Involvement of the γ-Phosphate of UTP in the Synergistic Inhibition of *Escherichia coli* Aspartate Transcarbamylase by CTP and UTP†

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Received October 18, 1993; Revised Manuscript Received January 27, 1994®

ABSTRACT: The allosteric control of *Escherichia coli* aspartate transcarbamylase (ATCase) involves synergistic feedback inhibition by CTP and UTP. Previously reported results [England, P., & Hervé, G. (1992) *Biochemistry 31*, 9725–9732] suggest that this phenomenon relies entirely on interactions between the two neighboring allosteric sites, which belong to the same regulatory dimer. Furthermore, it has been demonstrated that UTP alone binds to the enzyme, but that it is only in the presence of CTP that this binding inhibits the catalytic activity. The properties of mutants in which the synergistic inhibition is totally abolished suggested that the terminal γ-phosphate of the pyrimidine triphosphate nucleotides may play a crucial role in promoting site–site interactions within the regulatory dimer. In the present work, kinetic studies and binding experiments by continuous-flow dialysis were performed, using combinations of diphosphate and triphosphate nucleotides. The results obtained show that the γ-phosphate of UTP is indeed essential for synergistic inhibition to occur, as UDP is unable to inhibit ATCase activity, whether alone or in combination with CTP. On the contrary, the γ-phosphate of CTP can be suppressed without modifying the inhibitory properties of this nucleotide and its synergy of action with UTP. These results indicate that the mutual effects of CTP and UTP on their respective binding are not symetrical and that the signals emitted upon binding of the two triphosphate pyrimidine nucleotides to the regulatory sites do not follow the same pathway and involve different mechanisms.

Aspartate transcarbamylase (EC 2.1.3.2) from *Escherichia coli* is one of the best known allosteric enzymes. It catalyzes the first committed reaction of the pyrimidine pathway, i.e., the carbamylation of the amino group of aspartate by carbamyl phosphate to produce *N*-carbamyl-L-aspartate and inorganic phosphate. Its structure and properties have been reviewed extensively (Allewell, 1989; Hervé, 1989; Kantrowitz & Lipscomb, 1990, 1992). *Escherichia coli* ATCase consists of two catalytic trimers and three regulatory dimers (Wiley & Lipscomb, 1968). It shows homotropic cooperative interactions between the catalytic sites for the utilization of its substrate. This phenomenon is linked to a concerted transition of the enzyme, from a conformation which has a low affinity for aspartate (T state) to a conformation that has a high affinity for this substrate (R state). The crystallographic structures of these two extreme conformations have been established with a resolution of 2.4 Å (Honzatko *et al.*, 1982; Ke *et al.*, 1984, 1988; Krause *et al.*, 1987).

The activity of ATCase is regulated through allosteric inhibition by CTP and UTP, the end products of the pyrimidine pathway, and stimulation by ATP, one of the end products of the purine pathway. This antagonism is assumed to contribute to maintaining a balance between the intracellular pools of pyrimidine and purine nucleotides. ATP, CTP, and most probably UTP bind competitively to the same allosteric sites in the N-terminal domains of the regulatory chains, approximately 60 Å away from the nearest catalytic site (Honzatko *et al.*, 1982). Each shows a heterogeneous pattern of binding, which results from negative cooperativity between the two allosteric sites that belong to the same regulatory dimer (Tondre & Hammes, 1974; Allewell *et al.*, 1975; England & Hervé, 1992). Numerous experimental results indicate that the nucleotide effectors of ATCase do not act by directly shifting the T ⇔ R equilibrium involved in the homotropic cooperative interactions between the catalytic sites for aspartate binding, but rather by local conformational changes, which extend from the regulatory sites to the catalytic sites located 60 Å away (for a review, see Thiry and Hervé (1978); Tauc *et al.*, 1982; Hervé *et al.*, 1985; Hsuanyu & Wedler, 1988; Allewell, 1989; Hervé, 1989; Lipscomb, 1992). The result of these conformational changes is an alteration of the rate constant for the binding of aspartate to the catalytic sites (Hsuanyu & Wedler, 1988). In addition, slight effects on the quaternary structure of the enzyme can also be observed (Stevens & Lipscomb, 1992), which have no influence on the T/R proportion in the case of ATP and only a barely detectable effect in the case of CTP (Hervé *et al.*, 1985).

