INORGANIC PYROPHOSPHATE AND CELLULAR CONTROL MECHANISMS: INHIBITION OF ASPARTATE TRANSCARBAMYLASE

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Aspartate transcarbamylase (E.C. 2.1.3.2) catalyzes the synthesis of N-carbamyl-L-aspartate from L-aspartate and carbamyl phosphate. This reaction constitutes the first step in the pyrimidine biosynthesis. The enzyme has recently been crystallized by Shepherdson and Pardee (1960). Gerhart and Pardee (1962) observed that under certain conditions the enzyme was inhibited by CTP and activated by ATP. On the basis of these feedback properties of aspartate transcarbamylase, they postulated that this enzyme plays an important role in biological control mechanisms.

The present report deals with the effect of inorganic pyrophosphate, PP_i, on this enzyme. In a comprehensive study on the effect of anions on aspartate transcarbamylase, it was noted that PP_i was a particulary good inhibitor. The details of this study will be published *in extenso* elsewhere.

Materials and Methods

The enzyme, obtained from a mutant of *E. coli*, was puri-

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fied according to a published procedure (Gerhart and Pardee, 1962, Shepherdson and Pardee, 1960). It gave a single protein peak when examined on Sephadex-G-100 and Sephadex-G-200, and the specific activity of the enzyme was approximately the same as that reported by Gerhart and Pardee (1962). All solutions of substrates and inhibitors were adjusted to pH 7.0, either by addition of NaOH or acetic acid. Recrystallized samples of PP_i and carbamyl phosphate were always used. The activity was followed by measuring carbamyl aspartate (Gerhart and Pardee, 1962). Control experiments were carried out measuring inorganic phosphate by the method of Lowry and Lopez (1946). These gave the same result as with the first method. The reaction period was usually 15 minutes at 25°C. The rates given, are essentially the same as the initial rates.

Results and Discussion

Fig. 1 shows the effect of aspartate concentration

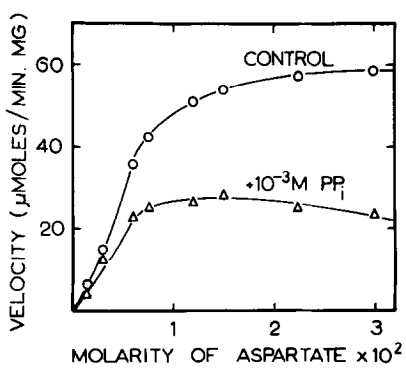

Fig. 1. Effect of aspartate concentration on the inhibition by PP_i. The reaction mixture contained: 8×10^{-4} M carbamyl phosphate, 10^{-3} M PP_i, when used, and 0.12 μg protein in a total volume of 0.5 ml. Aspartate concentration varied as shown. The buffer was: 0.05 M imidazole, 0.03 M acetate and 0.001 M EDTA, pH 7.0. Temperature 25°C.

on the activity, measured in the absence and presence of PP_i, and at a constant concentration of carbamyl phosphate. It is evident that increasing concentrations of aspartate will not abolish the inhibition caused by PP_i. On the contrary, with increasing aspartate concentrations the inhibition seems to increase. Fig. 2 shows the effect of carbamyl phosphate concentration on the inhibition. At high concentrations of carbamyl phosphate there is very little inhibition by PP_i. Thus, the inhibition caused by PP_i is competitive only with carbamyl phosphate, and not with aspartate. PP_i, therefore, competes with carbamyl phosphate for the same site on the enzyme.

The rates in Fig. 1 do not follow Michaelis-Menten kinetics, i.e. plots of 1/v vs 1/s will not give straight lines. This is in agreement with earlier observations for this enzyme with aspartate (Gerhart and Pardee, 1964). However, in the case of carbamyl phosphate in Fig. 2, plots 1/v vs 1/s give straight lines which cross each

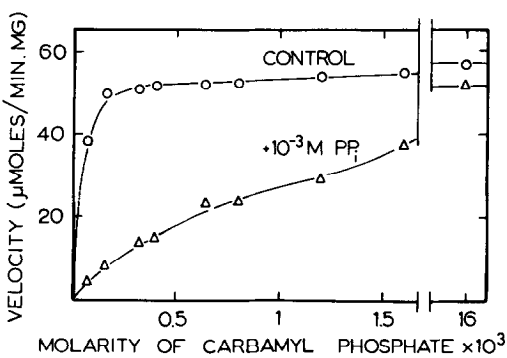

Fig. 2. Effect of carbamyl phosphate concentration on the inhibition by PP_i. The reaction mixture consisted of: 1.5×10^{-2} M aspartate, 10^{-3} M PP_i, when added, and 0.12 μg protein in a total volume of 0.5 ml. Carbamyl phosphate varied as indicated. The buffer was: 0.05 M imidazole, 0.03 M acetate and 0.001 M EDTA, pH 7.0. Temperature 25°C.

other on the 1/v axis, indicating full competitive inhibition. The K_i for PP_i was found to be 6×10^{-5} M at 25°C.

According to Gerhart and Pardee (1964) the native enzyme consists of four subunits which can be dissociated by heating the enzyme at 60°C for 4 minutes. The effect of PP_i on the heated enzyme has also been investigated. The inhibitory action of PP_i is not lost by heating the enzyme. The K_i for PP_i is approximately the same for the heated as for the native enzyme. The inhibition caused by PP_i is, therefore, not an allosteric effect, analogous to the ATP and CTP effect, described by Gerhart and Pardee (1962), but a true competitive relationship with carbamyl phosphate. Since PP_i constitutes an integral part of both di- and trinucleotides, it might be thought that these molecules also could bind to the carbamyl phosphate site. Preliminary studies have shown, however, that this probably does not occur to any large extent. Concerning this aspect, it should also be noted that the CTP inhibition, observed by Gerhart and Pardee (1962), was reversed by aspartate and not by carbamyl phosphate (Gerhart, 1962).

The findings reported in the present communication show that PP_i is a very effective inhibitor of aspartate transcarbamylase. This observation would suggest that PP_i may play an important role in cellular control mechanisms as an inhibitor of certain enzymes. PP_i is universally distributed in biological materials; it is produced in every major metabolic process required for growth in a cell, such as biosynthesis of nucleic acids, proteins, lipids, cell walls, etc.. In periods of rapid growth the intracellular concentration of PP_i would be expected to

rise above the normal level. This increase could, according to the present work, result in a decrease in rate of synthesis of pyrimidines, and thereby also biosynthesis of nucleic acids and growth. The inhibitory effect of PP_i could be overcome either by increasing the concentration of carbamyl phosphate, or decreasing the PP_i concentration. Therefore, of great significance in this respect is the amount of inorganic pyrophosphatase present in the cell. It would seem that this catabolic enzyme must have an important role in growth regulating processes. Recent studies in this laboratory have shown that large variations occur in the level of inorganic pyrophosphatase in mammalian cells in tissue culture (Eker).

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