A Model for Nucleotide Regulation of Aspartate Transcarbamylase†

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ABSTRACT: A number of nucleotides and related compounds are found to affect the velocity of the reaction catalyzed by aspartate transcarbamylase of *Escherichia coli*. Cytosine is an inhibitor but does not produce the same maximal effect as cytidine. Both adenosine and adenosine monophosphate are allosteric activators; at saturating concentrations, the maximal effect of AMP is less than half that of ATP. The shape of the adenosine activation curve is atypical. Both inosine and inosine triphosphate inhibit the enzyme. The effect of ITP is qualitatively similar to but weaker than the inhibitory effect of GTP. The nucleosides 3-methylcytidine and 7-methylinosine are noninhibitory. Under conditions of limiting inhibition by CTP (1 mM), the further addition of GTP slightly reduces the observed inhibition. Based on these results and previous studies of the nucleotide sensitivity of aspartate transcarbamylase, a model is proposed for the role of nucleotide binding in the regulatory mechanism. It is postulated that an electrophilic portion of the regulatory site interacts with the most basic ring nitrogen of each base: N-3 in the case of CTP, N-7 in the case of GTP or ITP, and N-1 in the case of ATP. For such interactions to occur, the nucleotide binding site must be capable of assuming at least two conformations. Consequently, the nucleotides can effect the state of the enzyme by having an enhanced affinity for one or the other of the conformations of the nucleotide binding site. A tentative identification is made of the postulated electrophilic element of each nucleotide binding site with the zinc ions present in aspartate transcarbamylase.

Aspartate transcarbamylase from *Escherichia coli* plays a key role in the regulation of cellular nucleotide levels, being subject to both activation and inhibition by nucleotide effectors at sites distinct from the enzyme active site (Gerhart and Pardee, 1962). In the present work, the effects on enzyme kinetics of several nucleosides and structurally similar molecules were systematically examined. Previous studies of this nature include the original work of Gerhart and Pardee (1962), the recent report of Goodrich and Cardeilhac (1970) that 2-thioUMP is a strong inhibitor of ATCase,[1] and several unpublished observations on 5-substituted cytidines included in a recent review article (Gerhart, 1970). In addition, studies of this nature are presently being carried out by Buckman.[2]

Unlike the compounds examined in the first two references mentioned above, the allosteric effectors studied here are not believed to play a significant role in the physiological regulation of enzyme activity. Most of the molecules studied are not normally present at the high concentrations necessary to obtain measureable effects. Rather, the attempt has been made to determine the structural requirements for allosteric effectors.

At least two models have been proposed for the quaternary conformational changes of ATCase induced by its allosteric effectors (Gerhart, 1970; Markus *et al.*, 1971); to our knowledge no proposal has been made for the specific nucleotide–enzyme interactions which form the basis for such changes. Based on the studies presented here, and on data concerning the structure of ATCase, a molecular mechanism is proposed for the allosteric control by nucleotides. A role for Zn^{2+} at the nucleotide binding site is also suggested.

Materials and Methods

Cytosine was purchased from City Biochemical Corp. All other nucleosides and nucleotides tested were purchased from Sigma Chemical Co. and were used without further purification. ATCase was isolated and catalytic subunit was obtained according to the procedures of Gerhart and Holoubeck (1967).

Enzyme activity was measured using the radioactive assay procedure developed by Porter *et al.* (1969). The reaction mixture contained 0.75–1.0 μg/ml of ATCase, 4.5 mM aspartate, 3.6 or 11.8 mM dilithium carbamyl phosphate, and varying concentrations of nucleoside or nucleotide. From a total volume of 0.5 ml, 150 μl samples were taken at 2, 4, and 6 min. The percent effect of each nucleoside or nucleotide on enzyme activity was determined using the formula

$$\% \text{ effect} = \frac{\text{slope (enzyme + effector)} - \text{slope (enzyme)}}{\text{slope (enzyme)}} \times 100\% \quad (1)$$

Either a 0.04 M phosphate or 0.05 M imidazole–acetate buffer (pH 7.0) was used in each case. In contrast to results reported for the catalytic subunit (Porter *et al.*, 1969), 0.2 M imidazole–acetate buffer is strongly inhibitory at the 4.5 mM aspartate concentration used in the assays, inhibiting the reaction ~60% relative to a 0.05 M imidazole–acetate buffer. This effect is probably due primarily to acetate competition for the aspartate site. Although these buffers are noninhibitory under the conditions employed in the assays (Kleppe, 1966), we have found that the presence of phosphate interferes with the binding of the nucleotide effectors of ATCase (results presented in the following section).