Wild *et al.* (1989) showed that CTP and UTP synergistically inhibit the activity of ATCase. Binding studies using continuous-flow dialysis demonstrated that UTP binds to ATCase in the absence of CTP, but that this binding does not induce any inhibition unless CTP is present (England & Hervé, 1992). The mechanism of this synergistic inhibition relies on positive and negative homotropic interactions between the two regulatory sites, which belong to the same regulatory dimer. The binding of CTP to one of these sites decreases the affinity of the second site for this nucleotide, but increases its affinity for UTP. Conversely, the binding of UTP to one of the two sites decreases the affinity of the second site for this nucleotide, but increases its affinity for CTP (England & Hervé, 1992). As far as these cooperative effects are concerned, there do not seem to be significant interactions between regulatory dimers in the whole enzyme since the same pattern of competition and negative and positive interactions

†This work was supported by the Centre National de la Recherche Scientifique and by funds from the Université Pierre et Marie Curie and the Ecole Normale Supérieure (Paris). The authors are indebted to Prof. David Evans for reading and improving this manuscript.

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® Abstract published in *Advance ACS Abstracts,* March 1, 1994.

0006-2960/94/0433-3913$04.50/0 © 1994 American Chemical Society

is observed, whether or not the regulatory dimers are associated to the catalytic subunits to form the holoenzyme. The only difference observed is a factor of 2 between the measured values of the dissociation constants (England & Hervé, 1992).

Recently, several site-directed mutations in the regulatory chains of ATCase were reported to abolish the synergistic inhibition of enzyme activity by UTP and CTP (Corder & Wild, 1989; Zhang & Kantrowitz, 1991, 1992). Examination of the crystallographic structure of the enzyme shows that the modified residues could interact with the terminal phosphate (γ-phosphate) of CTP or UTP (Kim *et al.*, 1987; Gouaux *et al.*, 1990; Stevens *et al.*, 1990), suggesting that some interactions of the enzyme with the γ-phosphate of the pyrimidine nucleotides might play an important role in the mechanism of synergistic inhibition. In order to investigate this possibility, kinetic inhibition experiments and binding studies by continuous-flow dialysis were performed using diphosphate and triphosphate pyrimidine nucleotides, alone and in combination. The results obtained show that the γ-phosphate of UTP, but not that of CTP, is necessary for the process of synergistic inhibition to occur.

MATERIALS AND METHODS

Materials

Enzyme. Native ATCase was overproduced from the plasmid pEK2 and expressed in *Escherichia coli* strain EK1104 strain (Nowlan & Kantrowitz, 1985). It was then purified to homogeneity by a method similar to that described by the aforementioned authors, although an additional chromatography step on Sepharose CL-6B was found to be necessary, prior to the isoelectric precipitation at pH 5.9, in order to totally eliminate the remaining nucleic acids and some impurities.

Chemicals. Carbamyl phosphate (dilithium salt), L-aspartate, CTP, CDP, and UDP (sodium salts) were purchased from Sigma Chemical Co. UTP (sodium salt) was obtained from Pharmacia, imidazole and Titriplex were from Merck, 2-mercaptoethanol was from Kodak, and Aquasol liquid scintillation cocktail was from NEN Research Products. [5-^{3}H]-CTP (23 Ci/mmol), [5-^{3}H]-UTP (10.2 Ci/mmol), and [5-^{3}H]-CDP (21 Ci/mmol) (ammonium salts) were from Amersham, while L-[U-^{14}C]aspartate (300 mCi/mmol) was from CEA-Saclay.

