BBA 66024

ACTIVATION OF ASPARTATE TRANSCARBAMOYLASE BY PURINE NUCLEOTIDES

HANNA CHROBOCZEK KELKER

Department of Botany and Plant Pathology, Michigan State University, East Lansing, Mich. 48823 (U.S.A.)

(Received April 29th, 1969)
(Revised manuscript received September 29th, 1969)

SUMMARY

1 A previous report demonstrated an increase in aspartate transcarbamoylase activity (carbamoylphosphate L-aspartate carbamoyltransferase, EC 2.1.3.2) upon incubation of an homogenate of *Escherichia coli* with ATP, GTP, phosphoenolpyruvate, amino acids and magnesium acetate. The object of this study was to ascertain whether enzyme synthesis *de novo* or enzyme activation was responsible for the increase in aspartate transcarbamoylase activity.

2 Utilizing density labeling and subsequent equilibrium density centrifugation it was demonstrated that the increase in aspartate transcarbamoylase activity was not due to synthesis *de novo* of this enzyme or to extensive completion of preexisting peptides.

3 It was shown that incubation of an *Escherichia coli* homogenate with ATP, GTP, phosphoenolpyruvate, amino acids and Mg^{2+} resulted in a decrease in the apparent K_m of aspartate transcarbamoylase for aspartate while the v_{max} changed little.

4 Crystalline aspartate transcarbamoylase behaved differently than the enzyme in crude homogenates. Incubation of the crystalline enzyme with ATP, GTP and Mg^{2+} caused a threefold increase in v_{max}. This is the first report of an allosteric effector altering the v_{max} of aspartate transcarbamoylase.

5 ATP, GTP and Mg^{2+} act in a synergistic manner and must be present together for maximum activation of aspartate transcarbamoylase. Previously published studies showed that ATP is an activator and GTP is an inhibitor of this enzyme.

6 Mg^{2+} modify the effect of purine nucleotides on aspartate transcarbamoylase activity. GTP in equimolar concentration with Mg^{2+} had little effect on enzyme activity, while ATP and equimolar Mg^{2+} together were much better activators than ATP alone.

7 The activation of aspartate transcarbamoylase by ATP and GTP in the presence of Mg^{2+} reported here may be of biological significance in regulating the relative pool size of pyrimidine nucleotides and purine nucleotides of *Escherichia coli*.

INTRODUCTION

Aspartate transcarbamoylase (carbamoylphosphate L-aspartate carbamoyltransferase,, EC 2 1 3 2) the first enzyme involved in pyrimidine biosynthesis is at a strategic point for regulation of the flow of metabolites towards synthesis of pyrimidines The enzyme is inhibited by the end products of the pathway in bacteria, fungi and higher plants, however there is no evidence that the activity of mammalian aspartate transcarbamoylase is regulated by feedback inhibition Feedback inhibition of aspartate transcarbamoylase was first demonstrated in bacteria by YATES AND PARDEE[1] using *Escherichia coli* and subsequent studies of GERHARDT AND PARDEE[2] demonstrated that *E coli* aspartate transcarbamoylase is inhibited specifically by cytidine derivatives Both uridine and cytidine derivatives are inhibitors of aspartate transcarbamoylase in a number of other bacteria[3] while uridine derivatives are the best inhibitors of aspartate transcarbamoylase from *Saccharomyces cerevisieae, Neurospora crassa*[5] and *Lactuca sativa*[3] Purines are involved in the regulation of aspartate transcarbamoylase activity in addition to the feedback inhibition by pyrimidines described above In *S cerevisieae,* adenine counteracts the inhibition *in vivo* of aspartate transcarbamoylase by uracil[6] A similar effect *in vitro* of adenine derivatives on feedback inhibition of aspartate transcarbamoylase from *S cerevisieae* was demonstrated by KAPLAN *et al* [4] ATP, which does not have an effect on aspartate transcarbamoylase activity relieves uracil inhibition Aspartate transcarbamoylase of *E coli* is activated by ATP and by dATP ATP also counteracts the inhibition of aspartate transcarbamoylase by CTP *in vitro* GTP and dGTP are inhibitors of this enzyme[2]