It was observed that the addition of nucleotides, which had been reported to inhibit the enzyme, frequently appeared to activate it. The activation was not always proportional to the nucleotide concentration and could be eliminated either

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[1] Abbreviations used are: ATCase, aspartate transcarbamylase; BrCTP, 5-bromocytidine 5′-triphosphate; pHMB, *p*-hydroxymercuribenzoate; MWC model, Monod–Wyman–Changeux model.

[2] T. Buckman (to be published, 1972).

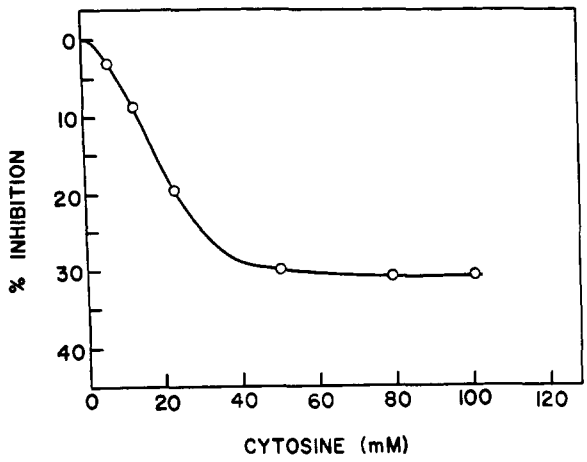

FIGURE 1: Inhibition of ATCase by cytosine. Assay conditions were 4.5 mM aspartate, 3.6 mM carbamyl phosphate, and 0.75 μg/ml of ATCase. Buffer: 0.04 M phosphate (pH 7.0), 2×10^{-3} M 2-mercaptoethanol, and 2×10^{-4} M EDTA. (% inhibition = −% effect, as defined in eq 1.)

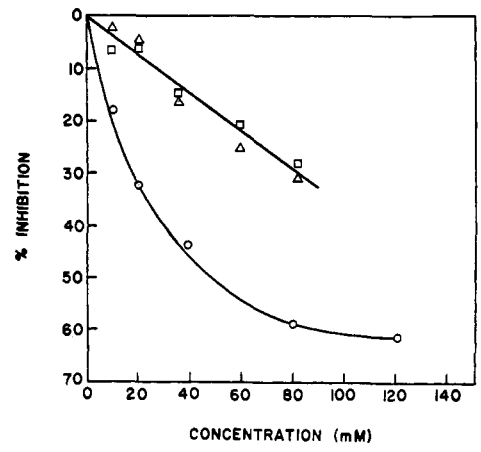

FIGURE 2: Inhibition by cytidine (○), 3-methylcytidine methosulfate (△) and sodium methosulfate (□). Assay conditions were 4.5 mM aspartate, 3.6 mM carbamyl phosphate, and 1.0 μg/ml of ATCase. Buffer was 0.05 M imidazole–acetate, 10^{-4} M EDTA, and 10^{-3} M 2-mercaptoethanol.

by pretreatment of the nucleotide with Chelex resin or by the addition of 2-mercaptoethanol and EDTA to the reaction mixture. It is therefore probable that this effect is due to the presence of heavy metal ions present as contaminants in the nucleotides (Gerhart and Pardee, 1962). In all cases, buffers contained 10^{-3} M 2-mercaptoethanol and 10^{-4} M EDTA. 2-Mercaptoethanol is noninhibitory at this concentration, although it does become inhibitory at much larger concentrations (0.1 M 2-mercaptoethanol caused a 37% inhibition under conditions as in Figure 2). Due to the critical dependence of enzyme activity on pH, samples of nucleoside or nucleotide were carefully neutralized before testing.

Results

Cytosine Derivatives. It has been reported that CTP, CDP, CMP, and cytidine, but not cytosine, are inhibitors of ATCase, each capable of producing the same maximal inhibition (Gerhart, 1970). In the present study, CTP was found to produce a maximal inhibition of about 65% at 4.5 mM aspartate, and the inhibition produced by cytidine approached this value. However, cytosine was also found to be inhibitory (Figure 1). Since Gerhart and Pardee only assayed the activity at 2 mM cytosine, we conclude that these results are not in conflict with their findings; at this concentration, the inhibition would be difficult to detect. Although the limiting value of the inhibition is difficult to ascertain due to the low solubility of cytosine, it appears to be significantly below 65% (approximately 31%). Ribose was also tested and found to be noninhibitory at concentrations up to 100 mM.

