Level, Engstrom, A., and Strandberg, B., Ed., New York, N. Y., Interscience, p 18.

Stadtman, E. (1966), *Advan. Enzymol. 28*, 41.

Winkler, R. (1969), Ph.D. Dissertation, Göttingen.

Role of an Allosteric Effector. Guanosine Triphosphate Activation in Cytosine Triphosphate Synthetase*

Alexander Levitzki† and D. E. Koshland, Jr.‡

ABSTRACT: The allosteric effector, GTP, acts to accelerate the synthesis of CTP by the enzyme, CTP synthetase, when glutamine is the nitrogen source.

$$\text{Glu-NH}_2 + \text{UTP} + \text{ATP} \xrightarrow{\text{GTP}} \text{Glu} + \text{CTP} + \text{ADP} + \text{P}_i$$

GTP accelerates the glutaminase activity of the enzyme but has little or no effect when NH_3 is the nitrogen source. Kinetic studies indicate that the role of GTP can be explained by its effect in accelerating the formation of glutamyl-enzyme from the E·Gln Michaelis complex. ATP and UTP, the substrates of the NH_3 to CTP reaction, also act as allosteric effectors for the glutaminase step in the enzyme mechanism. GTP binding was found to be noncooperative in the dimer, negatively cooperative in the tetramer at high temperatures, and positively cooperative in the tetramer at low temperatures.

Allosteric effectors are known to exert their control on an enzyme without being consumed themselves. It is now widely accepted that such effectors operate through conformational changes although the precise mechanism of these changes is not known. The similarity of these changes to a large number of other biological processes such as the peeling of a repressor from DNA, the activation of a nerve receptor molecule, and the induction of conformation changes in transport proteins suggests a widespread occurrence of properties analogous to those of the allosteric effector.

It is of particular interest, therefore, to examine the mode of reaction of an allosteric effector in a system in which the individual components have been analyzed extensively. In such a system it may be possible to dissect the overall activation or inhibition and to determine the precise mechanism by which the effector exerts its effect on the reaction pathway. CTP synthetase offered an unusual opportunity for such an examination since both the covalent chemistry of the individual steps in the reaction catalyzed by the enzyme (Levitzki and Koshland, 1971a) and the structure of the enzyme have been studied extensively (Long *et al.*, 1970). In this paper, we shall describe the role of the allosteric effector, GTP, in terms of the known chemistry and structure of the enzyme.

Experimental Section

All compounds used were of the highest purity commercially available. The ATP analog with a nitrogen atom in the β,γ position (ADPNP) was kindly donated by Dr. Ralph Yount of Washington State University, Pullman, Wash. [^{3}H]-GTP and [^{14}C]GTP in 50% ethanol were obtained from New England Nuclear Corp. Over 94% of the radioactivity was identified as GTP using thin-layer chromatography (tlc) on PEI-cellulose[1] (Randerath, 1964).

CTP synthetase was purified from *Escherichia coli* B using a modification (Levitzki and Koshland, 1970) of the method described earlier (Long and Pardee, 1967; Long *et al.*, 1970). Protein was determined by the method of Lowry *et al.* (1951). Inorganic phosphate was determined according to Fiske and Subbarow (Leloir and Carolina, 1957). Nucleotide concentrations were determined spectrophotometrically (National Academy of Sciences, 1960). γ-Glutamyl hydroxamate was determined using the $FeCl_3$–HCl reaction (Pamiljans *et al.*, 1962) and ammonia was determined using the glutamic dehydrogenase submicro method (Levitzki, 1970).

Binding Measurements by Equilibrium Dialysis. Equilibrium dialysis was carried out in dialysis cells of the design of Englund *et al.* (1970) having a volume of 30 μl in each compartment. Their aliquoting method (Englund *et al.*, 1970) was used except that aliquots of 3 μl were taken instead of 4 μl. This was found to increase the reproducibility of the sampling so that an accuracy of better than 1% was obtained. The membranes used were size 20 dialysis tubing (Union Carbide) boiled three times for 5 min in 1 mM EDTA and stored in 0.1 mM EDTA at 4°.

Binding Measurements Using Paulus Filtration Method. The binding by the Paulus filtration method (Paulus, 1969) was found to be extremely useful. However, the method must be modified for some cases since [^{14}C]GTP binds to Diaflo membranes (UM-10) in the absence of protein. This binding to the membrane follows a Michaelis–Menten isotherm with an apparent dissociation constant of 1.25×10^{-4} M. The fraction of [^{14}C]GTP bound is 20% of the amount

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† Present address: Department of Biophysics, The Weizmann Institute of Science, Rehovot, Israel.

‡ To whom to address correspondence.