In this study a synergistic activation of *E coli* aspartate transcarbamoylase by ATP and GTP in the presence of Mg^{2+} is reported The three components, ATP, GTP and Mg^{2+} must be present together for maximum activation Modification of the ATP and the GTP effect by Mg^{2+} is also described The increase in aspartate transcarbamoylase activity in a cell free system of *E coli* previously reported by SINGH[7] was found to be caused by the activation of the enzyme by the components of the incubation mixture, ATP, GTP and Mg^{2+} and not due to synthesis *de novo* or completion of preexisting unfinished peptides of aspartate transcarbamoylase

MATERIALS AND METHODS

Bacterial strain E coli K_{12} 411-189, obtained from Dr J C Gerhardt was used throughout this work This strain was diploid with respect to that portion of the genome (approximately one third) containing gene or genes coding for aspartate transcarbamoylase production This strain has a defect in orotidylate decarboxylase, and has an absolute requirement for histidine and a partial requirement for leucine

Growth media The growth medium MS-56 was utilized (J C GERHARDT, personal communication) It contained the following compounds in g/l of distilled water Na_2HPO_4, 4 4, KH_2PO_4, 2 6, $MgSO_4$ 7 H_2O, 0 02, Ca $(NO_3)_2$ 4 H_2O, 0 014, $FeSO_4$ 7 H_2O, 0 05, $(NH_4)_2SO_4$ 4 H_2O, 4, uracil, 0 008, D-glucose, 2, L-leucine, 0 05, L-histidine, 0 07 A solid medium was prepared by adding 2% agar to MS-56

MS-56 medium containing [^{2}H]water was prepared by diluting the stock solutions of salt with the purified [^{2}H]water of density 1 10 g/cm^3 Final concentration of [^{2}H]water was approx 67 atom percent

Maintenance, growth of the mutant and preparation of the 30 000 × g supernatant of E coli homogenate Cultures of *E coli* were maintained on MS-56 agar supplemented with 0 06 g/l of uracil The inoculum for large scale growth of cells was prepared by inoculating liquid MS-56 from an agar slant This culture was grown on a shaker at 37° until there were approx 2 10^9 bacteria/ml Subsequently the culture was diluted 33-fold in a large volume of MS-56 This culture was aerated with sterile compressed air and grown at 37° until the cell number per ml reached approx 7 10^8 At this stage of growth cells were partially derepressed for aspartate transcarbamoylase synthesis The cells were harvested by continuous flow centrifugation The preparation of the 30 000 × *g* supernatant solution of an *E coli* homogenate was as described by SINGH[7]

Assay of aspartate transcarbamoylase activity Activity of aspartate transcarbamoylase was assayed by the colorimetric method described by GERHARDT AND PARDEE[2] Assay conditions, unless otherwise specified, were as follows 3 6 mM carbamoyl phosphate, 5 mM aspartate, 40 mM potassium phosphate buffer (pH 7 0) and the aspartate transcarbamoylase preparation were incubated in a volume of 0 5 ml for 30 min at 30° Carbamoyl aspartate was asayed colorimetrically as described by GERHARD AND PARDEE[2] Activity of aspartate transcarbamoylase was expressed as nmoles of carbamoyl aspartate synthesized per min

Assay of α-amylase activity α-Amylase was assayed according to SHUSTER AND GIFFORD[8] as modified by CHRISPEELS AND VARNER[9]

Protein determination Protein content was determined according to LOWRY *et al* [10] using egg albumin as a standard

Incubation of a 30 000 g supernatant solution of an E coli homogenate leading to an increase in aspartate transcarbamoylase activity Incubation of the 30 000 *g* supernatant solution of *E coli* homogenate was performed as described by SINGH[7] The complete system contained the following compounds in μmoles potassium phosphate buffer (pH 7 4), 50, magnesium acetate, 60, ATP, 10, GTP, 10, phosphoenolpyruvate, 5, 50 μgrams of each of 20 amino acids, an aliquot of 30 000 × *g* supernatant solution of *E coli* and enough distilled water to make a volume of 1 ml As a control an equal aliquot of 30 000 × *g* supernatant solution of *E coli* was incubated with enough 0 01 M phosphate buffer (pH 7 4) containing 0 06 M KCl (designated as standard buffer) to make a volume of 1 ml The incubation was for 10 min at 37 and was terminated by placing the tubes in an ice bath Aliquots of the control and the complete system were dialyzed at 4 for 3 h against 125 ml of standard buffer with two changes of buffer prior to assay for aspartate transcarbamoylase activity