Both labeled and unlabeled triphosphate nucleotides were controlled for purity by the method described by Beck and Howlett (1977). Spectrophotometry was used to determine the exact concentrations of unlabeled solutions (at pH 7.0): ϵ(CTP) = ϵ(CDP) = 9000 at 271 nm and ϵ(UTP) = ϵ(UDP) = 10 000 at 262 nm.

Buffer. All continuous-flow dialysis and enzyme assay experiments were performed in buffered solutions containing 5×10^{-2} M imidazole (pH 7.0), 10^{-3} M 2-mercaptoethanol, and 10^{-4} M EDTA (ethylenediaminotetraacetic acid).

Methods

Binding Studies by Continuous-Flow Dialysis. The binding properties of the pyrimidine nucleotides were studied by the continuous-flow dialysis method (Colowick & Womack, 1969) at 4 °C, using the apparatus described by England and Hervé (1992). The range of ligand concentrations used was as wide as possible within the limits imposed by the method used. The ATCase concentration and the smallest concentration of labeled ligand used at the start of the measurement were imposed by the technical requirement that 95% of the ligand must be bound. When the binding of one labeled nucleotide was studied in the presence of another competing unlabeled nucleotide, the range of concentrations used had to be in the range of the apparent dissociated constant of the first one and not in the range of the subsequently calculated real macroscopic dissociation constant. In each case, 15 mM carbamyl phosphate was added to both the enzyme sample and the circulating buffer in order to avoid binding of nucleotides to the active sites of the enzyme. Addition of ligand increments, sampling, and counting of the radioactive samples were performed as previously described (England & Hervé, 1992). The Scatchard plots were fit using KaleidaGraph. The values found for CTP and UTP alone are in agreement with the values previously reported using traditional methods. The values and standard deviations reported here result from 6–10 determinations in each case.

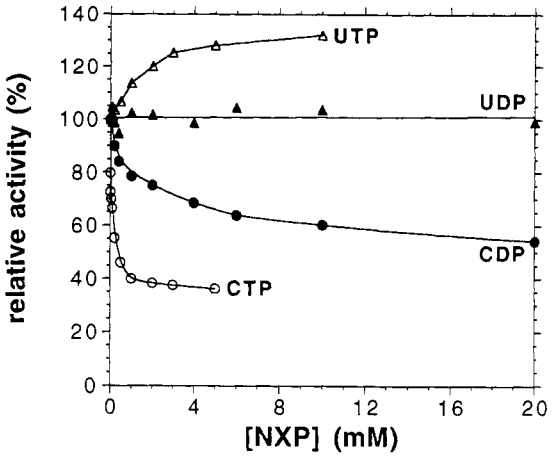

FIGURE 1: Effect of CDP and UDP on the activity of ATCase. The enzymatic activity was assayed as described in Materials and Methods at 37 °C, in the presence of 15 mM carbamyl phosphate, 5 mM aspartate, and variable concentrations of CTP (○), UTP (△), CDP (●) or UDP (▲).

Enzyme Assay Using [^{14}C]Aspartate. The ATCase activity was determined as previously described (Perbal & Hervé, 1972) at 37 °C, in the presence of the above-mentioned buffer and 10^{-2} M carbamyl phosphate.

Protein Concentration. The enzyme concentration was determined by the method of Lowry *et al.* (1951) using bovine serum albumin as a standard, taking into account the 20% overestimate given by this method for ATCase (Kerbiriou *et al.*, 1977).

RESULTS

(1) Effect of CDP and UDP on the Activity of ATCase. The influence of increasing concentrations of CDP and UDP on the rate of the reaction catalyzed by ATCase was determined and compared with the effects produced by CTP and UTP. The results obtained are shown in Figure 1. As previously shown by Gerhart and Pardee (1962), CDP behaves as an allosteric inhibitor of the catalytic activity of ATCase. However, it takes a 40-fold higher concentration of CDP than CTP to promote 50% inhibition of the enzyme activity. The shape of the dose/response curve (Figure 1) suggests that the lower efficiency of CDP only reflects a lower affinity for the regulatory sites, a conclusion that is consistent with the results of the binding experiments (Table 1). On the other hand, UDP has no effect at all on the enzymatic activity, even at high concentrations (Figure 1), in contrast with the slight activation that is induced by UTP under the same experimental conditions (England & Hervé, 1992).

