

Structure and Mechanisms of *Escherichia coli* Aspartate Transcarbamoylase[†]

WILLIAM N. LIPSCOMB[‡] AND EVAN R. KANTROWITZ^{*,§}

[‡]*Department of Chemistry and Chemical Biology, Harvard University, 12 Oxford Street, Cambridge, Massachusetts 02138, United States, and* [§]*Department of Chemistry, Boston College, Merkert Chemistry Center, Chestnut Hill, Massachusetts 02467-3860, United States*

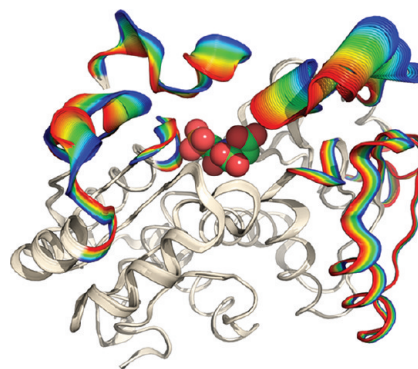
RECEIVED ON JUNE 27, 2011

CONSPECTUS

Enzymes catalyze a particular reaction in cells, but only a few control the rate of this reaction and the metabolic pathway that follows. One specific mechanism for such enzymatic control of a metabolic pathway involves molecular feedback, whereby a metabolite further down the pathway acts at a unique site on the control enzyme to alter its activity allosterically. This regulation may be positive or negative (or both), depending upon the particular system. Another method of enzymatic control involves the cooperative binding of the substrate, which allows a large change in enzyme activity to emanate from only a small change in substrate concentration. Allosteric regulation and homotropic cooperativity are often known to involve significant conformational changes in the structure of the protein.

Escherichia coli aspartate transcarbamoylase (ATCase) is the textbook example of an enzyme that regulates a metabolic pathway, namely, pyrimidine nucleotide biosynthesis, by feedback control and by the cooperative binding of the substrate, L-aspartate. The catalytic and regulatory mechanisms of this enzyme have been extensively studied. A series of X-ray crystal structures of the enzyme in the presence and absence of substrates, products, and analogues have provided details, at the molecular level, of the conformational changes that the enzyme undergoes as it shifts between its low-activity, low-affinity form (T state) to its high-activity, high-affinity form (R state). These structural data provide insights into not only how this enzyme catalyzes the reaction between L-aspartate and carbamoyl phosphate to form N-carbamoyl-L-aspartate and inorganic phosphate, but also how the allosteric effectors modulate this activity.

In this Account, we summarize studies on the structure of the enzyme and describe how these structural data provide insights into the catalytic and regulatory mechanisms of the enzyme. The ATCase-catalyzed reaction is regulated by nucleotide binding some 60 Å from the active site, inducing structural alterations that modulate catalytic activity. The delineation of the structure and function in this particular model system will help in understanding the molecular basis of cooperativity and allosteric regulation in other systems as well.



Early History

Biosynthetic control by a feedback inhibition mechanism was established in 1956 by Yates and Pardee¹ for pyrimidine nucleotides and by Umberger² for isoleucine. Specifically for pyrimidine nucleotide biosynthesis, Yates and Pardee¹ showed that the formation of N-carbamoyl-L-aspartate was feedback-inhibited by the end product of the pathway, CTP, in a specific and potent manner.

The enzyme responsible for the formation of N-carbamoyl-L-aspartate is aspartate transcarbamoylase (E.C.2.1.3.2, aspartate carbamoyltransferase, ATCase), first purified and

crystallized in 1960 by Shepherdson and Pardee.³ Although the catalytic reaction was actually deduced in 1955–1956^{4–6} (Figure 1). Shepherdson and Pardee³ estimated the molecular weight of the *Escherichia coli* enzyme to be approximately 220 000 ± 40 000 Da, based upon sedimentation velocity measurements. Heating the enzyme at 60 °C for 4 min was sufficient to render the enzyme insensitive to feedback inhibition without loss of catalytic activity.⁷ Then, in 1964, Gerhart and Pardee⁸ reported that the catalytic and regulatory sites were distinct. In a 1963 summary, Gerhart and Pardee⁹ described CTP inhibition and ATP activation