Equilibrium density centrifugation The stock solution of cesium formate of density 1 70 g/cm³ was mixed with the standard buffer containing aspartate transcarbamoylase and α-amylase Total volume of this mixture was 4 5 ml and its final density was 1 295 g/cm³ The amount of stock solution needed to obtain the desired density was calculated according to VINOGRAD[11] The contents of the tubes were covered with 0 3 ml of paraffin oil The tubes were centrifuged in a pre-cooled SW-39L rotor of a Spinco Ultracentrifuge for 67 h at 39 000 rev /min One drop fractions were collected The refractive index was determined on every fifth fraction with a Bausch and Lomb Abbe-type refractometer The corresponding densities were determined with the aid of a standard curve The standard curve was prepared by measuring the refractive index of a series of cesium formate solutions of known density The density

was determined by weighing cesium formate solutions in a 100 μl pipette. Aspartate transcarbamoylase and α-amylase were assayed alternately in the remaining fractions.

Test for the novo synthesis of aspartate transcarbamoylase. Density labelling of preformed aspartate transcarbamoylase was achieved by growing *E. coli* on [^{2}H]water as described above. An aliquot (0.1 ml) of the dialyzed 30 000 $\times$ *g* supernatant of *E. coli* grown on [^{2}H]water was incubated either with the components of the complete system containing [^{1}H]amino acids, ATP, GTP, phosphoenolpyruvate and Mg^{2+}, or, as a control, 0.1 ml of the same preparation was incubated with 0.9 ml of standard buffer, as described above. After 3 h of dialysis against standard buffer an aliquot of the dialyzed solution was taken for the assay of aspartate transcarbamoylase activity. The remaining solution was diluted with 1.5 ml of standard buffer and a measured volume taken for equilibrium density centrifugation.

RESULTS

Equilibrium density centrifugation of aspartate transcarbamoylase and its application as a test for synthesis de novo of aspartate transcarbamoylase

[^{1}H]Aspartate transcarbamoylase, obtained from *E. coli* grown on [^{1}H]water, and [^{2}H]aspartate transcarbamoylase, from *E. coli* grown on [^{2}H]water, were well resolved by equilibrium density centrifugation in a cesium formate gradient. The equilibrium position of [^{1}H]aspartate transcarbamoylase is marked by the broken line on Fig. 1. After centrifugation of the mixture of [^{1}H]aspartate transcarbamoylase and [^{2}H]aspartate transcarbamoylase approx. 105% of [^{2}H]aspartate transcarbamoylase activity was recovered in the peak of the higher density and 88% of [^{1}H]-aspartate transcarbamoylase activity was recovered in the peak of the lower density.

As demonstrated in Table I, the activity of aspartate transcarbamoylase in the supernatant prepared from *E. coli* grown on [^{2}H]water was increased 100% upon preincubation with the components of the complete system. This is in agreement with the report of SINGH[7].

Subsequent equilibrium density centrifugation of the preincubated [^{2}H]aspartate transcarbamoylase revealed that the distribution of aspartate transcarbamoylase activity on the gradient remained the same relative to the equilibrium position of an α-amylase marker regardless of whether the enzyme was preincubated with the buffer or with the complete incubation mixture (Figs. 1a and 1b). Since there was no change

TABLE I

ACTIVITY OF [^{2}H]ASPARTATE TRANSCARBAMOYLASE AFTER PREINCUBATION WITH [^{1}H]AMINO ACIDS. RECOVERY OF ASPARTATE TRANSCARBAMOYLASE ACTIVITY FROM THE GRADIENT

Activity of aspartate transcarbamoylase was measured as described in MATERIALS AND METHODS.

	Activity $\times 10^3$ *(μmoles of carbamoyl aspartate per min)*		
	In 1 ml of incubation mixture	*Put on the gradient*	*Recovered from the gradient*
Control	55.7	30.9	36.4 (109%)
Complete	113.0	78.6	40.3 (51%)