Table 1: Binding of Pyrimidine Nucleotides to ATCase; Dissociation Constants Measured at 4 °C[a]

	in the presence of	apparent constants K_{d1} (μM)	apparent constants K_{d2} (μM)	calculated real macroscopic K_d values
UTP binding	no other nucleotide	810 ± 200	nd	
	1mM CDP	850 ± 250	nd	K_{d1} = 65 μM
CTP binding	no other nucleotide	9 ± 0.5	260 ± 65	
	3 mM UDP	12 ± 1	330 ± 80	
CDP binding	no other nucleotide	82 ± 9	1650 ± 400	
	3 mM UTP	120 ± 15	1930 ± 500	K_{d1} = 65 μM
	3 mM UDP	107 ± 16	2180 ± 560	K_{d2} = 65 μM

[a] The apparent dissociation constants were obtained by fitting the Scatchard plots with the program KaleidaGraph. The real macroscopic constants were calculated as described in the text and are indicated only when they are different from the apparent constants. K_{d1} and K_{d2} are, respectively, the dissociation constants for the high-affinity and low affinity sites. nd: not detectable. The range of ligand concentrations used was on the order of the apparent dissociation constants and as wide as possible within the limits imposed by the experimental method used (see Methods).

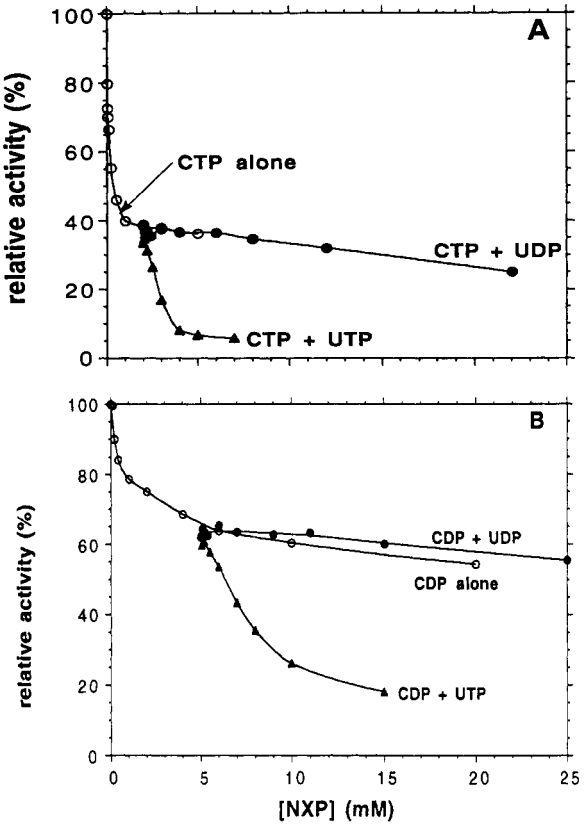

FIGURE 2: Effect of combinations of triphosphate and diphosphate nucleotides on the activity of ATCase. The enzymatic activity was assayed as described in the legend to Figure 1, in the presence of varying total nucleotide concentrations ([NXP]): (A) 2 mM CTP alone (○), 2 mM CTP + varying UTP (▲), or 2 mM CTP + varying UDP (●); (B) 5 mM CDP alone (○), 5 mM CDP + varying UTP (▲), or 5 mM CDP + varying UDP (●).

