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Ornithine carbamoyl transferase, a wide spread enzyme in nature, catalyses the carbamylation of ornithine to citrulline and serves as a key enzyme in the recycling of ornithine for urea production.

The enzyme has been found to be decreased in hepatomas (Tung and Cohen, 1950; Greenstein, 1954) and carcinoma (Sarapkina, 1966). However, Kondo, Kurino, Harabayashi and Kobayashi (1961) have reported an increase in ornithine carbamoyl transferase activity in malignant tumours. In the present study also the enzyme was found to be low in tumour than in the corresponding normal tissue.

The enzyme has been reported to be unstable to freezing (Cohen and Grisolia, 1950; Rogers and Novelli, 1959). Pea seedling preparation (Kleczkowski and Cohen, 1964) is however, stable in ammonium sulfate for two months. Similarly the enzymes from *Mycoplasma luminis* (Schimke, Berlin, Sweeney, Carrol, 1966) and *S. faecalis* and bovine liver (Marshall and Cohen, 1972a) have been found to be quite stable. In contrast to these the Rumex enzyme was found to be very unstable and could not be stabilized by any of the treatments. Addition of ornithine also had no influence on its stability.

The enzyme seems to be highly specific, normally it catalyses citrulline formation with L-ornithine and carbamyl phosphate. Reichard (1957) showed that only L-isomer of DL-ornithine is utilized by rat liver enzyme. Similar observations have been made by Rogers and Novelli (1962) and Reichard (1959) in the case of E. coli. D-ornithine was however, found to show activity only when very high concentration of enzyme was used (Burnett and Cohen, 1957). L-ornithine was about 1000 times more active than D-ornithine. Pea seedling (Kleczkowski and Cohen, 1964) enzyme was found to be 8% active with D-ornithine at optimum pH while at higher pH activity rose to 17% of that with L-isomer. Marshall and Cohen (1972b) showed that the rate with D-ornithine was only 3% of that with L-ornithine. They have also shown that at more alkaline pH lysine is also carbamylated to form homocitrulline with bovine enzyme. As regards the carbamyl donor it has been reported that carbamyl phosphate can be utilized in case of beef liver (Pietra, Rogliani, Procaccini and Rogliani, 1957) rat liver (Della, 1957) and wheat seedling (Kleczkowski and Jolanta, 1968) enzymes. In the present case the enzyme was found to utilize only L-ornithine and the presence of D-ornithine (from DL-mixture) did not have any effect on the reaction velocity. Other carbamyl compounds like carbamyl-glutamate,-aspartate or -arginine could not replace carbamyl phosphate.

The enzyme from S. faecalis (Knivett, 1954c) Pleuropneumonia organisms (Smith, 1957), rat liver (Reichard, 1957), serum (Reichard and Reichard, 1958) and Pseudomonas (Stalon, Wiame, Ramos and Pierard, 1967) have been found to catalyse a reversal of the reaction, though the equilibrium has been found to be more towards citrulline synthesis (Knivett, 1954c; Reichard, 1959). In contrast Laishley and Bernlohr (1968) have reported that in B. licheniformis the back reaction is not catalysed by any of the two ornithine carbamoyltransferases present in the tissue. The Rumex enzyme also did not show any reversal.

Ornithine carbamoyltransferase from several sources has been found to have pH optimum between 8.0-9.0 (Burnett and Cohen, 1957; Ravel, Grona, Humphreys and Shive, 1959; Caravaca and Grisolia, 1960; Rogers and Novelli, 1962; Kleczkowski and Cohen, 1964; Bernlohr, 1966; Bastarrachea and Ortega, 1967; Grillo and Bedino, 1968; Marshall and Cohen, 1972a). In Pleuropneumonia like organisms the pH optimum was found to be 5.5 (Smith, 1957) whereas in ox liver it was 6.5-7.5 (Joseph, Baldwin and Watts, 1963). Tris has been reported to be inhibitory at higher concentrations (Joseph, Baldwin and Watts, 1963; Ravel, Grona, Humphreys and Shive, 1959). They have suggested that the inhibitory power of tris can be explained if it is assumeed that the neutral species of tris is inhibitory. The pH

optima for the Rumex enzyme was found to be 9.0-9.5 using Tris-HCl buffer and Tris was found to have some inhibitory effect at higher concentrations. However, the activity was found to be higher in case of Tris buffer compared to bicarbonate buffer at the same pH. The study of the first order rate constants at different pH's gave pK values of 7.7 and 8.5 with ornithine and carbamyl phosphate respectively as the variable substrates. The energy of activation was found to be 13.2 KCal. which compares well with that reported by other workers viz. 13.1 KCal. (Burnett and Cohen, 1957; Joseph, Baldwin and Watts, 1963), 13.3 KCal. and 20.5 KCal. (Ravel, Grona, Humphreys and Shive, 1959).