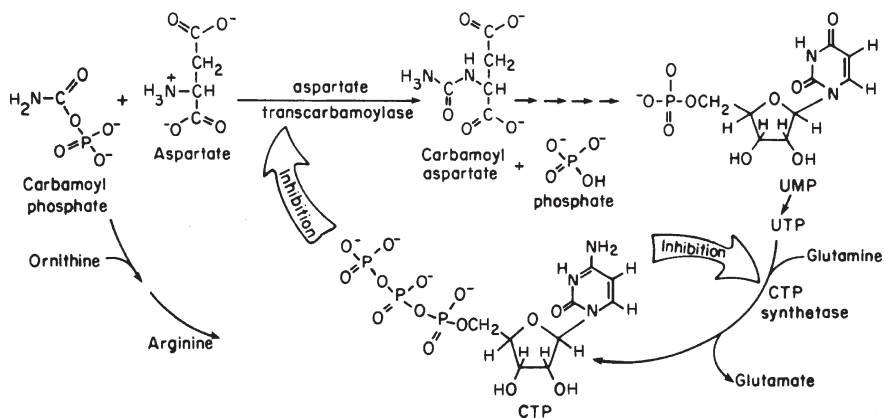


FIGURE 1. Aspartate transcarbamoylase (ATCase) catalyzes the committed step, the condensation of carbamoyl phosphate and aspartate to form carbamoyl aspartate and inorganic phosphate, in pyrimidine nucleotide biosynthesis for *E. coli*. Carbamoyl aspartate continues through the pathway leading to the formation of the nitrogenous base in pyrimidine nucleotides. ATCase is feedback inhibited by CTP and the combination of CTP plus UTP.⁴⁷

and correctly suggested that these nucleotides affected substrate affinity in an indirect manner.

A more exact molecular weight of 310 000 Da was determined in 1965.^{10,11} Gerhart and Schachman¹¹ demonstrated that the ATCase holoenzyme could be dissociated into two types of subunits, one with a molecular weight of ~96 000 Da and the other with a molecular weight of ~30 000 Da. Furthermore, the larger subunit retained catalytic activity (the catalytic subunit) while the smaller subunit exhibited no activity while it contained the nucleotide binding sites (regulatory subunit). However, these same studies¹¹ incorrectly proposed that the holoenzyme was composed of two catalytic subunits and four regulatory subunits.

The aspartate saturation curve for *E. coli* ATCase is not hyperbolic as expected for an enzyme that obeys Michaelis–Menten kinetics; rather the saturation curve is cooperative,⁷ exhibiting a pH dependent Hill coefficient.^{8,12} In 1965, the first model for the cooperative kinetic properties of ATCase was proposed by Monod, Wyman, and Changeux,¹³ which required that the enzyme be in a dynamic equilibrium between a low-activity, low-affinity T state and a high-activity, high-affinity R state. The existence of such a dynamic equilibrium was demonstrated for a mutant ATCase in 2007.¹⁴

In 1971, Collins and Stark¹⁵ synthesized a specific and potent inhibitor of ATCase, *N*-phosphonacetyl-L-aspartate (PALA). A comparison of the molecular formulas of carbamoyl phosphate (CP) plus L-aspartate (Asp) with the bisubstrate “transition state analogue” PALA is shown in Figure 2. The ability of PALA to enhance the activity of ATCase at low concentrations of aspartate, in the presence of a saturating concentration of CP, indicates that PALA is able to shift the