(2) Synergistic Inhibition by CDP and UTP. The influence of all possible combinations of diphosphate and triphosphate nucleotides on the activity of ATCase is shown in Figure 2. Only the UTP + CTP and UTP + CDP combinations are able to promote the synergistic phenomenon. The maximal value of the overall inhibition is always approximately 95%, but when CDP is used instead of CTP, higher concentrations of UTP must be used to attain maximal inhibition (by extrapolating the experimental curve, it appears that about 30 mM UTP is needed when the concentration of CDP is 5 mM). In contrast, UDP is totally unable to induce any synergistic phenomenon, both in the presence of CTP or CDP, showing that the γ-phosphate of UTP is essential for that process to occur.

(3) Effect of UTP on the Binding of CDP. In order to correlate the above-described regulatory properties with the

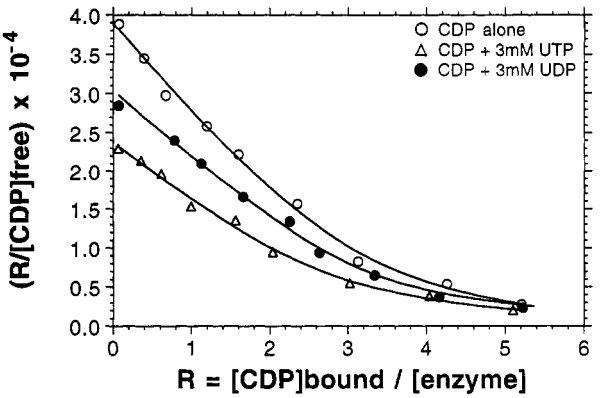

FIGURE 3: Scatchard plot of the binding of CDP to ATCase. The binding was measured at 4 °C, as described in Materials and Methods, alone (○) or in the presence of 3 mM UTP (△) or 3 mM UDP (●). The ATCase concentration used was 13 mg/mL.

ability of the different pyrimidine nucleotides to bind to the regulatory sites and promote cooperative interactions, continuous-flow dialysis experiments were performed. The study of CDP binding shows that, as in the case of CTP, two apparent classes of sites can be distinguished, whose dissociation constants differ by approximately 20-fold (Table 1). As in the case of the triphosphate nucleotide, negative cooperativity is the most likely explanation of the heterogeneous binding pattern of CDP (Tondre & Hammes, 1974; Allewell *et al.*, 1975; England & Hervé, 1992). The presence of UTP has a negative effect on the apparent affinity of CDP for the enzyme (Figure 3), in contrast with the positive effect that has been reported on that of CTP (England & Hervé, 1992). If it is assumed that only direct competition for the allosteric sites of the enzyme between the two nucleotides is responsible for the negative effect of UTP on the binding of CDP, one can calculate the values of apparent K_{d1} and K_{d2} for CDP in the presence of UTP, according to

$$\text{app } K_{dCDP(UTP)} = \text{real } K_{dCDP}(1 + [UTP]/K_{dUTP}) \quad (1)$$

where app $K_{dCDP(UTP)}$ is the calculated apparent K_d for CDP in the presence of UTP. Given that the K_d for UTP is 810 ± 200 μM in the absence of any other nucleotide, the calculated values for CDP are 390 and 7750 μM for K_{d1} and K_{d2} respectively. In reality, the measured values in the presence of UTP are 120 and 1930 μM, i.e., approximately 3 times lower than the calculated values. This difference between the calculated and measured values indicates that positive site–site interactions partially compensate for the negative effect of direct competition, in the same way as what has been described previously for the effect of UTP on the binding of CTP (England & Hervé, 1992).

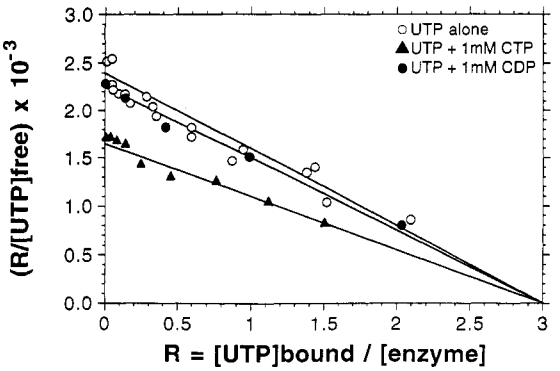

FIGURE 4: Scatchard plot of the binding of UTP to ATCase. The binding was measured at 4 °C, as described in Materials and Methods, alone (○) or in the presence of 1 mM CTP (▲) or 1mM CDP (●). The ATCase concentration used was 40 mg/mL.

