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Studies on Plant Aspartate Transcarbamylase

Purification and Properties of the Enzyme from Mung-Bean *(Phaseolus aureus)* Seedlings

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Aspartate transcarbamylase is purified from mung bean seedlings by a series of steps involving manganous sulphate treatment, ammonium sulphate fractionation, DEAE-cellulose chromatography, followed by a second ammonium sulphate fractionation and finally gel filtration on Sephadex-G 100. The enzyme is homogeneous on ultracentrifugation and on polyacrylamide gel electrophoresis. It functions optimally at 55 °C. It has two pH optima, one at 8.0 and the other at 10.2. The enzyme follows Michaelis-Menten kinetics with L-aspartate as the variable substrate. However, it exhibits sigmoid saturation curves at both the pH optima when the concentration of carbamyl phosphate is varied. The enzyme is allosterically inhibited by UMP at both the pH optima. Increasing phosphorylation of the uridine nucleotide decreases the inhibitory effect. The enzyme is desensitized to inhibition by UMP on treatment with *p*-hydroxymercuribenzoate, gel electrophoresis indicating that the enzyme is dissociated by this treatment; the dissociated enzyme can be reassociated by treatment with 2-mercaptoethanol. The properties of the mung bean enzyme are compared with the enzyme from other sources.

Aspartate transcarbamylase catalyzes the initial reaction unique to the *de novo* pathway of pyrimidine biosynthesis *i.e.*, the carbamylation of L-aspartate by carbamyl phosphate to give N-carbamyl aspartate. The enzyme from *Escherichia coli* has been crystallized [1] and is now available in large quantities [2]. It is subject to feedback inhibition by the end products of the pathway. This inhibition plays an important role in the regulation of pyrimidine nucleotide biosynthesis [3,4]. It has for these reasons attracted widespread attention as a model allosteric enzyme [5,6].

The observations on the partially purified enzyme from various plant sources indicate that the enzyme is maximally inhibited by UMP [7–11], unlike the *E. coli* enzyme which is inhibited by CTP. The end-product inhibition characteristics of the plant enzyme differs greatly from that of the *E. coli* enzyme. The enzyme from mung bean seedling is purified to homogeneity in order to understand the nature of substrate and end-product interactions and to evaluate the role of this enzyme in plant metabolism. Contrary to the observations of Ong and Jackson [9], homotropic interactions with carbamyl phosphate in the absence of the allosteric inhibitor and the desensitization of the enzyme to UMP inhibition were observed during the course of this investigation.

The present communication describes the isolation of a homogeneous aspartate transcarbamylase from mung bean seedlings and a comparison of its molecular and kinetic properties with the enzyme from other sources.

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Enzyme. Aspartate transcarbamylase or carbamyl phosphate: L-aspartate carbamyl transferase (EC 2.1.3.2).

MATERIALS AND METHODS

Materials

L-Aspartic acid, carbamyl aspartic acid, dilithium carbamyl phosphate, diacetyl monoxime, DEAE-cellulose (coarse), uridine, cytosine, UMP, UDP, CMP, TTP, AMP, ADP, ATP, GMP, GTP, thymidine, adenosine, *p*-hydroxymercuribenzoate and Tris were obtained from Sigma Chemical Co. (St. Louis, Mo., U.S.A.). 2′(3′)-UMP was obtained from Nutritional Biochemicals (Cleveland, Ohio, U.S.A.). Diphenylamine-4-sulphonic acid (sodium salt) was obtained from Fluka A.G. (Buchs, Switzerland). Sephadex G-25, G-100 and G-200 were products of Pharmacia

(Uppsala, Sweden). All other chemicals were of the highest purity available. Mung bean seeds were purchased from the local market.

Methods

Carbamyl phosphate was purified by the method of Gerhart and Pardee [12].

Enzyme Assay

A standard assay mixture consisting of 5 mM carbamyl phosphate (solution freshly prepared), 10 mM L-aspartic acid (neutralised with 0.5 N NaOH to the pH of the buffer indicated), 0.1 M Tris-HCl pH 8.0 or sodium carbonate-sodium bicarbonate buffer pH 10.2, enzyme and water to make up a total volume of 1 ml, was incubated at 30 °C for 20 min. The reaction was initiated by the addition of carbamyl phosphate followed by L-aspartate. It was terminated by the addition of 0.3 N perchloric acid (1 ml) and the tubes were centrifuged at $1000 \times g$ for 5 min to remove the denatured proteins. The carbamyl aspartate formed was estimated by the colorimetric method of Gerhart and Pardee [12]. Protein was determined by the method of Lowry *et al.* [13] with bovine serum albumin as the standard.