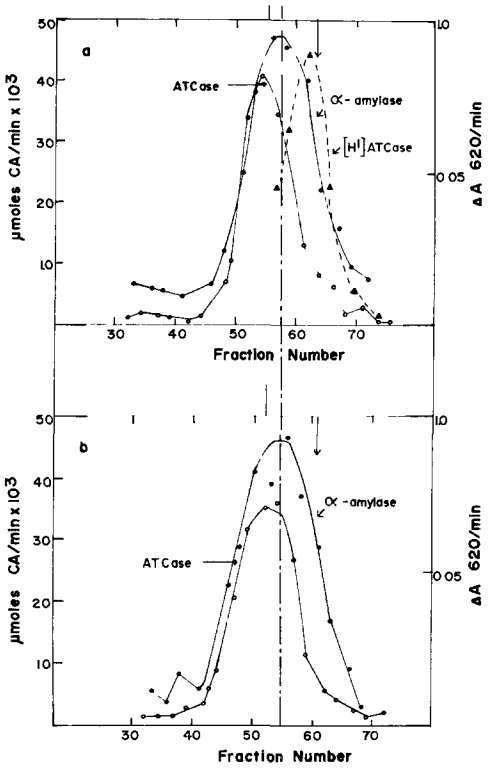

Fig. 1. Distribution of aspartate transcarbamoylase (ATCase) activity from *E. coli* grown on [^{2}H]water after centrifugation on a cesium formate gradient. (a) Enzyme preincubated with the standard buffer. (b) Enzyme preincubated with the complete incubation mixture. The broken line indicates the equilibrium position of [^{1}H]aspartate transcarbamoylase. Assay conditions were as described in MATERIALS AND METHODS. The change in $A_{620\ m\mu}$/min was taken as a measure of α-amylase activity. The direction of decreasing density is from left to right. CA, *N*-carbamoyl aspartate.

in density nor in peak symmetry upon incubation of aspartate transcarbamoylase with [^{1}H]amino acids we conclude that neither appreciable completion of preexisting partially formed aspartate transcarbamoylase nor synthesis *de novo* of aspartate transcarbamoylase occurred in this system.

All of the aspartate transcarbamoylase activity was recovered from the gradient in the case of the control (Table I). However, only one-half the activity was recovered after centrifugation of aspartate transcarbamoylase preincubated with the complete system. Since the recovery of aspartate transcarbamoylase is good after centrifugation of the mixture of [^{1}H]aspartate transcarbamoylase and [^{2}H]aspartate transcarbamoylase it is unlikely that the preferential loss of [^{1}H]aspartate transcarbamoylase was responsible for the loss of aspartate transcarbamoylase activity from the gradient. The aspartate transcarbamoylase preparation was dialyzed prior to the assay for only 3 h as described by SINGH[7]. The increase in aspartate transcarbamoylase activity, described below, is due to the activation by ATP, GTP and Mg^{2+}. Therefore the loss of the aspartate transcarbamoylase activity from the samples pre-

incubated with the complete system is, most likely, due to the dissociation of the activating compounds during prolonged centrifugation

The effect of pretreatment with the complete incubation mixture on the properties of aspartate transcarbamoylase from the 30 000 × g supernatant

The properties of aspartate transcarbamoylase in this system were examined to ascertain whether the enzyme became activated upon pretreatment with the components of the complete incubation mixture, that is, ATP, GTP, Mg^{2+}, phosphoenolpyruvate and amino acids

The dependence of reaction velocity on aspartate concentration was changed as a result of preincubation with the complete system from sigmoidal to hyperbolic (Fig 2a) The difference in reaction rate of the control and of aspartate transcarbamoylase pretreated with the complete incubation mixture was the greatest at low aspartate concentrations At saturating aspartate concentrations the difference is slight The increase in aspartate transcarbamoylase activity reported by SINGH[7] and in this study was measured at 5 mM aspartate, the concentration at which the difference in reaction rate is 100% A Lineweaver–Burk plot of the control and of aspartate transcarbamoylase pretreated with the complete incubation mixture shows that preincubation leads to a change from an upward curving to a nearly linear plot (Fig 2b) These changes in the kinetics of aspartate transcarbamoylase indicate that the enzyme became activated during incubation with the complete system

Heating at 60° for 10 min, a treatment which causes a change from sigmoid to hyperbolic kinetics of crystalline aspartate transcarbamoylase[2,12] increased the reaction rate of the control sample of aspartate transcarbamoylase by 50% (measured at 5 mM aspartate) but heating had little effect on the reaction rate of aspartate transcarbamoylase pretreated with the nucleotides and other components of the complete mixture (Fig 2a)