[1] Abbreviations used are: polyethylenimine, PEI; 6-diazo-5-oxonorleucine, DON; β,γ-NH-ATP, ADPNP; *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid, Hepes.

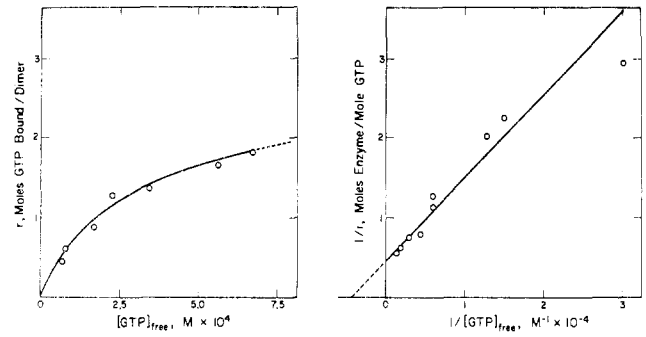


FIGURE 1: Binding of [^{14}C]GTP to the CTP synthetase dimer. Experimental conditions were as follows: 0.43 nmole of enzyme dimer was mixed with different [^{14}C]GTP concentrations in the presence of 35 mM β-mercaptoethanol, 1 mM EDTA, 100 mM glutamine, 0.01 M $MgCl_2$, and 0.02 M imidazole acetate (pH 7.2). Similar results were obtained when glutamine was omitted from the binding experiment.

applied at low [^{14}C]GTP concentrations and is 10% of the amount applied at high [^{14}C]GTP concentrations. It was found that dialysis membranes size 20 (Union Carbide) could replace the Diaflo membranes since they do not bind nucleotides (Table I). The membranes were treated as described above. The protein–ligand mixtures were prepared by mixing 0.25 ml of protein solution (4×10^{-7}–8×10^{-7} M dimer, 44–88 μg of protein/ml) and 0.25 ml ligand solution. Then, 0.35 ml of the mixture was applied to each channel; 35 psi of nitrogen pressure was applied for 3 hr until all the solution was pushed through the membrane. The membranes were then rinsed with ethylene glycol from the bottom as described earlier (Paulus, 1969). For each ligand solution a control without protein or with the same amount of egg albumin was prepared and run in parallel. A manifold (Model MF 2 Amicon) allowed us to process three apparati with eight channels each at a single time. Dialysis membranes were found to retain 0.14–0.16% of the radioactivity applied (Table I). This fraction is independent of the ligand concentration and of the nature of the ligand; it reflects the fraction of the volume actually retained by the membrane. The Diaflo membranes retain 0.5% of the radioactivity even for a ligand which is not actively bound to the membrane such as an amino acid (Paulus, 1969). In both cases the amount of radioactivity bound to the membrane is subtracted from the reading.

TABLE I: Retention of [^{3}H]GTP to Dialysis Membranes.[a]

Concn (M)	[^{3}H]GTP Applied (nmoles)	[^{3}H]GTP Bound to Membrane (nmole)	% Bound
4×10^{-5}	14	0.0228	0.163
6×10^{-5}	21	0.0305	0.145
8×10^{-5}	28	0.0480	0.171
1×10^{-4}	35	0.040	0.140
2×10^{-4}	70	0.110	0.156
6×10^{-4}	210	0.302	0.144

[a] [^{3}H]GTP solution (0.35 ml; 203 cpm/nmole) containing: 0.01 M $MgCl_2$, 0.02 M imidazole acetate (pH 7.2), 0.004 M sodium phosphate (pH 7.4), 2 mM EDTA, and 70 mM β-mercaptoethanol. Filtration was carried out at 24° for 2.5 hr. Dialysis membranes (size 20) were washed from the bottom three times with 5 ml of ethylene glycol as described above. Each sample was counted at least 20,000 cpm.

TABLE II: Activation of the Glutamine Reaction by GTP.[a]

Substrate	$S_{0.5}$ −GTP	$S_{0.5}$ +GTP	k_{cat}[b] −GTP	k_{cat}[b] +GTP
Glutamine	1.0×10^{-3}	1.6×10^{-4}	42	300
NH_3	5.3×10^{-3}	5.3×10^{-3}	270	270

[a] The glutamine assay mixture was the standard assay mixture: 7.5×10^{-4} M ATP, 7.5×10^{-4} M UTP, 0.01 M $MgCl_2$, 6×10^{-3} M glutamine, 0.02 M imidazole acetate (pH 7.2), and 0.04 unit of CTP synthetase. GTP concentration after addition was 5.0×10^{-4} M (saturating). The ammonia reaction mixtures contained 7.5×10^{-4} M ATP, 7.5×10^{-4} M UTP, 0.01 M $MgCl_2$, 0.025 M $(NH_4)_2SO_4$, 0.02 M Hepes buffer (pH 8.15), and 0.04 unit of CTP synthetase. The kinetic parameters were obtained from Lineweaver–Burk plots. [b] Moles of CTP formed per mole of enzyme sites per minute.