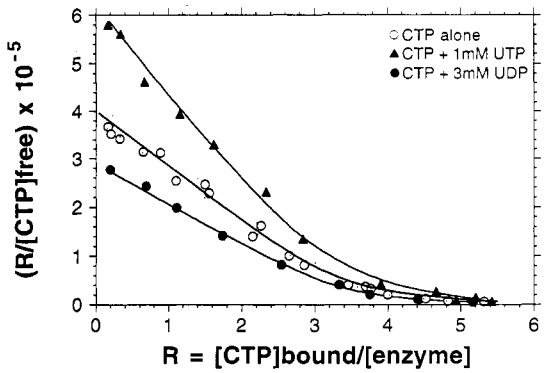

FIGURE 5: Scatchard plot of the binding of CTP to ATCase. The binding was measured at 4 °C, as described in Materials and Methods, alone (○) or in the presence of 1 mM UTP (▲) or 3 mM UDP (●). The ATCase concentration used was 2 mg/mL.

These positive site–site interactions are the basis of a positive cooperative phenomenon between the allosteric sites, which results in an increase in the real affinity of CDP. It is possible to calculate the real macroscopic dissociation constants of CDP in the presence of UTP, using the following equation:

$$\text{real } K_{\text{dCDP(UTP)}} = \frac{\text{app } K_{\text{dCDP(UTP)}}}{1 + ([\text{UTP}]/K_{\text{dUTP}})} \quad (2)$$

The real K_{d1} and K_{d2} values thus obtained are, respectively, 25.5 and 410 μM (Table 1). Therefore, the presence of saturating UTP increases the real affinity of CDP for the regulatory sites by 3–4-fold.

(4) Effect of CDP on the Binding of UTP. Continuous-flow dialysis experiments were also performed in order to investigate the influence of CDP on the binding of UTP. The result obtained is shown in Figure 4. Here again, the use of eq 1 shows that the direct competition of CDP and UTP to bind to the regulatory sites is compensated for by homotropic positive cooperative interactions. CDP provokes an increase in the real affinity for UTP. The use of eq 2 to calculate the real K_d for UTP in the presence of 1 μM CDP gives a value of 65 μM, as compared to 810 ± 200 μM for UTP alone (England & Hervé, 1992). Thus, the binding of CDP increases by about 10-fold the affinity of the regulatory sites for UTP.

(5) Effect of UDP on the Binding of CTP and CDP. The fact that UDP has no effect whatsoever on the enzymatic activity, neither when it is alone nor in the presence of CTP or CDP, raises the question of whether this diphosphate nucleotide is able to bind at all to ATCase. Direct study of the binding properties of UDP would not be an efficient way to answer this question, as the dissociation constant of this nucleotide is above the empirical upper limit of dissociation constants measurable by continuous-flow dialysis (approximately 5 mM). Indeed, the K_d of UDP can be expected to be approximately 10 mM, assuming that the ratio $K_{\text{dUDP}}/K_{\text{dUTP}}$ is approximately similar to the ratio $K_{\text{dCDP}}/K_{\text{dCTP}}$. UDP binding can be studied indirectly. In the presence of 3 mM UDP, the binding of CTP and CDP is slightly decreased (Figures 3 and 5). This indicates that UDP is able to bind competitively to the allosteric sites, although it is unable to promote any sort of inhibition of the enzymatic activity. The apparent K_d values for CTP and CDP are related to the real K_d values in the presence of UDP (real $K_{\text{dCXP(UDP)}}$) by the following equation:

$$\text{app } K_{\text{dCXP(UDP)}} = \text{real } K_{\text{dCXP(UDP)}}(1 + [\text{UDP}]/K_{\text{dUDP}}) \quad (3)$$