In order to further determine the specificity of the interaction, 3-methylcytidine was tested for allosteric activity. This compound is a positively charged species, and was purchased as 3-methylcytidine methosulfate. Since many anions have been found to inhibit ATCase (Kleppe, 1966), a control experiment was done to test the inhibition due to sodium methosulfate. A comparison of the inhibition produced by cytidine, 3-methylcytidine methosulfate, and sodium methosulfate (Figure 2) indicates that all or nearly all of the inhibition due to 3-methylcytidine methosulfate can apparently be attributed to the methosulfate anion. Within experimental error, 3-methylcytidine has no effect on the activity of ATCase. In addition to blocking the cytidine N-3 the methyl group also introduces a steric effect and reduces the basicity of the C-4 amino group.

As a check that the observed nucleoside inhibition is due to an allosteric mechanism rather than direct competition, cytidine was tested as a possible inhibitor of the catalytic subunit. Assay conditions were identical with those described in Figure 2, except that the native enzyme was replaced by 1 μg/ml of catalytic subunit, and samples were taken at 45-sec intervals. No inhibition was observed at concentrations of cytidine sufficient to produce the maximal effect in the native enzyme (120 mM cytidine).

CTP and GTP. A study was made of the simultaneous inhibition by a pyrimidine nucleotide, CTP, and a purine nucleotide, GTP. CTP was present in phosphate buffer at a concentration just sufficient to produce maximal inhibition (1 mM), and the GTP concentration was varied from 0 to 12 mM. The extent of the inhibition decreased monotonically from 65 to 58% as the GTP concentration was increased.

Inosine Triphosphate, Inosine, and 7-Methylinosine. ITP was found to inhibit ATCase, the inhibition qualitatively resembling that produced by GTP (Figure 3). The limiting inhibition was ~28% compared with 37% for GTP. Although the maximal effect is buffer independent, the nucleotide concentration needed to produce a half-maximal effect is greater in the phosphate buffer.

The inhibitory effect of the corresponding nucleoside, inosine, was also studied (Figure 4). As in the case of cytidine, the presence of one or more phosphate groups is not essential to achieve inhibition.

The effect of 7-methylinosine on enzyme activity is shown in Figure 4. The nucleoside was obtained from Sigma at a quoted purity of 85–90%. 7-Methylinosine may, therefore, be a very weak activator of ATCase, although the presence of impurities in the compound precludes a definite conclusion on this point. In addition to blocking the interaction of one of the ring nitrogens, methylation at position 7 considerably alters the electronic structure of the nucleoside, lowering the pK_a of the NHCO group in the pyrimidine portion of the purine so that the nucleoside exists primarily as a zwitterion at neutral pH (Lawley and Brookes, 1961; Jones and Robins, 1963; Michelson and Pochon, 1966).

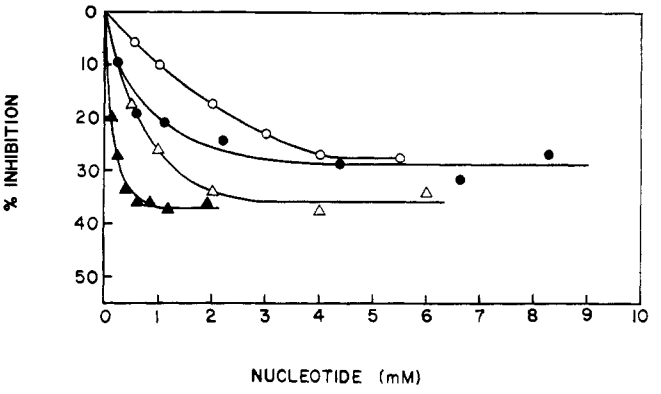

FIGURE 3: Inhibition due to ITP in 0.04 M phosphate (pH 7.0) (○), GTP in 0.04 M phosphate (pH 7.0) (△), ITP in 0.05 M imidazole–acetate (pH 7.0) (●), and GTP in 0.05 M imidazole–acetate (pH 7.0) (▲). Buffers also contained 10^{-3} M 2-mercaptoethanol and 10^{-4} M EDTA. Assay conditions were 4.5 mM aspartate, 3.6 mM carbamyl phosphate, and 0.75 μg/ml of enzyme.