Results

Activation of the Glutamine Reaction by GTP. The gross effect of GTP on the glutamine reaction was tested by examining the kinetics of the glutamine reaction in the complete absence of GTP and in the presence of saturating amounts of GTP. The results are shown in Table II. In the absence of GTP, glutamine shows Michaelis–Menten kinetics with an $S_{0.5}$ of 1×10^{-3} M. In the presence of GTP at saturating levels

TABLE III: Glutaminase Activity of CTP Synthetase.[a]

Substrates	Species of Enzyme	k_{cat}[b] Glutamine	k_{cat}[b] γ-Glutamyl-NHOH
Mg^{2+}	Dimer	5.0	0.74
Mg^{2+} + GTP	Dimer	62	1.60
Mg^{2+} + ADPNP + UTP	Tetramer	28	1.36
Mg^{2+} + ADPNP + UTP + GTP	Tetramer	290	9.60
E. coli B glutaminase		38,000[c]	6350[c]

[a] Reaction mixtures contained the substrates specified in the table in the following concentrations: 1×10^{-2} M Mg^{2+}, 1×10^{-3} M GTP, 1×10^{-3} M ADPNP, 1.5×10^{-3} M UTP, 1×10^{-2} M Glu-NHOH or 1×10^{-2} M Glu-NH_2, and 0.02 M imidazole acetate (pH 7.2) in a final volume of 1.0 ml. The enzyme (0.75 unit, specific activity 6 units/mg) was added after the reaction mixtures were incubated at 38° for 10 min. The blank rate of decomposition of γ-glutamyl hydroxamate in the absence of enzyme was found to be 0.039 mole/min and was always subtracted from the measured initial velocities. [b] Moles of substrate turned over per mole of enzyme site per minute. [c] Data from Hartman (1968).