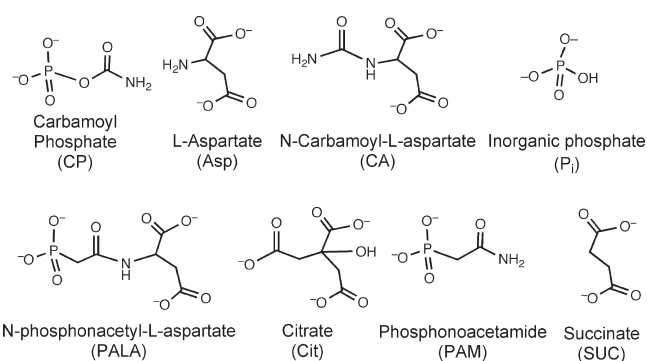


FIGURE 2. Structures of carbamoyl phosphate (CP), L-aspartate (Asp), *N*-carbamoyl-L-aspartate (CA), phosphate (P_i), the bisubstrate analogue *N*-phosphonacetyl-L-aspartate (PALA), the *N*-carbamoyl-L-aspartate analogue citrate (Cit), the CP analogue phosphonoacetamide (PAM), and the Asp analogue succinate (SUC).

equilibrium from the T to the R state, a finding that will be important for the determination of the structure of the R state of the enzyme.

The Quaternary Structure of ATCase

Structural studies in the Lipscomb Laboratory began on the *E. coli* enzyme in 1967 following a colloquium by J. C. Gerhart, during which the details of a new purification procedure for the holoenzyme as well as the catalytic and regulatory subunits¹⁶ was presented. In 1968, K. Weber determined the amino acid sequence of the regulatory subunit¹⁷ and used this information to calculate a molecular weight of ~17 000 Da. These data implied that the holoenzyme was composed of six regulatory chains. This fact was combined with the subunit molecular weights^{10,11} and crystallographic studies of two crystal forms¹⁸ to deduce the quaternary structure of ATCase. One of the crystal forms requires a

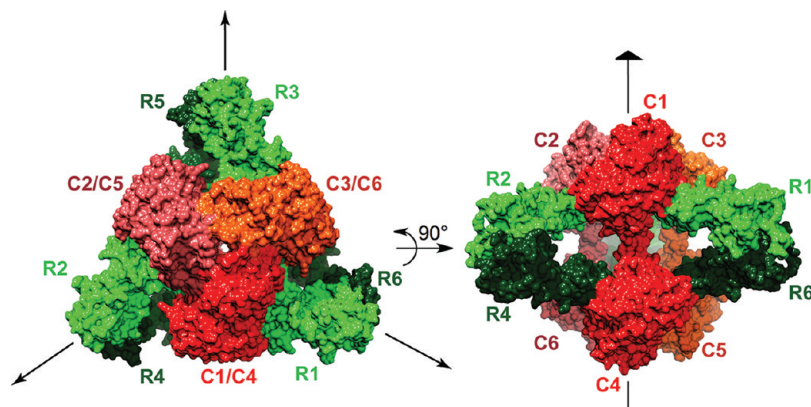


FIGURE 3. Schematic representation of the quaternary structure of ATCase. Catalytic chains C1–C2–C3 and C4–C5–C6 correspond to the two catalytic trimers, while the regulatory chains R1–R6, R2–R4, and R3–R5 correspond to the three regulatory dimers. This figure was drawn with Chimera.⁴⁸

molecular 3-fold axis, whereas the other requires at least one non-coincident 2-fold axis in the molecule, thus necessitating a c_6r_6 quaternary structure for the holoenzyme.¹⁸ The relative arrangement of the c_3 and r_2 units in c_6r_6 was found in the (idealized) R32 T-state crystal form at 5.5 Å resolution.¹⁹ The symmetry of c_6r_6 is D_3 (Figure 3, one 3-fold axis and three 2-fold axes) or the slightly lower C_3 (one 3-fold axis) in the presence of the allosteric inhibitor CTP or CTP plus UTP.²⁰

The Three-Dimensional Structure of ATCase

The resolution of the structure of the unliganded *E. coli* ATCase improved to 2.6 Å.^{21,22} Simultaneously, structural studies were pursued of the T-state enzyme in the presence of ATP or CTP,²² the R-state enzyme in the presence of PALA (Figure 2) at 2.1 Å resolution,²³ and the R-state enzyme with ATP or CTP bound,²⁴ along with additional studies of the T and R states with a variety of substrates and substrate analogues bound.