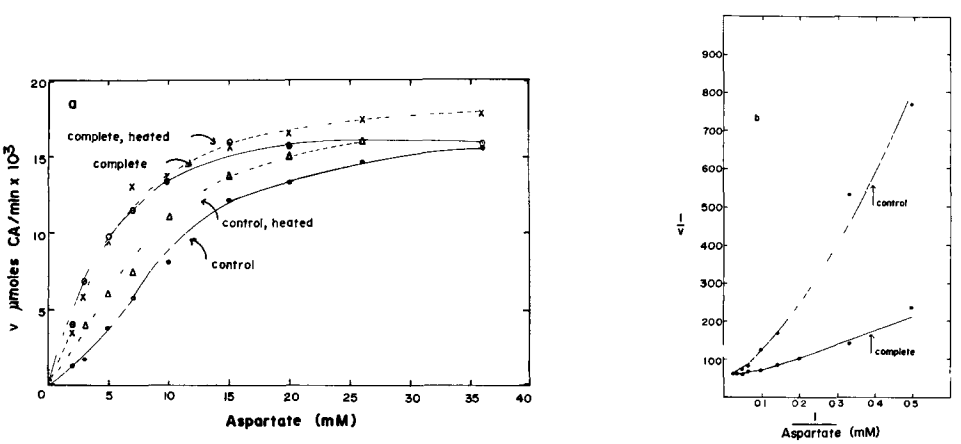

Fig 2 Dependence of reaction rate of aspartate transcarbamoylase (ATCase) on aspartate concentration (a) Crude aspartate transcarbamoylase pretreated with buffer (control) with complete incubation mixture (complete) and the same samples after the enzymes have been heated (b) Double reciprocal plots of complete and control Crude *E coli* homogenate (8 mg of protein) was incubated as described in MATERIALS AND METHODS Aliquots of the dialyzed solution diluted to a final protein concentration of 0 08 mg/ml were assayed for aspartate transcarbamoylase activity, as described in MATERIALS AND METHODS, but aspartate concentration was as indicated Heating was for 10 min at 60° at pH 7 0 CA, *N*-carbamoyl aspartate

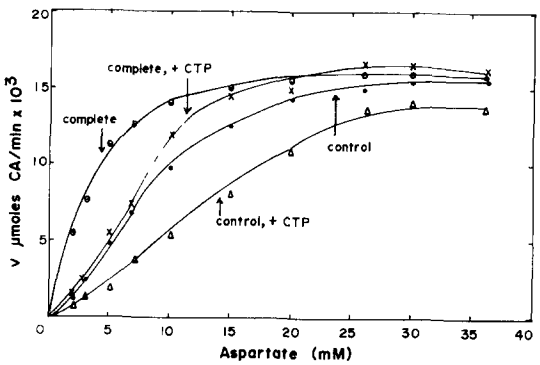

Fig 3 Effect of CTP on the reaction rate of aspartate transcarbamoylase (ATCase) as a function of aspartate concentration Crude aspartate transcarbamoylase (8 mg of protein) was pretreated with buffer (control), or with the complete incubation mixture (complete) as described in MATERIALS AND METHODS Aliquots of the dialyzed solution were assayed for aspartate transcarbamoylase activity with aspartate concentration as indicated CTP concentration, when indicated, was 2 mM CA, *N*-carbamoyl aspartate

Inhibition of aspartate transcarbamoylase activity by 2 mM CTP was observed both in the case of the control enzyme and enzyme which had been pretreated with the complete incubation mixture (Fig 3) However the inhibition of the control sample of aspartate transcarbamoylase as compared with the activated enzyme was lower when measured at aspartate concentrations below 5 mM and higher above that concentration