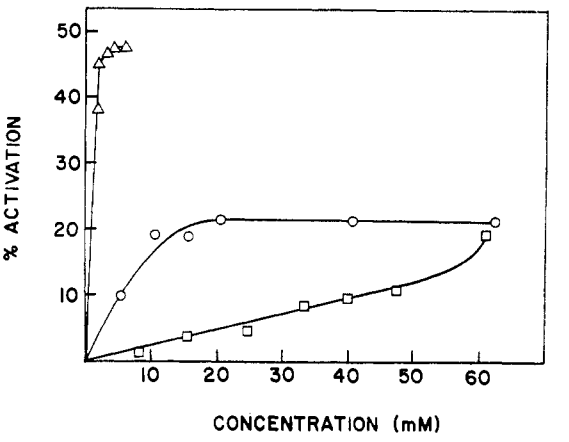

FIGURE 5: Effect of adenosine (□), AMP (○), and ATP (△) on ATCase activity. Assay conditions were as in Figure 4, except that a larger carbamyl phosphate concentration (11.8 mM) was used in order to minimize the competitive effect of AMP at the carbamyl phosphate site. The level maximum obtained indicates that the competitive effect was unimportant at AMP concentrations up to 60 mM.

Adenosine Derivatives. The effects of ATP, AMP, and adenosine on ATCase activity were examined to determine the role of the phosphate groups in the activation mechanism. Although neither AMP nor adenosine was mentioned as allosteric activators in a recent review article (Gerhart, 1970), this omission probably reflects the fact that, as in the case of cytosine, only low concentrations were studied. In contrast to the rule for cytidine derivatives, AMP was not capable of producing the same maximum effect as ATP. A saturating concentration of ATP produced an activation of about 47%, compared to the 21% activation maximum produced by saturating AMP at the same substrate levels and buffering conditions (Figure 5).

The effect of adenosine on ATCase activity is unusual (Figure 5). Although a very weak activation is observed at the lower concentrations, there is a sudden sharp increase in activity at levels near the saturating concentration of adenosine in solution (about 0.085 M). In each case where a large activity was observed there was visible precipitation of adenosine in the reaction mixture. Furthermore, the activity was not quite constant during the six minutes of the assay, but tended to increase slightly. While the initial portion of the activation curve may reflect allosteric activation, the stronger activation at higher adenosine concentrations is probably the result of a different mechanism.

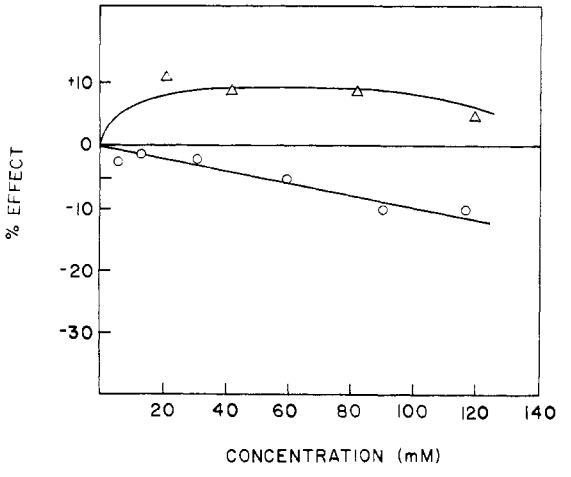

FIGURE 4: Effect of inosine (○) and 7-methylinosine (△) on ATCase activity. Assay conditions were 4.5 mM aspartate, 3.6 mM carbamyl phosphate, and 1.0 μg/ml of ATCase. Buffer was 0.05 M imidazole–acetate (pH 7.0), 10^{-4} M EDTA, and 10^{-3} M 2-mercaptoethanol.

Discussion

A relatively simple model has been developed which predicts the different allosteric effects of the various nucleotides on the assumption that all of the nucleotide binding sites are equivalent. The model includes the provision that the nucleotide binding sites can undergo conformational transitions which are coupled to overall conformational transitions of the enzyme. The ability of the various nucleotides to affect enzyme activity is thus a consequence of a differing affinity for the different conformations of the binding site.

Specifically, the conformational states of the nucleotide binding site can be characterized as follows. Each site is considered to be composed of two parts, designated A and B, and the relative orientation of A and B is variable. Part A binds completely the ribose triphosphate moiety, as well as having some affinity for the base, perhaps by a hydrophobic ring interaction. Part B consists of a strongly electrophilic moiety, X, capable of binding to a basic ring nitrogen of the nucleotide, and possibly a hydrogen-bond receptor, Y (Figure 6). The more contracted conformation of the binding site favors a less active enzyme conformation, and *vice versa*.