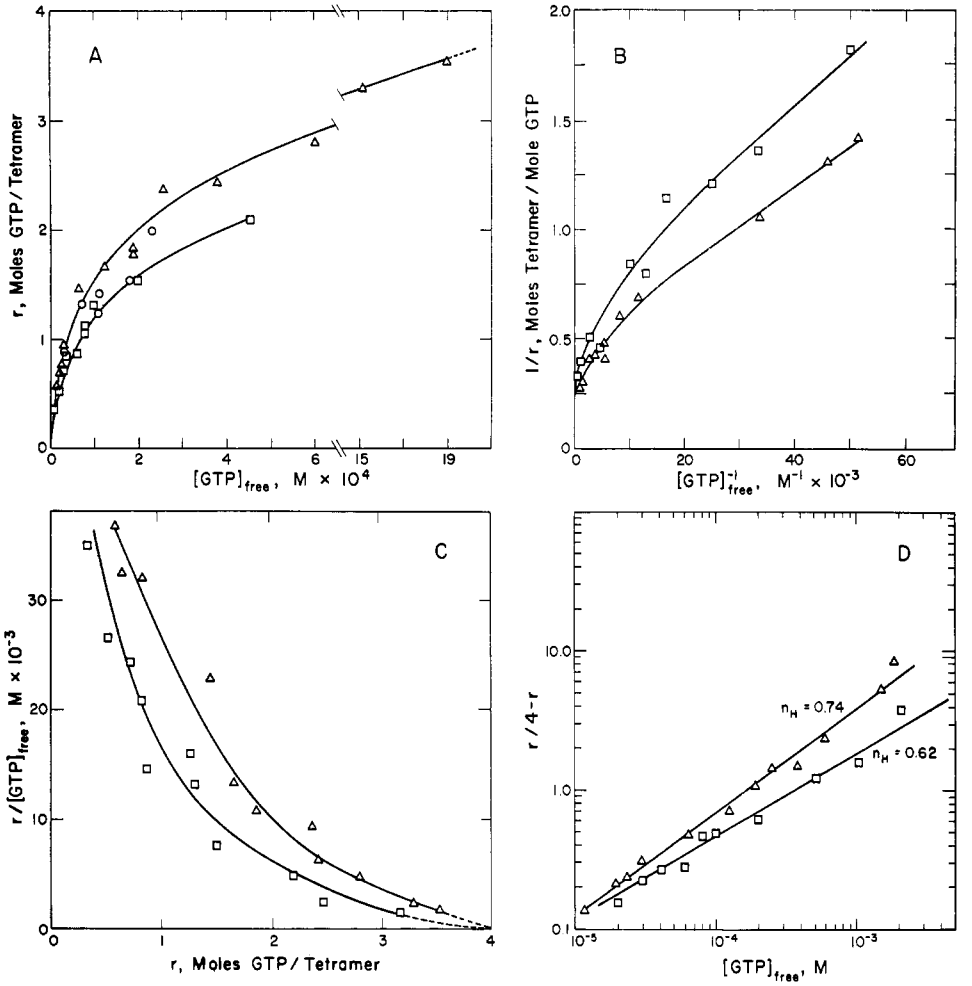


FIGURE 2: Binding of [^{14}C]GTP to DON-labeled CTP synthetase and native CTP synthetase as the tetramer. DON-enzyme, devoid of glutamine activity, was prepared as described earlier (Long *et al.*, 1970). The solution contained the following final concentrations: 0.012 M $MgCl_2$, 0.02 M imidazole acetate, 1.2 mM UTP, 1.5 mM ATP, 35 mM β-mercaptoethanol, 0.5 mM EDTA, and 1.444 × 10^{-6} M enzyme tetramer in the filtration experiments and 2.24 × 10^{-5} M enzyme tetramer in the equilibrium dialysis experiments. The native enzyme experiments employed the same conditions except ATP was replaced by 2.2 mM $ADPCH_2P$. (△) DON–enzyme, binding determined by filtration; (○) DON–enzyme, binding determined by equilibrium dialysis; (□) native enzyme with $ADPCH_2P$, binding determined by filtration.

this $S_{0.5}$ drops to 1.6 × 10^{-4} M. The same addition of GTP increases the turnover number (k_{cat}) of the enzyme by a factor of approximately 7. While having this profound influence on the glutamine reaction, the GTP has no visible effect on the ammonia reaction. Both the $S_{0.5}$ and k_{cat} remain unchanged for the NH_3 reaction in the presence and absence of GTP (Table II).

In the absence of the normal acceptors ATP and UTP it has been shown that glutamine will react with the enzyme to form a glutamyl-enzyme (Levitzki and Koshland, 1971a). This intermediate hydrolyzes to liberate glutamic acid and regenerates the enzyme. In the dimer which exists in the absence of ATP and UTP the only possible glutamine reaction is this hydrolysis process and the effect of GTP on this activity is examined in Table III. It is seen that GTP increases the turnover number of the enzyme by about tenfold for glutamine and about twofold and sevenfold when glutamylhydroxyamic acid is used as a substrate.

When ATP and UTP are present in the reaction mixture, the product CTP will be formed from the glutamine as the nitrogen source. To avoid this, the analog of ATP, ADPNP, was substituted for ATP. The latter is isosteric and isoelectronic with ATP (Larsen *et al.*, 1969). Indeed, it was found that ATP and ADPNP have identical binding constants with the enzyme (Levitzki and Koshland, 1971a). However, the nitrogen analog does not activate UTP and this makes it possible to study glutaminase activity in the tetramer as well as the dimer. The results of Table III show that the same activating effect of GTP occurred in the tetramer as in the dimer and the quantitative relations of activation are similar. It is also evident that the process of binding the ligands ATP and UTP and the concomitant aggregation to the tetramer (Long *et al.*, 1970; Levitzki and Koshland, 1971b) also affect the turnover number of the enzyme which is appreciably higher in the tetramer. It is of interest that the CTP formation in the presence of ATP is 150 moles/min per mole of enzyme sites, identical with the glutaminase activity in the presence of ADPNP. Apparently the NH_3 is consumed as soon as it is produced in the tetramer.

In the table is also included the turnover number of an efficient glutaminase from the same bacteria (Hartman, 1968) which is noted to be far higher than that of the activity of CTP synthetase. Quite clearly, CTP synthetase is not designed to act as a glutaminase but this hydrolytic activity arises to provide ammonia as the nitrogen source in this enzyme.

Binding of GTP. Binding of GTP was studied in several different states of the tetramer. The individual values were checked both by equilibrium dialysis and the filtration technique of Paulus as modified here (see Experimental Section).

TABLE IV: Intrinsic Association Constants of GTP to CTP Synthetase Dimer and Tetramer.[a]

Parameter	Dimer, 25°	Tetramer, 25°C	
		Enzyme with $ADPCH_2P$ + UTP	DON–Enzyme with ATP + UTP
K_1'	0.47×10^4	$1.71 (\pm 0.60) \times 10^4$	$1.04 (\pm 0.29) \times 10^4$
K_2'	0.47×10^4	$0.835 (\pm 0.21) \times 10^4$	$0.49 (\pm 0.1) \times 10^4$
K_3'		$0.213 (\pm 0.12) \times 10^4$	$0.83 (\pm 0.58) \times 10^4$
K_4'		$0.311 (\pm 0.09) \times 10^4$	$0.26 (\pm 0.08) \times 10^4$
K_4'/K_1'	1.0	0.18	0.25
$S_{0.5}$ (M)	2.12×10^{-4}	4.0×10^{-4}	2.5×10^{-4}
n_H	1.0	0.59	0.62

[a] $K_1' \ldots K_4'$ are intrinsic association constants. n_H is the Hill coefficient at 50% saturation.