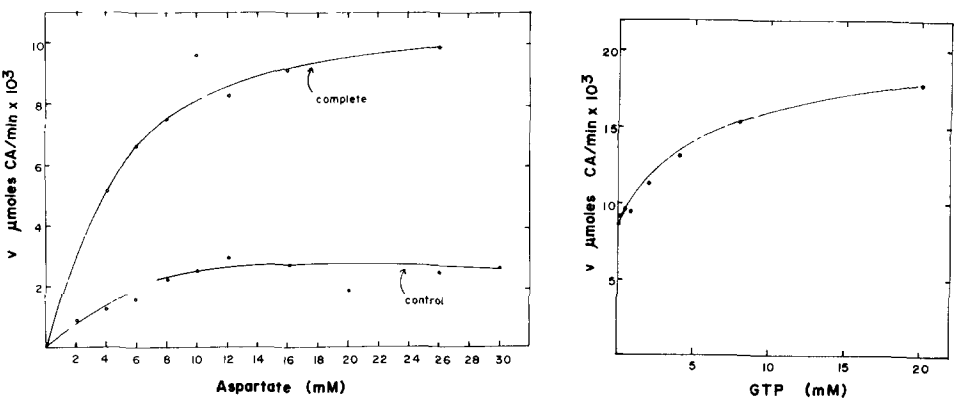

Fig 4 Dependence of reaction rates of aspartate transcarbamoylase (ATCase) on aspartate concentration Purified aspartate transcarbamoylase was pretreated with buffer (control) or with complete incubation mixture (complete) Purified aspartate transcarbamoylase (2 μg of protein) was preincubated with buffer or the complete incubation mixture, as for Fig 1, and subsequently dialyzed against 20 mM phosphate (pH 7 0), 2 mM mercaptoethanol, 0 2 mM EDTA Aliquots of the diluted dialyzed solution, containing 0 06 μg or protein were assayed for aspartate transcarbamoylase activity as described in MATERIALS AND METHODS but with aspartate concentration as indicated CA, *N*-carbamoyl aspartate

Fig 5 Activation of purified aspartate transcarbamoylase (ATCase) by GTP in the presence of ATP and Mg^{2+} Assay conditions were as follows 3 6 mM carbamoyl phosphate, 5 mM aspartate 0 1 mM EDTA, 40 mM (2-(*N*-morpholino) propanesulfonic acid (pH 7 0) (N E GOOD, unpublished), and 0 5 μg of enzyme protein ATP and magnesium acetate were both 2 mM CA, *N*-carbamoyl aspartate

Effect of components of the incubation mixture on crystalline aspartate transcarbamoylase

Treatment of crystalline *E coli* aspartate transcarbamoylase (obtained from Dr J C Gerhardt) with the complete incubation mixture followed by 3 h of dialysis caused approximately a 3-fold increase in v_{max}, as shown in Fig 4 The same increase in v_{max} was observed when crystalline aspartate transcarbamoylase was preincubated with only ATP, GTP and magnesium acetate v_{max} was increased from 3 5 to 10 2 μ-moles *N*-carbamoyl aspartate/min $\times 10^3$ when aspartate transcarbamoylase was incubated with ATP, GTP and magnesium acetate If GTP and magnesium acetate, or ATP and magnesium acetate were omitted, the observed v_{max} were 6 4 and 5 4 μmoles *N*-carbamoyl aspartate/min $\times 10^3$, respectively The simultaneous requirement for ATP, GTP and Mg^{2+} was confirmed by studies of the effect of these compounds on aspartate transcarbamoylase activity when they were added directly to the assay mixture As is shown in Fig 5, the activity of aspartate transcarbamoylase measured in the presence of 2 mM ATP and 2 mM magnesium acetate was stimulated by GTP

ATP, GTP and Mg^{2+} (all 2 mM) added directly to the assay resulted in a decrease in the apparent K_m for aspartate but not in a change in v_{max} However, inability to reproduce the increase in v_{max} by adding the activating compounds directly to the assay mixture is apparently caused by a change in the properties of the enzyme on storage Activation of aspartate transcarbamoylase upon pretreatment with ATP, GTP and Mg^{2+} decreased with time of storage After 8 days of storage the increase in v_{max} due to pretreatment with ATP, GTP and Mg^{2+} was not threefold but twofold Aging of the enzyme limited the attempts to obtain a sound value for the apparent K_m for aspartate in the presence of ATP, GTP and Mg^{2+} Aspartate transcarbamoylase was previously reported to be a stable enzyme GERHARDT AND HOLOUBEK[13] reported that activity of aspartate transcarbamoylase, and its inhibition by CTP did not change during 2 years of storage at 4° A change of properties of native aspartate transcarbamoylase has been reported by BETHELL *et al* [14], but only after 2 years of storage at −20°

The effect of ATP, GTP and Mg^{2+} on the electrophoretic mobility of purified aspartate transcarbamoylase was studied Electrophoresis on acrylamide gel gives excellent separation of the 'native' (oligomeric form of the enzyme) and of the catalytic subunit (kindly provided by Dr J C Gerhardt) Using this technique it was possible to show that treatment with ATP, GTP and Mg^{2+} did not alter the electrophoretic mobility of native enzyme We can conclude that purine nucleotides did not cause irreversible breakdown of the enzyme into subunits

Studies of the effect of purine nucleotides on aspartate transcarbamoylase activity in the 30 000 × g supernatant solution of an E coli homogenate

The effect of purine nucleotides and of Mg^{2+} on aspartate transcarbamoylase activity was examined with the objective of gaining a better understanding of activation of aspartate transcarbamoylase by ATP, GTP and Mg^{2+} The results are summarized below