The secondary structural elements of one catalytic and one regulatory chain are shown in Figure 4. Each catalytic chain is composed of two folding domains: the Asp domain, primarily involved in the binding of aspartate, and the CP domain, primarily involved in the binding of carbamoyl phosphate. Each regulatory chain is also composed of two folding domains: the Zn domain, primarily involved in the binding of the zinc cofactor, and the AI domain, primarily involved in the binding of allosteric effectors. The Asp and CP domains of the catalytic chain, as well as the AI domain of the regulatory chain, are each classified as α/β .²⁵ The Zn domain of the regulatory chain is classified as rubredoxin-like with a metal (zinc or iron) bound that contains usually two CX(*n*)C motifs.²⁵

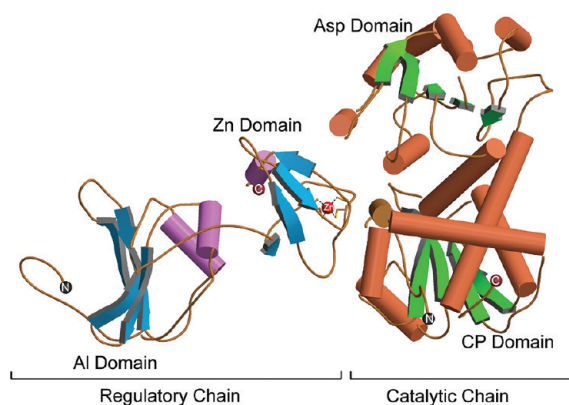


FIGURE 4. Secondary structural elements of one catalytic and one regulatory chain of *E. coli* ATCase in the T-state. The N- and C-termini of the catalytic and regulatory chains are indicated, as well as the Zn atom in the regulatory chain that is coordinated tetrahedrally to four cysteine residues. Each catalytic chain is composed of an aspartate (Asp) and a carbamoyl phosphate (CP) domain. Each regulatory chain is composed of an allosteric (AI) and a zinc (Zn) domain. This figure was drawn with MOLSCRIPT⁴⁹ using data from PDB entry 1ZA1.

The Structure of the ATCase in the T and R States

When both substrates are present at physiological concentrations, they convert ATCase from the T form to the R form (Figure 5).^{21,26,27} The same change in quaternary structure can also be induced by the binding of PALA. The changes in the molecular structure between the T and R states include an elongation of 11 Å along the molecular 3-fold axis. In this transformation, there is a rotation of one catalytic trimer c_3 relative to the other c_3 by 12° and a rotation of each of the three regulatory dimers about the approximate 2-fold axes by 15°. Thus, the molecular expansion occurs only along the 3-fold axis, with no elongation along the three molecular 2-fold directions. The positions of the domains of the

