krebs cycle and the respiratory chain, which are both controlled by the adenylate system. On the other hand oxalacetate can be directly transformed into aspartate which is the starting compound for a number of biosyntheses. The PC reaction leads subsequently from the standpoint of energy conservation to two opposite results, the production and the consumption of ATP. As a result of this double function PC shows an unusual kinetic behavior. PC must have a satisfactory activity in the presence of high ATP concentration in order to produce oxalacetate for the biosyntheses which are favored by a high ATP level. On the other hand the enzyme must still produce enough oxalacetate at a low ATP concentration, which can be caused by a decrease of the concentrations of Krebs cycle intermediates to conserve the ATP level. Because the Krebs cycle is not directly involved in the production of aspartate, the activity of PC can be regulated independently of the energy level by a simple feedback inhibition of aspartate. An inhibition of the oxalacetate synthesis does not cause a decrease of the energy production by the Krebs cycle because aspartate itself can enter the mitochondria and there be a source for the oxalacetate production. PC in yeast (Palacián et al., 1966) and phosphoenolpyruvatcarboxylase in Salmonella typhimurium (Maeba and Sanwal, 1965) which has the same function as PC, are also inhibited by aspartate. A similar inhibition of PC isolated from animal mitochondria has not been reported. This fact emphasizes the differences between the functions of PC in the metabolism of microorganism and of animal.

REFERENCES

Atkinson, D.E., and Walton, G.M., J. Biol. Chem., 242, 3239 (1967)
Atkinson, D.E., and Fall, L., J. Biol. Chem., 242, 3241 (1967)

Haslam, J.M., and Krebs, H.A., Biochem. J., 107, 659 (1968)
Keech, D.B., and Utter, M.F., J. Biol. Chem., 238, 2609 (1963)
Maeba, P., and Sanwal, B.D., Biochem. Biophys. Res. Commun., 21, 503 (1965)
Palaciàn, E., de Torrontegui, G., and Losada, M., Biochem. Biophys. Res. Commun., 24, 644 (1966)
Stan, H.-J., and Schormüller, J., in preparation
Umbarger, H.E., Science, 123, 848 (1956)
Yates, R.A., and Pardee, A.B., J. Biol. Chem., 221, 757 (1956)