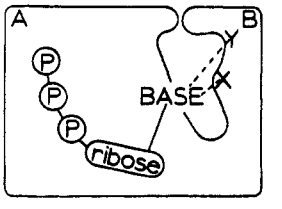

FIGURE 6: The proposed nucleotide binding site of ATCase. It is considered to be composed of two parts, A and B, whose relative orientation is contracted when the enzyme is in the T state (as shown); expanded when the enzyme is in the R state.

The interactions of CTP, GTP, ITP, UTP, and ATP with the proposed nucleotide binding site are illustrated in Figure 7. UTP is unable to affect the allosteric equilibrium since it does not interact with part B of the site. Specifically, the predominating tautomeric form of UTP is protonated at N-3 at neutral pH and has no protons in position to interact with Y. The interactions postulated for CTP and ATP with part B of the site are similar to the base pairing interactions observed in nucleic acids. ATP binds in its anti conformation to an expanded conformation of the site, while CTP binds to a contracted form. The purine nucleotides GTP and ITP behave as inhibitors since, as in the case of UTP, the pyrimidine rings of these nucleotides cannot interact with part B of the binding site. Instead, for these nucleotides N-7 can bind to X in the contracted conformation of the site.

The model outlined above does not constitute a complete description of the heterotropic interactions of ATCase. It does address itself to the central question of the mode of nucleotide binding and the role this binding plays in allosteric control. The model appears to be consistent with a large number of experimental observations. In the remainder of this paper evidence is presented in support of the specific proposals made.

Equivalance of Nucleotide Binding Sites. According to the model presented above, both activation and inhibition of ATCase by nucleotides can be explained on the basis of a set of equivalent binding sites, rather than on separate activating and inhibitory binding sites. The model is, therefore, consistent with the fact that ATP has been shown to decrease the binding of CTP competitively (Changeux *et al.*, 1968). Several studies, summarized by Gerhart (1970), have suggested the existence of two classes of regulatory sites: three "strong" CTP binding sites and three "weak" CTP binding sites. These studies do not necessarily indicate that the binding sites are structurally inequivalent and could reflect, for example, a negative interaction between pairs of bound nucleotides. More recently, Hammes *et al.* (1970) have shown that if carbamyl phosphate is added to reduce the nonspecific binding of BrCTP to the carbamyl phosphate binding site, native ACTase binds six equivalents of BrCTP per molecule. Finally, the interaction of both ATP and CTP with the same binding site might also help to explain why the two nucleotides affect several properties of the enzyme similarly; *e.g.*, the rate of digestion by proteolytic enzymes (McClintock and Markus, 1968), and changes in the electron spin resonance pattern of spin-labeled enzyme (Buckman, 1970), and the rate of dissociation of ATCase into subunits by heat or sodium dodecyl sulfate (Colman and Markus, 1972).

Structure of Nucleotide Effectors. A common characteristic of all of the inhibitors tested is the presence of a basic ring nitrogen capable of binding to an electrophilic protein residue, and located three atoms away from the glycosidic bond. Specifically, the CTP N-3 and the N-7 of GTP and ITP are the most basic ring positions at neutral pH. In contrast, UTP (in which N-3 is protonated), 3-methylcytidine, and 7-methylinosine are very poor inhibitors. As pointed out in the previous section, the difference in inhibitory strength between cytidine and 3-methylcytidine could result from the change in electronic structure of the C-4 group (amino → imino), or from the steric hindrance of the methyl group, as well as from the direct blocking of N-3. However, by assuming the involvement of a ring nitrogen in the inhibitory mechanism, the ability of both purine and pyrimidine nucleotides to inhibit ATCase can be explained in terms of a single nucleotide binding site. The interaction of both GTP and CTP at the same enzyme site is also consistent with the inhibition obtained in the presence of both nucleotides, [GTP + 1 mM CTP] $\leq$ [1 mM CTP]. This result can be explained by assuming that at sufficiently large concentrations, GTP displaces CTP from the binding site, resulting in decreased inhibition.