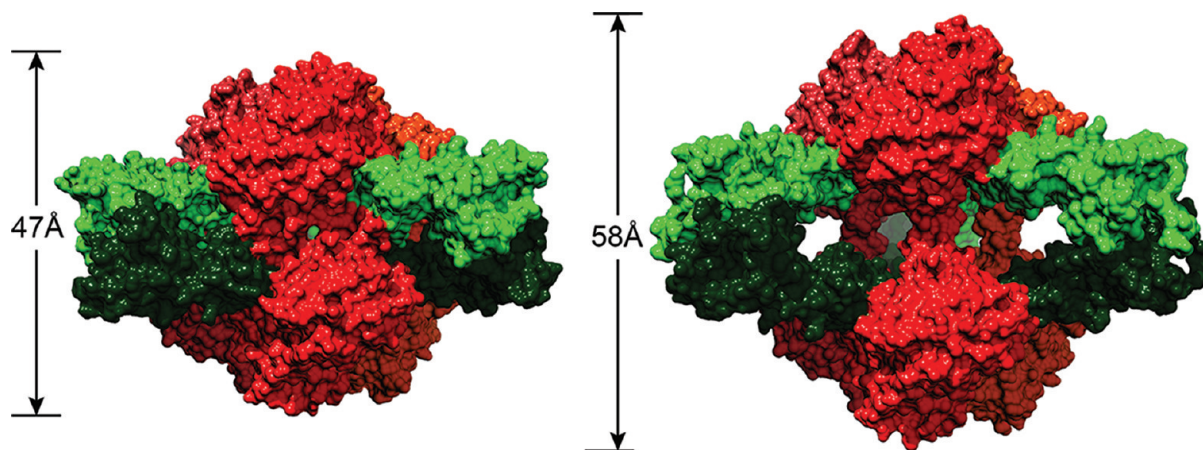


FIGURE 5. ATCase holoenzyme in the T (left) and R (right) structures. The catalytic chains are shown in shades of red, and the regulatory chains are shown in shades of green. The length of the molecule along the 3-fold axis is shorter in the T form than in the R form. This figure was drawn with Chimera.⁴⁸

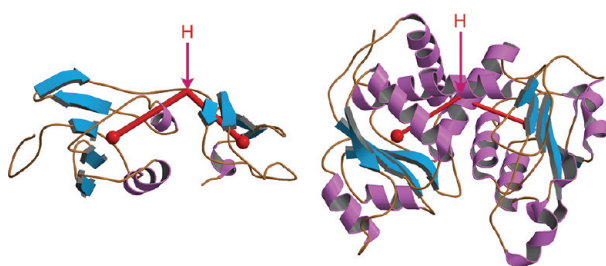


FIGURE 6. Secondary structure of a regulatory chain (left) and a catalytic chain (right) of ATCase. The angle between the domains is measured from the center of mass of each domain (red dot) and a hinge point (H).

catalytic and regulatory chains undergo structural alterations relative to each other. The two domains of the catalytic chain close by 6.8° , while the two domains of the regulatory chain open by 1.7° between the T and R states (Figure 6).

The interactions between the R-state enzyme and PALA are shown in Figure 7. Ser80 and Lys84 from an adjacent catalytic chain interact with PALA,²³ and thus each active site is composed of residues from two adjacent chains. Site-specific mutagenesis²⁸ and hybrid experiments²⁹ confirm that the residues from the adjacent catalytic chain are required for catalytic activity.

The R-state structure with bound CP and succinate, an analogue of the substrate Asp, is shown in Figure 8.³⁰ The interactions between the enzyme and the natural substrate CP are virtually identical to those between the enzyme and the phosphonate portion of PALA. However, the amide of CP interacts with the side chain of Gln137, and the oxygen between the phosphorus and the carbonyl carbon interacts with Arg54. Using molecular modeling, Asp was docked into the enzyme·CP complex. The lowest energy pose has the amino group of Asp within 2.8 \AA of the carbonyl carbon of CP, positioned correctly for the nucleophilic attack, which

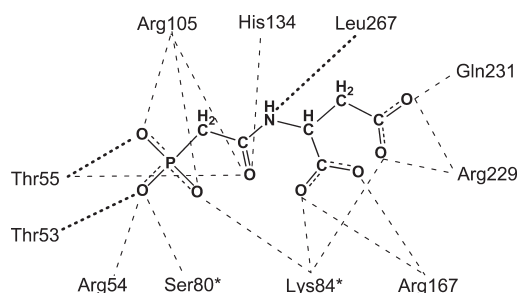


FIGURE 7. Interactions between PALA and ATCase in the R-state.²⁷ Dotted lines correspond to interactions with the backbone of the protein. An asterisk after the residue number indicates that it is donated into the active site from the adjacent chain.