One unit of enzyme activity was defined as the amount of enzyme which produced 1 μmol carbamyl aspartate in 20 min at 30 °C at pH 8.0 or at pH 10.2. Specific activity was expressed as units/mg protein.

Polyacrylamide-Gel Electrophoresis

Disc gel electrophoresis of the enzyme sample using 7.5% polyacrylamide gels was performed according to the method of Davis [14] employing riboflavin instead of ammonium persulphate [15] to effect polymerization of the gels. Electrophoresis was carried out in 0.1 M Tris-glycine buffer pH 8.6 at room temperature with a current of 6 mA per tube. About 40 to 200 μg protein was used and the gels after electrophoresis were stained overnight with amido black and destained by washing with 7% acetic acid.

Ultracentrifugal Analysis

Sephadex G-100 eluates (Table 1) exhibiting constant and high specific activities were pooled and concentrated by dialysis against sucrose for 24 h at 5 °C. The concentrated solution containing about 10 mg protein/ml was used for ultracentrifugal analysis. The ultracentrifugal runs were carried out in a single-sector 12-mm cell at 20 °C in a Beckman model E analytical centrifuge equipped with schlieren optics. Photographs were taken using a bar angle of 45° at 8-min intervals after the maximum speed of 59780 rev./min was attained. Sedimentation coefficient was calculated from the measurements made directly from the schlieren patterns by the method of Schachman [16].

Preparation of Mung-Bean Aspartate Transcarbamylase

All operations were carried out at 0—5 °C. All centrifugations were performed at $12000 \times g$ for 10 min in a Sorvall RC-2B centrifuge.

Crude Extract. Mung bean seeds (100 g), surface sterilized by washing with 0.02% $HgCl_2$, and soaked for 16 h in running tap water, were germinated for 48 h in the dark at 30 °C. The seedlings were washed thoroughly with distilled water, chilled and blended with 0.1 M Tris-HCl buffer pH 8.0 (125 ml) for 2 min in a precooled Waring blendor. The homogenate was filtered through cheese-cloth and the filtrate centrifuged. The resulting supernatant solution was designated as crude extract (Table 1).

Manganous Sulphate Treatment. Manganous sulphate (1 M) was added to the crude extract such that the final concentration of $MnSO_4$ was 0.01 M and stirred for 30 min. The precipitated nucleoproteins were removed by centrifugation (Table 1).

First Ammonium Sulphate Fractionation. Solid ammonium sulphate was added to the manganous sulphate supernatant solution to 45% saturation with gentle stirring. The precipitate was discarded after centrifugation. The supernatant solution was raised to 60% saturation by a further addition of ammonium sulphate and the precipitated protein was dissolved in 0.01 M Tris-HCl buffer pH 8.0. It was desalted by passage through a column of Sephadex G-25 (1.6 × 30 cm) previously equilibrated with 0.01 M Tris-HCl buffer pH 8.0 (Table 1).

DEAE-Cellulose Column Chromatography. The desalted enzyme was loaded on to a DEAE-cellulose column (2 × 10 cm) equilibrated previously with 0.01 M Tris-HCl buffer pH 8.0. The column was then washed successively with 0.01 M Tris-HCl buffer pH 8.0, 0.025 M KCl and 0.05 M KCl in water. The enzyme was eluted with 0.15 M KCl solution (Table 1).

Second Ammonium Sulphate Fractionation. The eluate was brought to 50% saturation by the addition of solid ammonium sulphate. The precipitate was discarded after centrifugation. The supernatant fraction was raised to 60% saturation by a further addition of solid ammonium sulphate. The precipitate obtained upon centrifugation was dissolved in 0.01 M Tris-HCl buffer pH 8.0 (Table 1).