On the basis of the isotope exchange data Reichard (1957) and Burnett and Cohen (1957) suggested that the reaction of ornithine carbamoyltransferase proceeds by a single displacement reaction rather than via the formation of an enzyme-carbamyl intermediate. A reaction of this sort is most simply explained by having the substrate bound simultaneously at separate sites on the enzyme. The results of Joseph, Baldwin and Watts (1963) also are in general agreement with the above findings. Kinetic data of the beef liver enzyme showed the independence between the dissociation constant of one substrate for both substrates and the finding eliminated the possibility that this reaction proceeds by a compulsory pathway in which the substrates are bound in a

particular order before formation of an intermediary complex and conversion into products occur. Steady state kinetics probably do not apply since Ka = Ká and Kb = Kb́. Thus their kinetic data suggested that ornithine and carbamyl phosphate have separate binding sites on the enzyme. In addition the dissociation constant Kp for the product, phosphate, was also found to be unaffected significantly by ornithine concentration. They also suggested that the binding of carbamyl phosphate to the enzyme can be attributed almost to the phosphate group. Identical binding constants for phosphate and carbamyl phosphate also appear to occur in the enzyme from L. lactis (Ravel, Grona, Humphreys and Shive, 1959).

However, the enzyme from S. faecalis (Kurtin, Bishop and Himoe, 1971) has been reported to follow steady state kinetics and gave a ping pong pattern where phosphate acted as a competitive inhibitor of carbamyl phosphate and citrulline did not inhibit the reaction. They have found that the enzyme from rat liver also displays a ping pong kinetic pattern. Indirect evidence for a ping pong kinetic pattern of Neurospora enzyme can also be interpreted from the observations in the literature. Davis (1961) reported that as compared to wild type enzyme, a mutant enzyme had a higher Km value for ornithine but a lower Km value for carbamyl phosphate. Since a simple interpretations of the observation would be that the enzyme follows a ping pong kinetic pattern

and the mutant enzyme is defective in its ability to bind ornithine.

Recently Marshall and Cohen (1972a,b,c) have again purified the enzyme from S. faecalis and bovine liver and studied the kinetics. From the kinetic data of the binding studies they have suggested an ordered sequential mechanism.

Studies reported in the present case for the Rumex enzyme did not entirely conform to a sequential or ping pong mechanism. The double reciprocal plots with respect to ornithine as variable substrate showed an intersecting pattern whereas the one with carbamyl phosphate showed parallel pattern. The phosphate was found to inhibit the reaction competitively with respect to carbamyl phosphate and non-competitively with respect to ornithine.

The most important finding was the sigmoidal substrate saturation response at low concentrations of both the substrates. A higher concentration of ornithine was, however, inhibitory. Hill coefficient value was 3.1 and 1.5 for ornithine and carbamyl phosphate respectively. The values of 1.08 and 1.7 respectively has, however, been reported for bovine liver enzyme (Grillo and Bedino, 1968).

Studies reported by Rogers and Novelli (1962) in E. coli gave no evidence for the requirement of any metal ion for this enzyme. In this respect the Rumex enzyme resembles the E. coli

enzyme. It was, however, inhibited by several metal ions. The enzyme also seems to involve -SH groups as demonstrated by the inhibition by pCMB. The enzyme from S. faecalis and bovine liver (Marshall and Cohen, 1972c) has also been found to involve -SH groups.

While studying the citrulline synthesis in rat liver mitochondria Charles, Tager and Slater (1967) demonstrated a stimulatory effect by glutamate or several TCA cycle intermediates possibly by furnishing ATP required for carbamyl phosphate formation. The Rumex ornithine carbamoyltransferase in the present case, however, was inhibited by several of the TCA cycle intermediates. Aspartate and glutamate, however, had no effect on reaction velocity. The inhibition by these organic acids was competitive with respect to ornithine and non-competitive for carbamyl phosphate.

Another interesting observation made in the present case was the inhibition of this enzyme by purine nucleotides but not by pyrimidine nucleotides. Kinetics of AMP inhibition gave the evidence that AMP may probably function as an allosteric inhibitor in controlling the activity of ornithine carbamoyltransferase and thereby regulating the entry of carbamyl phosphate for pyrimidine synthesis.

Preliminary attempts made to demonstrate whether there is any difference between the enzyme from normal and tumour tissue with regard to AMP inhibition indicated that at concentrations where tumour enzyme was almost completely inhibited the normal enzyme was not affected. It was, however, inhibited partially at very high concentrations of AMP. This needs further exploration.