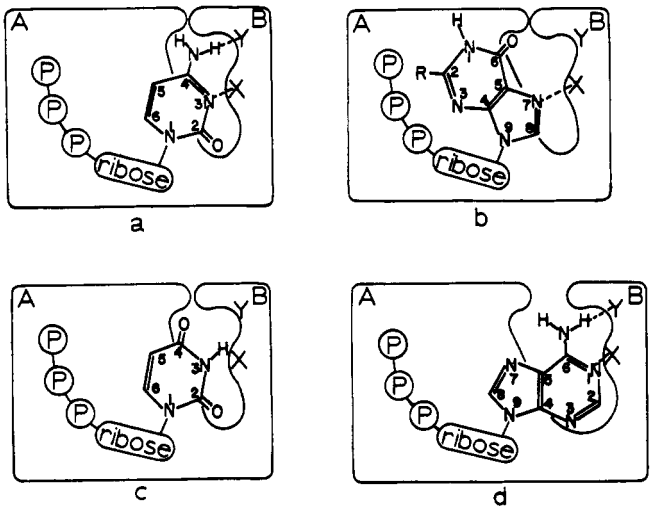

FIGURE 7: The proposed interaction of several nucleotides with the regulatory site of ATCase: (a) CTP bound to a contracted conformation of the site; (b) ITP (R = H) or GTP (R = NH_2) bound to the same conformation; (c) UTP, unable to interact with part B of the site; and (d) ATP, bound to an expanded conformation of the binding site.

The similarity between the inhibition curves of GTP and ITP suggests that the C-2 amino group of the former affects the interaction without being essential to it. If the inhibitory mechanism involves the formation of a bond between an electrophilic element of the protein and N-7 as postulated, GTP should be the better inhibitor, as observed; any positive charge acquired by N-7 would tend to be stabilized by the inductive effect of the amino group. The greater stability of 7-methylguanosine than 7-methylinosine has been explained on the basis of this effect (Jones and Robins, 1963).

The arguments given above suggest that ATP might also be an inhibitor due to an N-7 interaction. There are, however, several important differences between ATP and the purine nucleotides which inhibit ATCase. The studies of Jones and Robins (1963) and references therein show that N-7 is the preferred position for both protonation and alkylation of guanosine, whereas N-1 is preferred in adenosine. Although the most basic ring nitrogen of GTP is N-7, the most basic center of ATP is N-1. The model is also consistent with the result reported to us by Buckman[2] that isoATP, in which the ribose is attached to the adenine N-3 rather than N-9, is an inhibitor.

Assuming that the nucleotide binding site contains an electrophilic element in position to interact with the CTP N-3, it is reasonable that the site could also interact with the *amino group* by hydrogen bonding. The interaction at N-3 tends to decrease the electron density of the amino group, enhancing the tendency for donation of a hydrogen bond. For example, the binding of metal ions to cytidine N-3 is accompanied by a large downfield shift in the nuclear magnetic resonance (nmr) spectrum of the amino protons as the shielding electrons move toward the ring (Wang and Li, 1966, 1968; Kan and Li, 1970a,b). Similarly, both the base-pairing interactions in double-stranded nucleic acids and the postulated mode of

binding of cytidine monophosphate to ribonuclease (Meadows *et al.*, 1969) involve the simultaneous hydrogen-bond acceptance by N-3 and donation by the C-4 amino group. The additional hydrogen-bonding interaction would also explain, in part, the greater affinity of the nucleotide binding site for CTP than for GTP.

Data on Nucleotide Conformation. The model is consistent with available data on nucleotide conformations. Both purine and pyrimidine nucleotides in solution have been shown to favor the anti conformation (Feldman and Agarwal, 1968; Schweizer *et al.*, 1968; Danyluk and Hruska, 1968). The adoption of this conformation places the ATP N-1 and the CTP N-3 in similar positions, facing away from the phosphate chain. Although the conformation in solution is not necessarily identical with the conformation when bound, a number of enzymes have been shown to be selective for the anti conformation, and will not accept nucleotides in the syn conformation as substrates (Ogilvie *et al.*, 1971; Kapuler *et al.*, 1970; Tavale and Sobell, 1970). A nucleotide binding site shaped approximately like *anti*-CTP would then be likely to accept *anti*-ATP with the pyrimidine portions of each binding to the same residues. In order to have the GTP or ITP N-7 interact with the same protein group as the cytidine N-3 or the ATP N-1, however, these nucleotides would have to be in conformations opposite to ATP, *i.e.*, the syn conformation. Calculations have shown that the barrier to rotation about the glycosidic bond is much lower for purine nucleotides than for pyrimidine nucleotides, and that the syn conformation may even be favored for guanosine in solution (Haschemeyer and Rich, 1967; Tinoco *et al.*, 1968). Therefore, the interaction of a single protein group with the CTP N-3, the ATP N-1 and the GTP N-7 is consistent with the conformational states of these nucleotides.