ATP and Mg^{2+} at equimolar concentration activate aspartate transcarbamoylase more than ATP alone ATP at saturating concentration activates aspartate transcarbamoylase by 25%, while ATP and Mg^{2+} at 2 mM activate the enzyme by 70% (aspartate transcarbamoylase activity was measured as described in the legend to Fig 6)

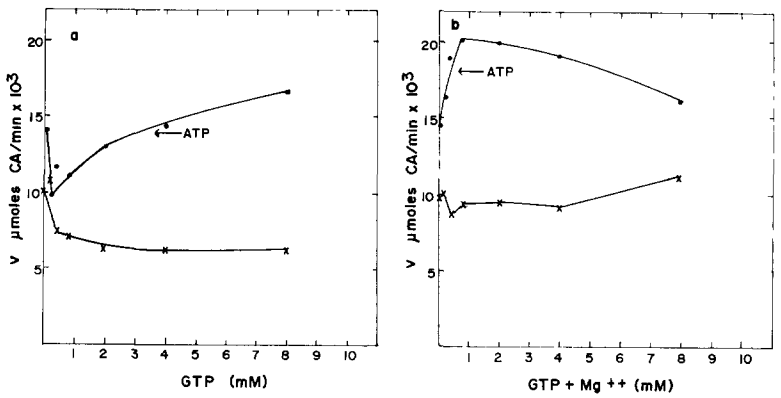

Fig 6 The effect of (a) GTP, or (b) GTP and Mg^{2+}, on aspartate transcarbamoylase (ATCase) activity in the presence or absence of ATP The reaction mixture contained 14 mM carbamoyl phosphate purified according to GERHARDT AND PARDEE[2], 1 mM EDTA, 40 mM potassium phosphate buffer, pH 7 0, 5 mM aspartate and crude aspartate transcarbamoylase preparation, 0 054 mg of protein /ml ATP, where indicated, was 8 mM ,and magnesium acetate equimolar to the indicated GTP concentration CA, *N*-carbamoyl aspartate

GTP alone is an inhibitor of aspartate transcarbamoylase (Fig 6a) The inhibitory effect of GTP is modified by Mg^{2+} As is shown in Fig 6b, GTP and Mg^{2+} at equimolar concentration have no effect on aspartate transcarbamoylase activity In the presence of ATP, GTP is inhibitory at low concentrations but is slightly stimulatory above 4 mM (Fig 6a) GTP and Mg^{2+} activate the enzyme at all concentrations (Fig 6b)

Aspartate transcarbamoylase from the 30 000 × *g* supernatant of an *E coli* homogenate was activated by ATP. The activation with a saturating amount of ATP was however less than the activation of the purified enzyme, 25% as compared with 100% However, a comparison of the properties of those two preparations is difficult not only because of the difference in their purity, but also because the mutant utilized in this study was harvested in a partially derepressed state, while the crystalline enzyme preparation was obtained from an *E coli* mutant completely derepressed for aspartate transcarbamoylase[13] A change in the degree of inhibition by pyrimidines as a function of the state of derepression has been observed for the aspartate transcarbamoylase of *S cerevisiae*[4]

DISCUSSION

The increase in aspartate transcarbamoylase activity observed under the conditions described by SINGH[7] and measured at 5 mM aspartate was confirmed in this study An examination of the properties of this system by density labeling and subsequent equilibrium density centrifugation showed that the increase in aspartate transcarbamoylase activity was not the result of synthesis *de novo* or of the completion of preexisting aspartate transcarbamoylase peptides Those results along with the results of the kinetic experiments demonstrate that aspartate transcarbamoylase became activated by components of the complete incubation mixture not removed

by dialysis Studies of the effect of the components of the incubation mixture on the crystalline aspartate transcarbamoylase demonstrate that the compounds involved in the activation of the enzyme are ATP, GTP and Mg^{2+}