Gel-Filtration on Sephadex G-100. The ammonium sulphate fraction was passed through a column of

Table 1. *Purification procedure for mung-bean aspartate transcarbamylase*
One unit of activity is defined as the amount of enzyme required to produce 1 μmol carbamyl aspartate in 20 min at 30 °C and pH 8.0

Fraction	Total protein	Total activity	Specific activity	Yield	Purification
	mg	units	units/mg protein	%	-fold
Crude extract	3200	106	0.03	100	1
Manganous sulphate supernatant	1500	100	0.07	94	2
First ammonium sulphate fraction	240	86	0.36	80	11
DEAE-cellulose eluate	120	80	0.67	75	20
Second ammonium sulphate fraction	30	37	1.2	34	37
Sephadex G-100 eluate	10	27	2.7	25	80

Sephadex G-100 (1 × 60 cm) equilibrated with 0.01 M Tris-HCl buffer pH 8.0. The enzyme was eluted with the same buffer and it appeared immediately after the void volume. Fractions showing highest specific activity were pooled and used as the enzyme. This procedure resulted in an 80-fold enrichment of the enzyme activity with a recovery of 25%.

Stability of the Enzyme Preparation

The purified enzyme was stable for 1 to 2 days at 0—4 °C and was inactivated if stored frozen. However, the second ammonium sulphate precipitate was stable for more than 2 months if stored at −20 °C.

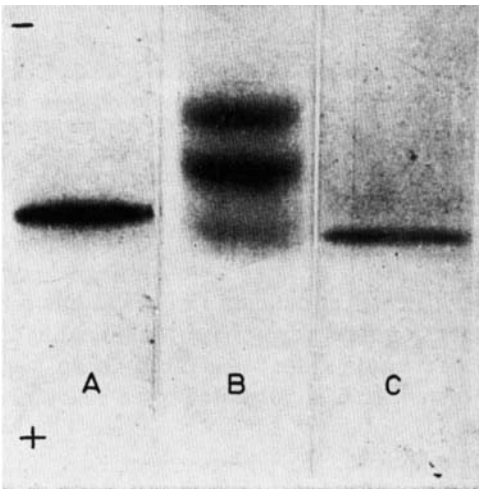

Fig. 1. *Polyacrylamide gel electrophoresis of mung-bean aspartate transcarbamylase.* Electrophoresis was carried out in 0.1 M Tris-glycine buffer pH 8.6. A current of 6 mA per tube was applied for 30 min. Migration was from top (cathode) to bottom (anode). (A) Purified mung bean aspartate transcarbamylase (60 μg). (B) Purified mung bean aspartate transcarbamylase (10 mg/ml) in 0.1 M Tris-HCl buffer was treated with 1.26 mg *p*-hydroxymercuribenzoate in the approximate molecular ratio of 1 : 50; 40 μg protein was used. (C) The treated enzyme from (B) was treated further with 2-mercaptoethanol; 40 μg 2-mercaptoethanol-treated enzyme was used

RESULTS

Criteria of Purity

Electrophoresis of the purified aspartate transcarbamylase in 0.1 M Tris-glycine buffer, pH 8.6 revealed a compact single protein band (Fig. 1 A). Analysis of a duplicate gel for enzyme activity indicated that the protein band corresponded with the enzyme activity. When electrophoresis was carried out at pH 4.3 in 4-alanine buffer, the protein did not move from the origin.

Analytical Ultracentrifugation

The sedimentation pattern of aspartate transcarbamylase (10 mg/ml) obtained in 0.01 M Tris-HCl buffer pH 8.0 is shown in Fig. 2. The purified enzyme exhibited a symmetrical peak, indicating that the enzyme was homogeneous.

The pattern after 48 min of centrifugation exhibited a slight bend. Preliminary ultracentrifugal studies using different concentrations of the protein indicated that this behaviour was not due to contaminating proteins but due to possible dissociation of the protein into low-molecular-weight species when subjected to prolonged high centrifugal force. The sedimentation coefficient for the mung bean aspartate transcarbamylase was calculated to be 5.62 S [16].