The binding curve for GTP to the dimer at 25° in the absence of ATP and UTP is shown in Figure 1. It seemed to give a straight line on a double-reciprocal plot and extrapolates to two sites on a Scatchard plot. Such a binding pattern indicates independent identical noninteracting sites with dissociation constant of 2×10^{-4}. When GTP is bound to the tetramer protein at 25° (Figure 2) induced in that form by the mixture of UTP and $ADPCH_2P$ the midpoint of the curve is altered somewhat giving an $S_{0.5}$ of 4×10^{-4} M but the curve is distinctly negatively cooperative. This is indicated both by the distinct downward curvature of the double-reciprocal plot and the Hill coefficient of 0.6 (Levitzki and Koshland, 1969). The curve fitting procedure (Cornish-Bowden and Koshland, 1970) can be applied to these data and the four constants are shown in Table IV. The binding of GTP to the DON–enzyme tetramer in the presence of ATP and UTP was also measured at 25° (Figure 2, Table IV). The binding of GTP to the native enzyme tetramer in the presence of $ADPCH_2P$ was found to be very similar to the binding of the ligand to the affinity-labeled DON–enzyme in the presence of ATP (Figure 2 and Table IV). The slight difference between the two curves may originate in the differences in conformations between the native and the DON–enzyme as was described earlier (Levitzki *et al.*, 1971).

When the GTP binds to DON-labeled enzyme in the form of the tetramer at 4°, the GTP binding is found to be positively cooperative (Figure 3 and Table IV). Thus, it is seen that GTP binding can be Michaelian, negatively cooperative, or positively cooperative depending on the conditions.

ATP and UTP up to 1.5×10^{-3} M each do not compete for the GTP site. Upon varying the ATP and UTP levels above their saturation level, the glutamine activity at a GTP concentration close to its $S_{0.5}$ remains constant.

Inhibition of the Ammonia Activity of DON-Labeled Enzyme by GTP. The ammonia activity of the native enzyme is not affected by GTP as indicated above (Table II). However, when the ammonia activity of DON-labeled enzyme is examined, it is found that GTP inhibits the activity and acts in a negative homotropic manner (*cf.* Figure 4).

Discussion

Role of GTP. GTP is an allosteric activator of the CTP synthetase reaction but it cannot be classed simply as an activator of the total reaction. Rather, its function is a specific activation of the glutamine reaction. Both glutamine and ammonia can serve as nitrogen sources in the overall CTP synthetase reaction and yet GTP has essentially no effect on the ammonia reaction. Since it has been shown that the role of glutamine is to form a glutamyl-enzyme liberating ammonia (Levitzki and Koshland, 1971a) which is then used as a nitrogen source, GTP must act in this glutamylation–deglutamylation cycles and not on the ammonia reaction subsequent to its generation.

Three phases of this reaction are shown in eq 1, 2, and 3.

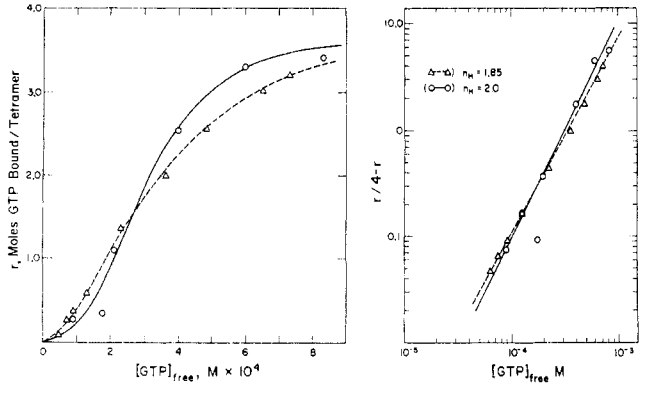


FIGURE 3: Binding of GTP to DON–CTP synthetase tetramer at 4°. Experimental conditions as described in Figure 2. (Δ) Binding determined by filtration; (O) binding determined by equilibrium dialysis.