It has been reported by GERHARDT AND PARDEE[2] that ATP is an activator and GTP is an inhibitor of aspartate transcarbamoylase activity KLEPPE AND SPAEREN[15] reported that Mg^{2+} inhibit the native form of aspartate transcarbamoylase The results of this study demonstrate that the effect on aspartate transcarbamoylase activity of ATP and GTP added separately or as a mixture is modified by Mg^{2+} ATP and Mg^{2+} in equimolar concentrations is a much better activator of aspartate transcarbamoylase in the 30 000 $\times$ *g* supernatant of *E coli* than ATP alone, while a previous report of KLEPPE AND SPAEREN[15] indicates that ATP and Mg^{2+} in equimolar concentration was only a few percent better activator than ATP It appears that the ATP and magnesium binding sites mutually interact and effect each others binding GTP and equimolar magnesium, which do not effect aspartate transcarbamoylase activity, either do not bind to the enzyme, or even if bound they do not effect the interaction between aspartate binding sites

The effect of ATP and GTP on aspartate transcarbamoylase activity in the presence of Mg^{2+} can be designated as a cooperative activation (by analogy to cooperative feedback inhibition, described by STADTMAN[16]) Mutual modification of the effect of each of these three compounds on aspartate transcarbamoylase activity suggests that ATP and GTP binding sites interact in the presence of Mg^{2+} No data have so far been reported concerning the binding of GTP to aspartate transcarbamoylase Studies of the binding of regulatory nucleotides to aspartate transcarbamoylase by CHANGEUX *et al* [17] indicated, but did not unequivocally prove, that ATP and CTP bind at the same site We cannot conclude from the data presented in this study what the relationship is between the binding sites of ATP and GTP However the mutual interaction between the two purine nucleotides can be most readily explained by a model allowing for simultaneous binding of ATP and GTP

The activation reported here is of considerable magnitude, being 2–4-fold depending upon experimental conditions The activation does not result in an irreversible change in the structure of the enzyme as evidenced by the failure to observe a change in the electrophoretic behaviour of the activated enzyme The increase in aspartate transcarbamoylase activity caused by ATP and GTP in the presence of Mg^{2+} may therefore be of biological significance A control mechanism of this type would lead to an increase in the biosynthesis of pyrimidines if a buildup of the pool of purine nucleotides occurred, and to an eventually balanced ratio if pyrimidines and purines necessary for the synthesis of nucleic acids

ACKNOWLEDGMENTS

This work was supported, in part, by a grant to Professor Robert S Bandurski from the U S Public Health Services (AM 05916) and, in part, by the Michigan Agricultural Experiment Station, Journal Article No 4562

The author wishes to express sincere thanks to Professor Robert S Bandurski for advice and encouragement during the course of this work Sincere thanks are expressed to Dr J C Gerhardt for the gift of crystalline aspartate transcarbamoyl-

ase Helpful discussions with Dr J Reimann and Dr P Filner are gratefully acknowledged

REFERENCES

1 R A YATES AND A B PARDEE, *J Biol Chem*, 221 (1956) 757
2 J C GERHARDT AND A B PARDEE, *J Biol Chem*, 237 (1962) 891
3 J NEUMANN AND M E JONES, *Arch Biochem Biophys*, 104 (1964) 438
4 J G KAPLAN, M DUPHIL AND F LACROUTE, *Arch Biochem Biophys*, 119 (1967) 541
5 W D DONACHIE, *Biochim Biophys Acta*, 82 (1964) 284
6 V W BURNS, *Biophys J*, 6 (1966) 787
7 R M M SINGH, *Biochem Biophys Res Commun*, 22 (1966) 684
8 L SHUSTER AND R H GIFFORD, *Arch Biochem Biophys*, 96 (1962) 534
9 M J CHRISPEELS AND J E VARNER, *Plant Physiol*, 42 (1967) 398
10 O H LOWRY, N J ROSEBROUGH, A L FARR AND R J RANDALL, *J Biol Chem*, 193 (1951) 265
11 J VINOGRAD, in S P COLOWICK AND N O KAPLAN, *Methods in Enzymology*, Vol 6, Academic Press, New York, 1963, p 854
12 P D WEITZMAN AND I B WILSON, *J Biol Chem*, 241 (1966) 5481
13 J C GERHARDT AND H HOLOUBEK, *J Biol Chem*, 242 (1967) 2886
14 M R BETHELL, K E SMITH, J C WHITE AND M E JONES, *Proc Natl Acad Sci U S*, 60 (1968) 1442
15 K KLEPPE AND U SPAEREN, *Biochim Biophys Acta*, 128 (1966) 199
16 E R STADTMAN, in *Advances in Enzymology*, Vol 28, Interscience, New York, 1966, p 41
17 J P CHANGEUX, J C GERHARDT AND H K SCHACHMAN, *Biochemistry*, 7 (1968) 531