Molecular Radius

The Stokes radius was determined by the method of Ackers [17]. The Sephadex G-200 column was cali-

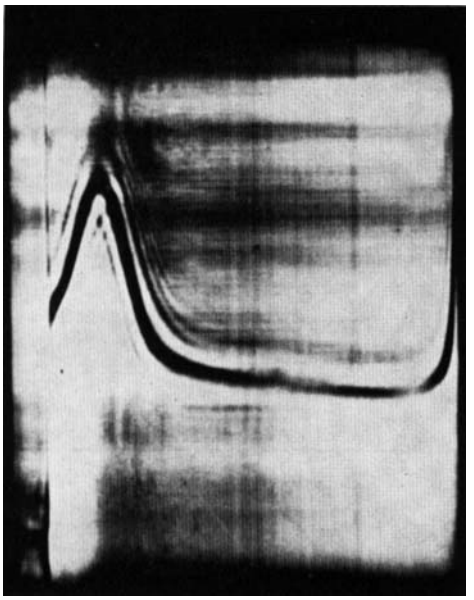

Fig. 2. *Sedimentation velocity pattern of mung-bean aspartate transcarbamylase in 0.01 M Tris-HCl buffer pH 8.0.* The photograph was taken at 32 min at a bar angle of 45° after a maximum speed (59780 rev./min) was attained. Protein concentration was 10 mg/ml

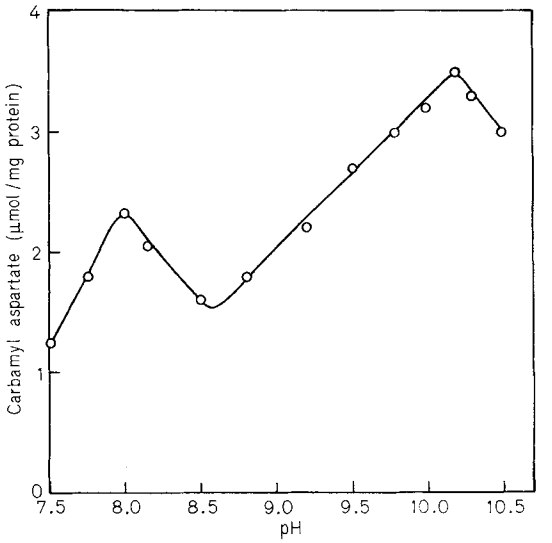

Fig. 3. *pH-activity profile of mung-bean aspartate transcarbamylase.* The buffers used were: Tris-HCl buffer (pH 7.5 to 8.0) and sodium carbonate-sodium bicarbonate buffer (pH 9.2 to 10.5). 90 µg enzyme was used in the standard assay mixture which was incubated for 20 min before measurement of carbamyl aspartate formed

brated with ox liver catalase (Stokes radius 5.22 nm) and yeast alcohol dehydrogenase (Stokes radius 4.6 nm). The Stokes radius for the mung bean aspartate transcarbamylase was calculated to be 5.22 nm.

Molecular Weight

The molecular weight of mung bean aspartate transcarbamylase was determined, by a combination of molecular sieve chromatography with sedimentation velocity data, according to the method of Siegel and Monty [18]. It was calculated to be approximately 128000.

pH Optimum

Mung bean aspartate transcarbamylase was found to exhibit two pH optima, when 10 mM L-aspartate and 5 mM carbamyl phosphate were used, one at pH 8.0 and a second higher activity optimum at pH 10.2 (Fig. 3). The two pH optima were found to shift towards 8.5 and 9.5, when the concentration of L-aspartate was increased to 40 mM. Most of the properties were studied at both the pH optima. In general the activity at pH 8.0 was found to be approximately 65% of the activity at pH 10.2.

Effect of Temperature on Enzyme Activity

The enzyme assays were carried out at temperatures indicated in Fig. 4. The optimum temperature was

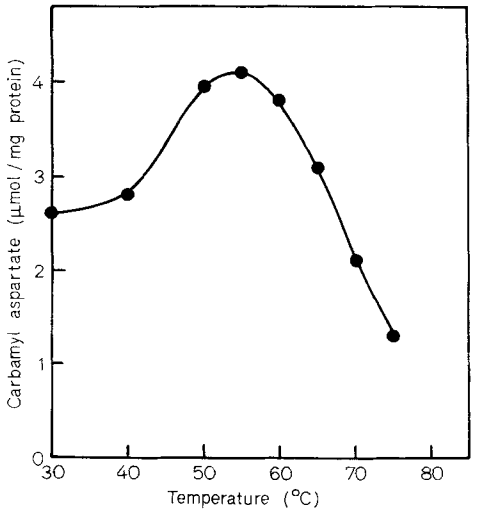

Fig. 4. *Effect of temperature on mung-bean aspartate transcarbamylase activity.* Enzyme activity (using 60 µg protein) was assayed at pH 8.0 for 20 min and at temperatures indicated in the figure

55 °C. However, all assays were carried out at 30 °C to enable comparison with aspartate transcarbamylase from other sources.