Dependence of the Maximal Effect on the Presence of Ribose and Phosphates. It is interesting to analyze the differing maximal effects of ATP and AMP in terms of the model proposed by Monod *et al.* for allosteric proteins (1965). Although a two-state model may not be adequate to completely describe the conformational states of ATCase, it has had considerable success in explaining much of the available data (Gerhart, 1970; Eckfeldt *et al.*, 1970). According to the MWC model, the saturation of an allosteric protein by substrates is a function of L_0, the intrinsic equilibrium constant between the R and T states. In the presence of allosteric activators or inhibitors, L_0 must be replaced by L' (Changeux and Gerhart, 1968) where

$$L' = \frac{\bar{T}}{\bar{R}} = L_0 \frac{\left(1 + \frac{[I]}{K_{TI}}\right)^n \left(1 + \frac{[A]}{K_{TA}}\right)^n}{\left(1 + \frac{[I]}{K_{RI}}\right)^n \left(1 + \frac{[A]}{K_{RA}}\right)^n} \qquad (2)$$

In the above expression, [I] is the inhibitor concentration [A] the activator concentration, n is the number of sites, K_{RI} the dissociation constant of [I] from the R state, etc. In the case of a saturating concentration of an activator, this becomes

$$L'_{sat} \rightarrow L_0 \left(\frac{K_{RA}}{K_{TA}}\right)^n \text{ for } [A] \gg K_{RA}, K_{TA}$$

The limiting effect of an allosteric ligand on the state function is therefore dependent only on the ratio of the dissociation constants from the R and T states. The difference in the maximal effects of AMP and ATP indicates that the removal of the pyrophosphate group does not alter the affinity of the nucleotide for the T and R states equally; ATP is more able to discriminate between these states than AMP. Since the phosphates are present in both activators and inhibitors, it is unlikely that the triphosphate binding site itself undergoes a conformational change (Gerhart, 1970). The role of the phosphate portion of ATP can be explained by assuming that allosteric activation is accompanied by a conformational change of the nucleotide binding site in which the *relative* position of the enzyme residues which bind the base and phosphate is altered. The model presented here makes a specific proposal for such a conformational change.

Although the maximal inhibition produced by cytidine was only a few per cent below that produced by CTP, the maximal effect of cytosine was considerably smaller. An application of the MWC model leads to the conclusion that the ribose is important for the ability of cytidine to discriminate between the T and R states. However, ribose itself was found to be completely noninhibitory. These facts strongly indicate that the portion of the binding site that binds ribose does not undergo a change of structure but a change of position relative to at least a part of the base binding site as the enzyme undergoes an R $\rightleftharpoons$ T transition.

The fact that cytosine is an inhibitor can be explained by assuming that what we have termed part A of the nucleotide binding site has some affinity for the base, perhaps due to a base stacking interaction, as well as for the ribose triphosphate moiety. It is interesting to note that the effect of removing the entire ribose triphosphate moiety from CTP is approximately equivalent to removing only two phosphate groups from ATP; in each case the maximal effect is reduced by a factor of $\sim$2. This reflects the fact that the allosteric equilibrium favors the T state in the absence of effectors.

Quaternary Conformational Changes of ATCase. The measurement of a number of physical properties of ATCase, including sedimentation coefficient, rate of *p*-hydroxymercuribenzoate attack (Gerhart and Schachman, 1968), and rate of digestion by trypsin (McClintock and Markus, 1968), have indicated that in the presence of carbamyl phosphate and succinate the enzyme has a more open quaternary structure (Gerhart, 1970). Recently, Markus *et al.* (1971) have shown that the presence of ATP also increases the reaction rate of ATCase with pHMB. It is a probable consequence of the proposed model that allosteric activation by ATP will lead to a more open enzyme structure since only the expanded conformation of the nucleotide binding site will bind ATP.