If UDP behaves as only a simple competitor of the binding of CTP and CDP, it should have the same effect on all of the apparent dissociation constants of these nucleotides, since the same concentration of UDP (3 mM) was used in all of the experiments. This is indeed what is observed: the measured K_d values of CTP and CDP in the presence of UDP are all approximately 30% higher than those in its absence [33% for K_{d1CTP}, 27% for K_{d2CTP}, 30% for K_{d1CDP} and 32% for K_{d2CDP}]. Hence, it appears that UDP has no positive effect on the real K_d values of CTP and CDP; it acts only as a competitor of these nucleotides for binding to the regulatory sites. Indeed, K_{dUDP} can be calculated using the following equation:

$$K_{\text{dUDP}} = \frac{[\text{UDP}]}{K_{\text{dCXP(UDP)}}/K_{\text{dCXP}} - 1} \quad (4)$$

This calculation leads to a value for K_{dUDP} of 9.83 ± 1.31 mM.

DISCUSSION

Previous work (England & Hervé, 1992) shows that the synergistic inhibitory effect of CTP and UTP on the ATCase activity is explained by cooperative interactions between the two allosteric sites within a regulatory dimer, without detectable interaction between the dimers. The binding of CTP to the first site decreases the affinity of the second site for this nucleotide, but increases the affinity of this same second site for UTP. Conversely, the binding of UTP to one of the regulatory sites decreases the affinity of the second site for this nucleotide, but increases its affinity for CTP. Thus, the regulatory dimer shows both negative and positive cooperativity between its two nucleotide binding sites, as well as competitive binding of these two nucleotides.

The results of kinetic experiments and the binding studies presented here are fully consistent, and they show that the γ-phosphate of UTP plays an important role in the mechanism of synergistic inhibition of ATCase by CTP and UTP. Indeed, if the interactions between this terminal phosphate and the regulatory sites of the enzyme are disrupted by replacing UTP with UDP, the synergistic behavior of the pyrimidine nucleotides is totally abolished. UDP is able to bind to ATCase, although with a very low affinity. However, upon binding to one of the two allosteric sites that belong to the same regulatory dimer, this nucleotide is unable to induce conformational modification of the adjacent site. Indeed, the replacement of UTP with UDP abolishes not only the positive cooperative interactions that are responsible for the UTP-dependent improvement of the real affinity of CTP (or CDP) but also the negative cooperative interactions that are responsible for

the heterogeneous pattern of binding of UTP. UDP binds to the six regulatory sites of the enzyme with the same affinity and acts as a simple competitive inhibitor toward CTP and CDP. These results are consistent with the likely hypothesis that it is the same conformational change that is responsible for both the positive and negative cooperative interactions between the regulatory sites. They also show that the amino acids that are directly in contact with the γ-phosphate of this nucleotide are involved in the pathway through which the positive and negative homotropic signals promoted by UTP binding are transmitted.

In contrast, the interactions between the regulatory sites of the enzyme and the γ-phosphate of CTP do not appear to be crucial. Indeed, the properties of CDP and CTP are qualitatively identical. Both nucleotides are able to inhibit the activity of ATCase, either alone or in synergy with UTP. They bind following a negatively cooperative pattern and induce positive site–site interactions for the binding of UTP. However, the affinity of CDP for the enzyme, as well as its inhibitory efficiency and the extent of its positive effect on UTP binding, is lower than that of CTP.

Taken together, the results obtained from the study of the properties of UDP and CDP show that the negative and positive homotropic cooperative signals, emitted upon CTP and UTP binding to the regulatory sites, are not symmetrical. It is not the same interactions between these two triphosphate nucleotides and the amino acid side chains of the regulatory sites that induce communication between the two regulatory sites of the regulatory dimer, and they act through different mechanisms involving different paths.