Effect of Substrate Concentration

When the initial velocities obtained at different concentrations of L-aspartate (Fig. 5) at fixed saturating

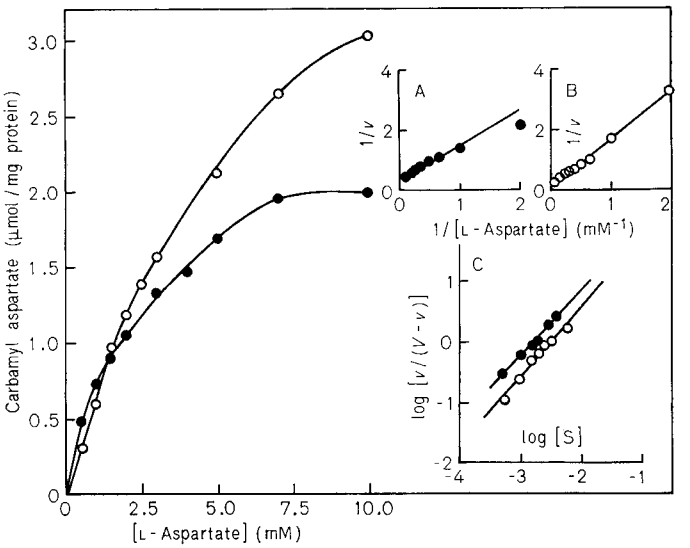

Fig. 5. *Effect of increasing concentration of aspartate on reaction velocity.* The enzyme assays were performed at the concentrations of aspartate indicated in the figure at a fixed concentration of carbamyl phosphate (5 mM) for 20 min. (●——●) Saturation curve at pH 8.0; (○——○) saturation curve at pH 10.2. Inset (A): Lineweaver-Burk plot of reciprocal of L-aspartate concentration *vs* reciprocal of velocity at pH 8.0. K_m and V were calculated to be 4 mM and 3.0 μmol carbamyl aspartate formed in 20 min per mg protein, respectively. Inset (B): Lineweaver-Burk plot at pH 10.2. K_m and V were determined to be 6.6 mM and 6.6 μmol carbamyl aspartate formed in 20 min per mg protein, respectively. Inset (C): Hill Plot. (●——●) at pH 8.0, $n = 1.0$; (○——○) at pH 10.2, $n = 1.1$

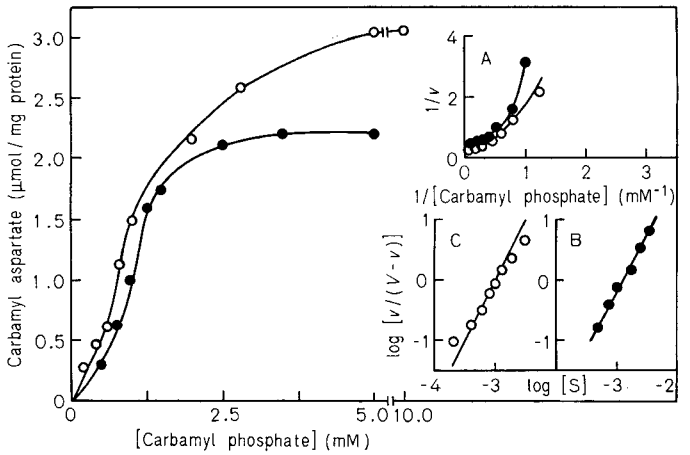

Fig. 6. *Effect of carbamyl phosphate concentration on reaction velocity.* Concentration of carbamyl phosphate was varied as indicated in the figure at a fixed concentration of L-aspartate (10 mM). (●——●) Saturation curve at pH 8.0; (○——○) saturation curve at pH 10.2. Inset (A): Lineweaver-Burk plots of reciprocal of carbamyl phosphate concentration *vs* reciprocal of velocity: (●——●) at pH 8.0, $[S]_{0.5} = 1.0$ mM; (○——○) at pH 10.2, $[S]_{0.5} = 1.0$ mM. Inset (B): Hill plot at pH 8.0, $n = 2.0$. Inset (C): Hill plot at pH 10.2, $n = 2.1$

concentration (5 mM) of carbamyl phosphate were plotted, hyperbolic saturation curves were obtained at pH 8.0 and at pH 10.2. The K_m and V values were calculated from the Lineweaver-Burk plots (inset A and B, Fig. 5) to be 4.0 mM and 3.0 μmol carbamyl aspartate formed in 20 min per mg protein at pH 8.0 and 6.6 mM and 6.6 μmol at pH 10.2, respectively. The n values were calculated from the Hill plot to be 1 at both the pH values (inset C, Fig. 5).