A Possible Role for Zinc in the Allosteric Mechanism. We tentatively identify the electrophilic element X postulated in the model with the zinc ions known to be present in ATCase (Rosenbusch and Weber, 1971a,b; Nelbach *et al.*, 1972). The primary evidence for this is the recent report that 2-thioUMP is a strong allosteric inhibitor (Goodrich and Cardeilhac, 1970). This result cannot be understood in terms of differences in the tautomeric equilibria of 2-thiouridine and uridine since the diketo form has been shown to predominate strongly in both (Sano, 1962). The striking difference between the ability of UTP and 2-thioUMP to inhibit ATCase can be explained if it is assumed that X is a good thiol agent as well as a strong electrophile. A zinc ion in position to bind to the CTP N-3 would also be in position to interact with the 2-thio group with a minimum of distortion (Figure 8).

Using nmr, Li and coworkers have studied the binding of

zinc ions to nucleosides in Me_2SO (Wang and Li, 1966, 1968; Kan and Li, 1970a,b). It was found that zinc ions bind well to the cytidine N-3; however, no binding to uridine was observed. Similar binding has been observed for Cu(II) ions (Eichorn *et al.*, 1966). Zinc ions also bind to adenosine and guanosine by becoming chelated between N-7 and the C-6 substituent group. It was also suggested that Hg^{2+} ions might be binding to adenosine N-1 (Kan and Li, 1970a,b). The affinity of the nucleosides in solution for zinc ions followed the same series as the inhibitory strengths of the corresponding nucleotides: cytidine > guanosine > uridine $\cong$ 0.

Although the possible binding of the protein-bound zinc would be determined by the location of the bound nucleoside relative to the zinc, these studies demonstrate the affinity of the ring nitrogens for zinc ions. The binding of zinc to nucleosides has been studied in water as well as in Me_2SO. Eichorn *et al.* (1966) concluded that similar complexes were formed in both media. An aprotic medium may be a better model for the bound nucleotide, however, since the pyrimidine N-3 is probably facing into the protein (Gerhart, 1970).

Based on a comparison of the binding of CTP by zinc and apo regulatory subunit, Rosenbusch and Weber (1971b) have concluded that the metal ion is not required for CTP binding. The model presented here is not in conflict with this general conclusion since the absence of zinc would eliminate only a single nucleotide–enzyme bond; the affinity of the ribose triphosphate for the enzyme would be unimpaired and the base would also retain some affinity for the apo regulatory subunit. It should also be noted that the apo regulatory subunit did have a slightly decreased affinity for CTP. Although Zn^{2+} is clearly not an absolute requirement for nucleotide binding, it may contribute partially to it.

Recent studies of the role on zinc in ATCase by Nelbach *et al.* (1972) suggest that the zinc may be located in the R:C domain of bonding. Based on this tentative conclusion and the model proposed here, the nucleotide effectors may act directly on the intersubunit contacts rather than on the regulatory subunit only. We emphasize, however, that the A and B portions of the binding site do not refer to the regulatory and catalytic subunits.

Aspartate Transcarbamylase from Other Organisms. Aspartate transcarbamylase from other organisms exhibits great variation in its sensitivity to nucleotides. Unfortunately, the effect of ATP on enzyme activity has usually not been determined, since feedback inhibitors were sought. Taking into account the paucity of data, it is interesting to note the following. In two cases in which ATP has been shown to be either a weak or strong inhibitor, *Saccharomyces cerevisiae* (Kaplan *et al.*, 1967) and *Pseudomonas fluorescens* (Neumann and Jones, 1964), respectively, UTP was found to be the strongest feedback inhibitor; in two cases in which ATP was found to be an activator, *E. coli* and more recently *Citrobacter freundii* (Coleman and Jones, 1971), CTP was the most powerful nucleotide inhibitor. The regulatory properties of *C. freundii* ATCase are, however, not identical with those of the *E. coli* enzyme. The pattern indicated above is consistent with the model proposed for *E. coli* ATCase. By developing sensitivity for CTP, the nucleotide binding site simultaneously develops an affinity for the pyrimidine portion of ATP, which we have shown to be a possible basis for the activation mechanism. The choice of CTP rather than UTP as the important feedback regulator of *E. coli* ATCase has been discussed by Gerhart (1970). In view of the above discussion, as well as the proposed model, UTP inhibition may have been sacrificed to achieve ATP activation as *E. coli* evolved.

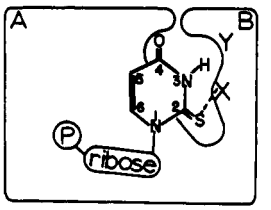

FIGURE 8: 2-ThioUMP bound to the nucleotide binding site (contracted conformation).

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