would lead to the formation of the proposed tetrahedral intermediate.³¹

Structural studies of the enzyme·PALA, enzyme·CP·succinate, and enzyme·P_i·citrate complexes were used to model the tetrahedral intermediate and the transition state of the reaction.³² The model of the tetrahedral intermediate bound in the active site of ATCase shows three additional interactions than are not observed between the enzyme and PALA (Figure 9). In addition, the model of the tetrahedral intermediate shows significantly more interactions than the summation of interactions between the enzyme and the various combinations of substrates and substrate analogues. Based on the tetrahedral intermediate model, the enzyme binds the tetrahedral intermediate and thus the transition state, significantly more strongly than the substrates or products.

In order to approximate and thereby observe the conformation of the R-state enzyme active site in the presence of products, the structure of the enzyme in the presence of citrate, an analogue of *N*-carbamoyl-L-aspartate (Figure 2) plus the product phosphate was determined after displacement

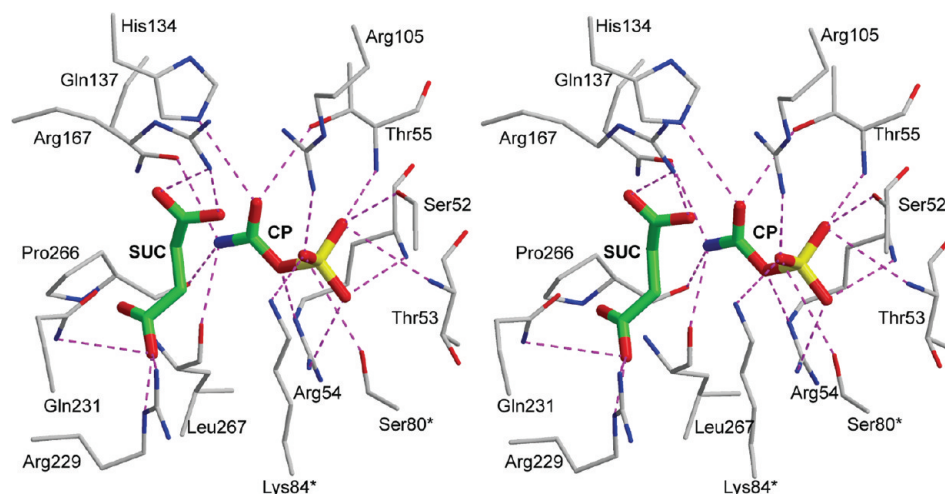


FIGURE 8. Stereoview of succinate (SUC) and CP bound in the active site of ATCase in the R state. An asterisk after the residue number indicates that it is donated into the active site from the adjacent chain. This figure was drawn with MOLSCRIPT.⁴⁹

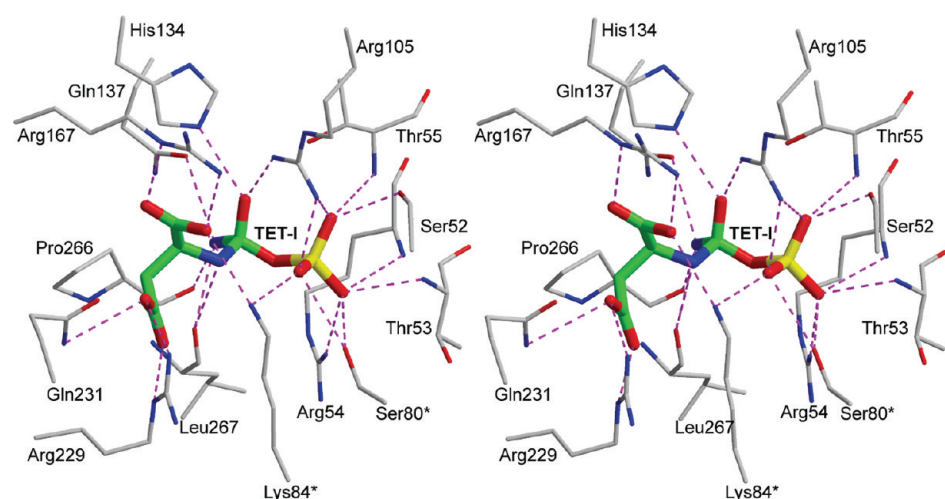


FIGURE 9. Stereoview of the proposed tetrahedral intermediate (TET-I) bound in the active site of ATCase in the R state. An asterisk after the residue number indicates that it is donated into the active site from the adjacent chain. This figure was drawn with MOLSCRIPT.⁴⁹