The variation of carbamyl phosphate concentration at fixed saturating concentration of L-aspartate (10 mM) resulted in sigmoid saturation curves at pH 8.0 as well as at pH 10.2 (Fig. 6), suggesting homotropic interactions between the enzyme and carbamyl phosphate. This was confirmed by replotting the data in the reciprocal form which gave parabolic curves and by calculating an n value of 2 at pH 8.0 and 10.2 from the Hill plot (insets, Fig. 6).

Substrate Specificity

The enzyme was specific for L-aspartate, as other L-amino acids such as ornithine, glutamate, asparagine and glutamine did not serve as substrates. The enzyme was competitively inhibited by succinate and maleate when tested at 1 mM, 2 mM, 10 mM and 20 mM concentrations. Hadacidine (*N*-formylhydroxyaminoacetic acid), another structural analogue of L-aspartate did not inhibit the enzyme reaction at a concentration of 10 mM.

Specificity of Feedback Inhibitors of Aspartate Transcarbamylase

The homogeneous mung bean aspartate transcarbamylase was feedback-inhibited by the end product of the biosynthetic pathway *viz.*, 5'-UMP.

The effect of various pyrimidine and purine derivatives on the mung bean enzyme at saturating concentrations of L-aspartate (10 mM) and carbamyl phosphate (5 mM) in 0.1 M Tris-HCl buffer pH 8.0 is summarized in Table 2. The inhibition was specific with respect to 5' position of uridine nucleotides since 2'(3')-UMP was completely without effect. The inhibition decreased with increasing phosphorylation of uridine, for UDP and UTP were not as effective as UMP.

Kinetic Properties of UMP Inhibition

Plots of the initial velocity of aspartate transcarbamylase at the two pH optima 8.0 and 10.2 *versus* the concentration of UMP are shown in Fig. 7. The curves which were clearly sigmoidal suggested that UMP was an allosteric inhibitor of the plant aspartate transcarbamylase exhibiting cooperative heterotropic interactions. This was confirmed by the Hill plots which gave an n value of 2 at pH 8.0 and at 10.2 (inset, Fig. 7).

Desensitization Experiments

Heat Treatment. In view of the observation that the *E. coli* enzyme could be desensitized by controlled heat teatment [12], the mung bean aspartate transcarbamylase was subjected to controlled heating for 5 min at 50 °C, 55 °C and 60 °C in the absence of the substrates. The enzyme was chilled rapidly to 0 °C. Heating at 50 °C did not result in loss of enzyme activity, while 20% and 50% loss of enzyme activity occurred at 55 °C and 60 °C, respectively. However, the full activity at 50 °C or the residual activities at 55 °C and 60 °C were completely sensitive to UMP inhibition suggesting that heat treatment failed to desensitize the enzyme. On the other hand, heating at 50 °C for longer periods of time resulted in loss of enzyme activity without desensitization.

Table 2. *Specificity of the feedback inhibition of the mung-bean aspartate transcarbamylase activity*
The reaction mixtures contained 5 mM carbamyl phosphate, 10 mM aspartate, 0.1 M Tris-HCl buffer pH 8.0, 80 μg enzyme preincubated with the compounds listed in the table at concentrations indicated for 15 min at 30 °C. The reaction was started by the addition of carbamyl phosphate followed by L-aspartate. The reaction mixtures were incubated at 30 °C for 20 min. Activity of the enzyme similarly preincubated was normalized to 100

Compound	Inhibition of enzyme activity by the compound at concn:	
	100 mM	1 mM
	%	
UMP	97	75
UDP	87	45
UTP	80	40
2'(3')-UMP	0	0
CMP	0	0
CDP	20	0
CTP	70	35
AMP	0	0
ADP	20	0
ATP	70	40
GMP	0	0
GTP	70	20
IMP	0	0
ITP	0	0
Uridine	0	0
Cytosine	0	0
Thymidine	0	0