As far as the binding of UTP and CDP is concerned, the calculated real macroscopic dissociation constants show that CDP increases the affinity for UTP by a factor 12 and that UTP increases the affinity for CDP by a factor 3. This inequality results either from the inaccuracy of these calculations or from the high apparent dissociation constants involved. This is unlikely since each apparent dissociation constant was obtained from 6–10 consistent determinations. The values found for CTP and UTP alone are in agreement with the values previously obtained using traditional methods. It is most likely that ATCase is much too complex to be assimilated to a simple thermodynamic box on the basis of only the scheme of interactions between regulatory sites, previously presented as the simplest explanation (England & Hervé, 1992). It might be that E_{UTP}^{CDP} is slightly different from E_{CDP}^{UTP}, a hypothesis that is not unreasonable since the crystallographic structure of ATCase shows some slight asymmetry that may not be entirely explained by crystal packing (W. Lipscomb, personal communication). Not much difference is required since the discrepancy observed is about 1 kcal/mol. These considerations do not hinder the conclusion of this work that the γ-phosphate of UTP, but not that of CTP, is essential for the process of synergy to occur.

A precise understanding of these different mechanisms requires a high-definition crystallographic structure of ATCase liganded by UTP, as well as further studies by site-directed mutagenesis. The amino acid replacements that have already been performed show that lysine 56 (Corder & Wild, 1989), aspartate 19 (Zhang & Kantrowitz, 1991), and histidine 20 (Zhang & Kantrowitz, 1992) are involved in the synergistic inhibition mechanism. Histidine 20 appears to interact directly with the γ-phosphate of CTP (and probably that of UTP), while lysine 56 and aspartate 19 are involved in maintaining this residue in an optimal position (Stevens *et al.*, 1990). Interestingly, the above-mentioned studies have shown that, by replacing any of these residues with alanine, the synergistic inhibition of ATCase by CTP and UTP is totally abolished, although the activity of these modified enzymes remains sensitive to feedback inhibition by CTP (Zhang & Kantrowitz, 1991, 1992). These results indicate that the modified residues are essentially involved in the transmission of homotropic signals between the allosteric sites, rather than the transmission of the heterotropic inhibitory signal propagated from the regulatory sites to the active sites of the enzyme.

Other amino acids might be specifically involved in the transmission of homotropic positive and negative interactions between regulatory sites, for example, threonine 82 and lysine 94, which have recently been shown to interact directly with the γ-phosphate of CTP and, therefore, probably also interact with that of UTP (Kosman *et al.*, 1993). No mutant involving threonine 82 has yet been reported, but mutants in which lysine 94 has been replaced with a glutamine (Zhang *et al.*, 1988) or a histidine (Wente & Schachman, 1991) have already been constructed, although their synergistic inhibition has not yet been studied. Although it appears that the region defined by His 20, Asp 19, and Lys 56 (Zhang & Kantrowitz, 1992), and the γ -phosphate of UTP (this work) plays an important role in the mechanism of CTP/UTP synergy, additional studies are needed for an understanding of these mechanisms. In this regard, attempts are underway to obtain mutant ATCases in which allosteric inhibition would be strongly altered, but whose pyrimidine nucleotide binding properties would be identical to those of the wild-type enzyme. Studies are currently being carried out on such mutants, which involve amino acid replacements located far from the regulatory sites of the enzyme.

The synergistic inhibition of ATCase activity by CTP and UTP cannot be explained solely by cooperative binding. Indeed, an increased concentration of CTP cannot mimic this synergy, and in the absence of CTP, the binding of UTP to the regulatory sites has no influence on the affinity of the catalytic sites for aspartate (England & Hervé, 1992). Two different mechanisms could be involved in the process of synergy, apart from the cooperative effects between the regulatory sites. First, in the presence of CTP, UTP binding could promote the transmission of a regulatory signal toward the nearest catalytic site. Alternatively, the effectiveness of the regulatory signal induced by the binding of CTP could be amplified in the presence of UTP. The influence of CTP and UTP on the activity of modified forms of ATCase obtained by site-directed mutagenesis favors the first hypothesis (to be published elsewhere).

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