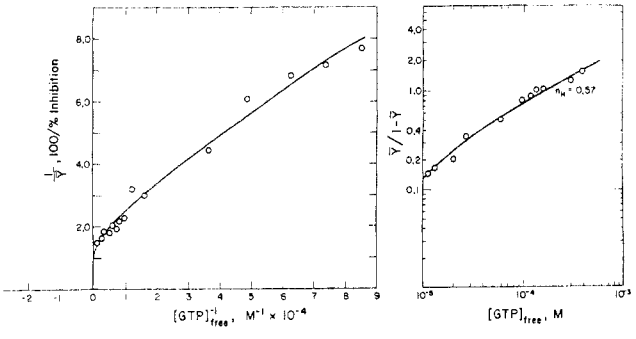


FIGURE 4: Inhibition of the ammonia activity of DON-labeled CTP synthetase by GTP. The ammonia activity was measured at pH 8.15 in 0.02 M Hepes (pH 7.2), 0.75 mM ATP, 0.75 mM UTP, 10 mM $MgCl_2$, and 25 mM $(NH_4)_2SO_4$ as a function of GTP in the assay mixture.

In the first step a glutamyl-enzyme is formed; in the second the glutamyl-enzyme hydrolyzes. These steps therefore comprise a glutaminase activity which fortunately can be studied

$$E + \text{Glu-NH}_2 \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \text{E-Glu-NH}_2 \quad (1)$$

$$\text{E-Glu-NH}_2 \xrightarrow{k_3} \text{E-Glu} + \text{NH}_3 \quad (2)$$

$$\text{E-Glu} + \text{H}_2\text{O} \xrightarrow{k_4} E + \text{Glu-OH} \quad (3)$$

under a variety of conditions (Table III).

When the details of the reactivity are examined, it is seen that GTP simultaneously serves to decrease K_M and increase k_{cat} (Table II). The three steps shown in eq 1, 2, and 3 are similar to the acylation and deacylation of serine enzymes and it can readily be shown that K_M is given by eq 4 and k_{cat} by eq 5 when $k_1 + k_2$ are large with respect to $k_3 + k_4$. The simplest mechanism which can explain the effect of GTP is

$$K_M = \frac{(k_2 + k_3)k_4}{k_1(k_3 + k_4)} = \frac{k_2 + k_3}{k_1(1 + k_3/k_4)} \quad (4)$$

$$k_{cat} = \frac{k_3k_4}{k_3 + k_4} = \frac{k_3}{1 + k_3/k_4} \quad (5)$$

that GTP increases k_3, *i.e.*, increases the rate of formation of covalent glutamyl enzyme. This will clearly increase k_{cat} and it will also decrease K_M if $k_2 > k_4$.[2] If glutamylation is the rate-determining step this condition will be fulfilled. Thus, activation of a single molecular event would explain both the decrease in K_M and the increase in k_{cat}.

Effect of Other Ligands of the Activation by GTP. A close examination of Table III reveals that the glutaminase activity of both the dimer and the tetramer species are activated 10- to 11-fold by GTP. However, the tetramer saturated with ATP and UTP has a higher glutaminase activity than the dimer. The conformational transitions occurring in the glutamine site are the sum of the changes induced by GTP and those induced by ATP and UTP. The conformational change induced by GTP in the dimer and tetramer results in the same relative change in activity, but the initial conformational state of the dimer is different from that of the tetramer. Thus, if we were to consider the area of the glutaminase activity as the "active site," ATP and UTP would be allosteric activators of the glutaminase reaction. Since the glutaminase activity is a prelude to the UTP-to-CTP conversion, the "allosteric effectors" of the first step become the substrates of the subsequent steps in the reaction. This synergistic effect of substrates in a multistep reaction was one of the earliest predictions of the induced-fit theory and has been demonstrated in the synthetase reactions by Hartman (1963) and McElroy *et al.* (1967). A general scheme for the glutamine activity of the enzyme and the role of GTP in activating the latter can now be written (Figure 5).

These interlocking conformational changes are particularly interesting in view of the effect of GTP on the NH_3 activity. The covalent changes occurring after the steps described in eq 1–3 above are given in eq 6–8 (Levitzki and Koshland, 1971a). Apparently, the conformation changes induced by GTP leave the amino acid residues performing these latter functions unchanged. The GTP-induced conformational changes can be decoupled from the region of the active site designed to carry out the steps after the release of NH_3. This involves some ingenious protein design since the NH_3, UTP, etc. sites must be immediately adjacent to the glutamine site as shown schematically in Figure 6.