Treatment with p-*Hydroxymercuribenzoate.* Another method of densitizing *E. coli* enzyme was by treatment with *p*-hydroxymercuribenzoate [12]. When the mung bean aspartate transcarbamylase was preincubated with different concentrations of this mercurial (indicated in Fig. 8) for 10 min at 30 °C in 0.1 M Tris-HCl buffer pH 8.0, no loss in enzyme activity was observed, but the sensitivity to UMP inhibition decreased with increasing concentrations of mercurial and at a concentration of 5 mM, UMP no longer inhibited the enzyme (Fig. 8). These results suggested that the treatment resulted in complete loss of sensitivity to UMP inhibition. Electrophoresis of the treated enzyme showed that the enzyme had dissociated and it could be reassociated by treatment with 2-mercaptoethanol (Fig. 1).

DISCUSSION

The purification of aspartate transcarbamylase from mung bean seedlings entails conventional steplike manganous sulphate treatment, ammonium sulphate fraction, DEAE-cellulose chromatography and gel filtration. The major difference in the purification

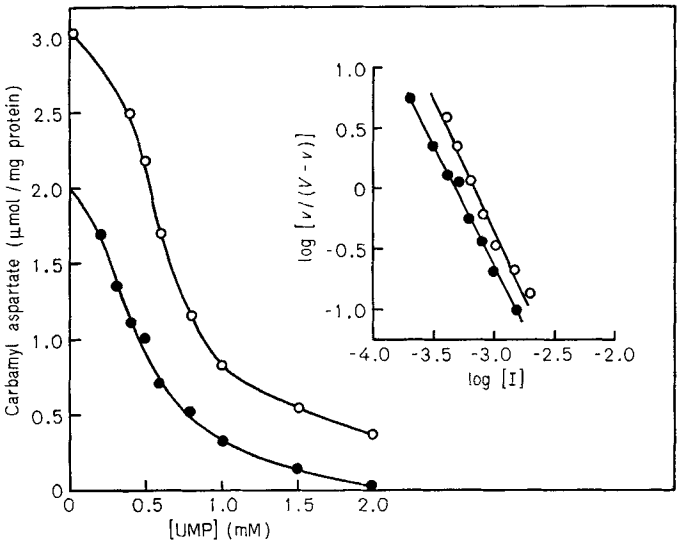

Fig. 7. *Inhibition of enzyme activity by increasing concentration of UMP.* The enzyme was preincubated with the different concentrations of UMP indicated in the figure for 15 min at 30 °C. (●——●) pH 8.0; (○——○) pH 10.2. Inset: Hill plots: (●——●) pH 8.0, $n = 2.0$; (○——○)pH 10.2, $n = 2.1$

procedure described in this paper from that of Ong and Jackson [9] lies in the use of a narrow range of ammonium sulphate fractions. The procedure adopted here is simple, rapid ,and is capable of yielding good recoveries. Processing the enzyme through the various steps resulted in an 80-fold purification and it seemed to be homogeneous on ultracentrifugation and on poly acrylamide gel electrophoresis. Ong and Jackson [9], while claiming a 200-fold purification, reported that the enzyme gave 8 to 10 bands on electrophoresis at pH 8.6 [9]. However, a comparison of our results with their data was difficult in view of several different values for V reported by them. The specific activity reported by them in the purification table (20.0) is much higher than the V (3.5) expressed in the same units. Even though the stability of highly purified aspartate transcarbamylase from the final step was only for 48 h, the second amonium sulphate precipitate could be stored in bulk quantities for 2 months at −20 °C and processed through the gel filtration step when necessary without loss of activity. The molecular weight (128000) of the purified enzyme was higher than that reported by Ong and Jackson (83000) [9] and by Yon (100000) [10]. Like the other plant aspartate transcarbamylases this enzyme also possessed the ability to exhibit allosteric behaviour although it had a lower molecular weight compared to the *E. coli* enzyme (300000).