This situation in the native enzyme is modified in the DON-labeled enzyme (Figure 4). The ammonia activity of the DON-modified enzyme is inhibited by GTP. It has already been shown that DON has profound cooperative effects (Levitzki

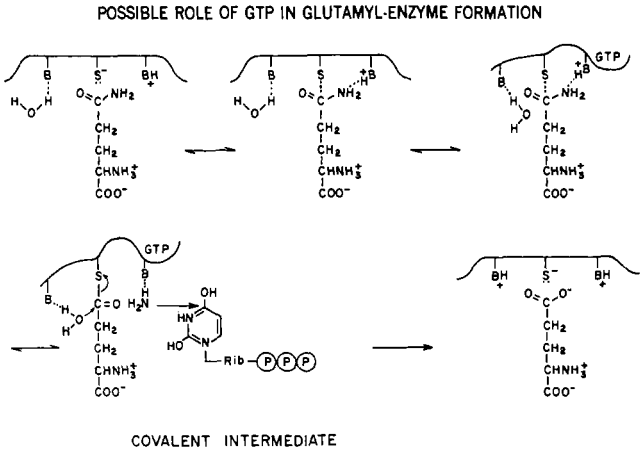
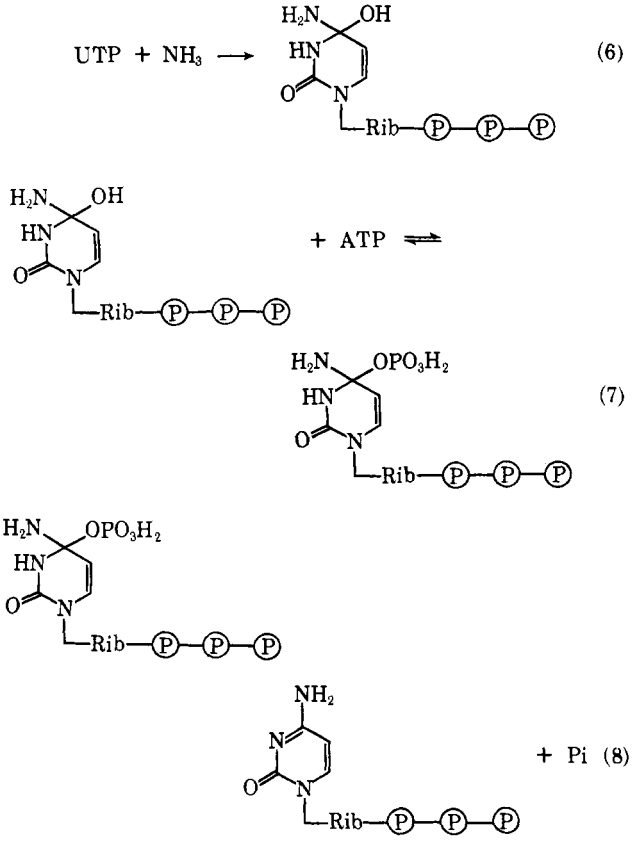


FIGURE 5: The mechanism of GTP activation. The glutaminase activity of CTP synthetase is described. The effect of GTP is shown to be exerted on the transformation of the Michaelis complex to the covalent glutamyl-enzyme intermediate.



[2] Some further limitations on the constants can be established if it is assumed that GTP only affects the k_3 step. In that case k_3/k_4 must be greater than 1 and k_2 must be at least $100k_3$.

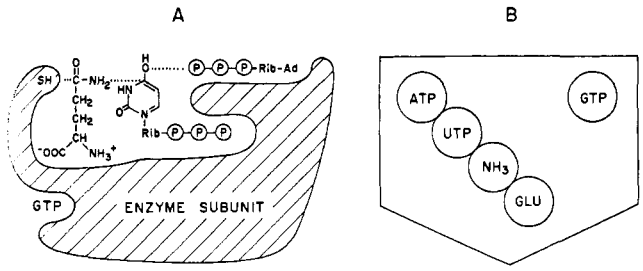


FIGURE 6: The active site of CTP synthetase. (A) The alignment of the substrate molecules interacting at one of the enzyme active sites. The allosteric site for GTP is shown to be remote from the active site. (B) A schematic representation of the active site and of the allosteric site.

et al., 1971) and it is not surprising that it in turn affects GTP interactions since the DON is a glutamine analog. However, these changes apparently are not entirely confined to the amino acids involved in the glutamine–GTP changes but also affect residues identified with the covalent steps of eq 6–8 above. The change is not great, but is sufficient so that GTP now inhibits the NH_3 reaction whereas in the native enzyme it has no effect. The inhibitory effect of GTP on the consumption of external ammonia may also be a property of the covalent glutamyl-enzyme. Since the steady-state fraction of sites covalently glutamylated is only 0.05–0.1 (Levitzki and Koshland, 1971a), glutamine alone has little effect on the ammonia reaction, in contrast to DON.