Bethel and Jones [19] classified the bacterial aspartate transcarbamylases into three classes depending on their molecular size and correlated it with kinetic classes described earlier by Neuman and Jones [8]. Classes A, B and C had Stokes radii of

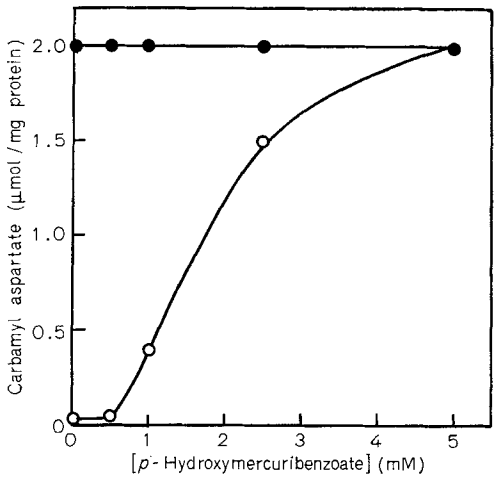

Fig. 8. *Desensitization of mung-bean aspartate transcarbamylase by* p-*hydroxymercuribenzoate towards inhibition by UMP.* The enzyme was preincubated with varying concentrations of *p*-hydroxymercuribenzoate for 10 min at 30 °C followed by preincubation of one set of tubes (○——○ with UMP (1 mM). The other set (●——●) was assayed without the addition of UMP

8.52, 6.48 and 3.95 nm, respectively. Mung bean aspartate transcarbamylases with a Stokes radius of 5.22 nm appeared to lie in between the polymeric *E. coli* enzyme (class B) and the catalytic subunit (class C) which is not inhibited by nucleotides. The mung bean aspartate transcarbamylase, although having a molecular radius less than the *E. coli* polymeric enzyme is allosterically inhibited by UMP.

The pH optima (pH 8.0 and pH 10.2) of the purified mung bean aspartate transcarbamylase were markedly

influenced by aspartate concentration. When the aspartate concentration was raised from 10 mM to 40 mM the optima shifted to pH 8.5 and pH 9.5. A close similarity existed between the plant enzyme and the *E. coli* enzyme as the crystalline *E. coli* enzyme also exhibited double pH optima, one at pH 8.5 and the other at 10.2, when 0.1 M L-aspartate was used [20]. Gerhart and Pardee [12] reported a pH optimum of around 7.0 for crystalline *E. coli* enzyme at 5 mM L-aspartate and suggested that the pH optimum was largely dependent on substrate concentration. The pH activity profile of the enzyme from mung bean seedlings also compared very well with that from wheat germ. The temperature optimum of the enzyme was 55 °C in agreement with the observation that many plant enzymes exhibit a high temperature optimum.

The effect of variation of L-aspartate concentration at saturating concentration of carbamyl phosphate on velocity yielded Michaelis-Menten kinetics. The K_m and V of the mung bean enzyme reported here were different from those reported by Ong and Jackson [9]. It is not possible at this time to offer a rational explanation for this difference.

The purified enzyme from mung bean seedlings exhibited sigmoid curves when initial velocities were plotted against varying concentration of carbamyl phosphate. In this respect it resembled the *E. coli* enzyme [21] rather than the enzyme from other plant sources [9, 10]. The reason for this could be the loss of substrate cooperativity during purification procedure [22]. Furthermore the homotropic interactions were observed at both the pH optima. However, substrate cooperativity with respect to aspartate could not be observed in the case of *E. coli* enzyme at pH 10.2 [20].

As already reported by earlier workers [9, 10] the purified mung bean aspartate transcarbamylase was also feedback-inhibited by UMP. The Hill coefficient of 2 at both pH optima suggested that negative heterotropic interactions were operative. Further work regarding the nature of these interactions is in progress. The inhibition by UMP was different from that of the *E. coli* enzyme in that the *E. coli* enzyme was inhibited by CTP. Further, the inhibition decreased with increasing phosphorylation of the uridine nucleotide whereas it increased with increasing phosphorylation of the cytidine nucleotides in the case of the *E. coli* enzyme, CTP being the most potent inhibitor [4].

While earlier workers [9, 10] were unsuccessful in their attempts to desensitize the plant aspartate transcarbamylase, the purified enzyme reported here could be desensitized to inhibition by UMP by treatment with 5 mM *p*-hydroxymercuribenzoate, lower concentrations causing partial desensitization. Desensitization in this way suggested the allosteric nature of UMP inhibition. Acrylamide gel electrophoresis of the treated enzyme and its comparison with the native enzyme indicated the formation of two new electrophoretically distinct components exhibiting decreased migration towards the anode compared to the mobility of the native enzyme. This preliminary work indicated that the enzyme could be dissociated. Further studies on the separation of dissociated subunits are in progress.

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