Homotropic Effects of GTP. The homotropic interactions of GTP are particularly instructive in regard to the general nature of such cooperative effects. When the protein is a dimer, GTP binds in a Michaelis–Menten manner at 25° indicating that the ligand-induced conformational changes within one subunit are not transmitted to neighboring subunits. It is clear in this case that GTP gives a hyperbolic curve because of a lack of transmission of the conformational change between subunits and not because of the absence of a conformational change since it activates the glutaminase activity of the enzyme dimer (Table III). In the tetramer, at 25°, the GTP-induced conformational changes are transmitted to neighboring subunits with a negatively cooperative effect (Figure 2), *i.e.*, the homotropic effect makes it more difficult for subsequent molecules of GTP to bind to neighboring subunits. At 4°, the GTP binding is positively cooperative (Figure 3) showing that the subunit contacts have been altered as a result of the temperature change.

Thus, the detailed role of GTP as an allosteric activator has been clarified. It acts almost exclusively on the glutamine step of the reaction and has no effect on the NH_3 reaction in the active enzyme. It apparently accelerates the glutamylation of the enzyme, affecting both the K_M and k_{cat} of this step. Its homotropic effects are negatively cooperative in the tetramer at 25°, positively cooperative at 4° in the tetramer, and non-cooperative in the dimer. The GTP binding seems also to differ slightly in the DON–enzyme when compared to the native one.

This behavior is in accord with the ligand-induced theory of cooperative interactions which postulates that the homotropic effects are transmitted through subunit contacts which determine the effectiveness of the transmission (Koshland *et al.*, 1966; Koshland, 1970). The different conditions apparently alter these contacts sufficiently so that in one case the transmission is decoupled ($K_{AB} = K_{BB} = 1$), in another case it leads to negative cooperativity (*e.g.*, $K_{AB} = 1$, $K_{BB} < 1$), and in the final case to positive cooperativity (*e.g.*, $K_{AB} = 1$, $K_{BB} > 1$). Although the nature of the GTP homotropic interactions varies, the mechanistic role of GTP in the heterotropic activation of the glutamine reaction remains unchanged.

Finally, the glutaminase activity serving as a direct conformational probe for the glutamine site itself enabled us to observe the cumulative nature of the conformational effects induced by the different ligands. The multiplicity of conformations required in this case to explain the diverse phenomena is most simply explained by a single initial structure which changes shape sequentially under the influence of the ligands. A model involving preexisting structures would be far more complicated to begin with because of the large number of states required. In addition, concerted conformational changes cannot explain the negative homotropic effects observed for GTP and DON. Thus, the induced shape of the total protein is determined by the type of ligand bound, the total number of ligands attached, and the nature of the subunit contacts which transmit these changes between subunits.

References

Cornish-Bowden, A. J., and Koshland, D. E., Jr. (1970), *Biochemistry 9*, 3325.

Englund, P. I., Huberman, J. H., Jovin, T. M., and Kornberg, A. (1970), *J. Biol. Chem. 244*, 3038.

Hartman, S. C. (1963), *J. Biol. Chem. 238*, 3024.

Hartman, S. C 1968), *J. Biol. Chem. 243*, 853.

Koshland, D. E., Jr. (1970), *in* The Enzymes, Vol. 1, Boyer, P. D., Ed., 3rd ed, New York, N. Y., Reademic Press, P 341.

Koshland, D. E. Jr., Nemethy, G., and Filmer, D. (1966), *Biochemistry 5*, 365.

Larsen, M., Willet, R., and Yount, R. G. (1969), *Science 166*, 1510.

Leloir, L. F., and Carolina, C. E. (1957), *Methods Enzymol.*, 843.

Levitzki, A. (1970), *Anal. Biochem. 33*, 335.

Levitzki, A., and Koshland, D. E., Jr. (1970), *Biochim. Biophys. Acta 206*, 473.

Levitzki, A., and Koshland, D. E., Jr. (1971a), *Biochemistry 10*, 3365.

Levitzki, A., and Koshland, D. E., Jr. (1971b), *Biochemistry 10*, 247.

Levitzki, A., Stallcup, W. B., and Koshland, D. E., Jr. (1971), *Biochemistry 10*, 3371.

Long, C. W., Levitzki, A., and Koshland, D. E., Jr. (1970), *J. Biol. Chem. 245*, 80.

Long, C. W., and Pardee, A. B. (1967), *J. Biol. Chem. 242*, 4715.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951), *J. Biol. Chem. 193*, 265.

McElroy, W. D., DeLuca, M., Travis, J. (1967), *Science 157*, 150.

National Academy of Sciences (1960), Publication No. 719.

Pamiljans, V., Kirshnaswamy, P. R., Dumville, G., and Meister, A. (1962), *Biochemistry 1*, 153.

Paulus, H. (1969), *Anal. Biochem. 32*, 91.

Randerath, K. (1964), *J. Chromatogr. Sci. 16*, 111.