of PALA from R-state crystals.³³ The carboxylate groups of citrate interact with His134, Arg167, Gln231, and Arg229 (Figure 10). Lys84, from the adjacent chain, interacts with both citrate and phosphate acting as a bridge between the two molecules. A phosphate oxygen is 3 Å away from the carbon in citrate that would correspond to the α -carbon in carbamoyl aspartate. These results imply that this complex represents a reasonable model for the products bound in the active site.³³

After the collapse of the oxyanion formed in the transition state, the products carbamoyl aspartate and phosphate become separate as seen in the model structure with citrate and phosphate bound (Figure 10). The weaker-binding carbamoyl aspartate departs the active site releasing Lys84, which may coincide with the opening of the 240s loop. The opening of the 240s loop is necessary to provide a path for the

release of carbamoyl aspartate followed by phosphate from the R-state active site, as the active site in the presence of the tetrahedral intermediate shows no opening to the solvent. After phosphate is released, the catalytic cycle can begin again with the binding of CP. Time-resolved X-ray scattering studies have shown that as long as substrates are available, the enzyme remains in the R state and only returns to the T state after the substrates are exhausted.³⁴

The Active Site and Mechanistic Aspects

The catalytic mechanism is ordered: CP binds before aspartate, and carbamoyl aspartate leaves before phosphate.³⁵ When CP,³¹ or CP analogues such as PAM (Figure 2), binds,³⁶ the enzyme remains in the T state and only tertiary-level conformational changes occur. The largest conformational change that occurs upon the binding of CP involves a

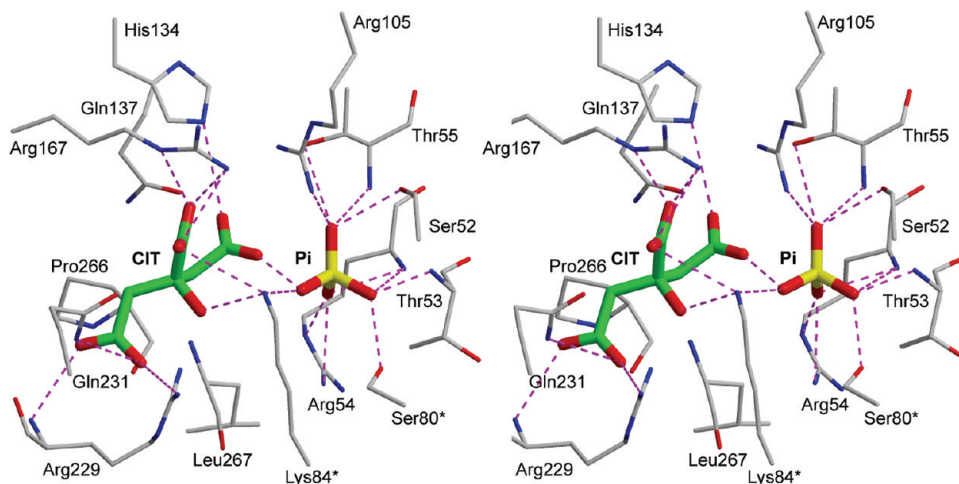


FIGURE 10. Stereoview of citrate (CIT) and phosphate (Pi) bound in the active site of ATCase in the R state. An asterisk after the residue number indicates that it is donated into the active site from the adjacent chain. This figure was drawn with MOLSCRIPT.⁴⁹

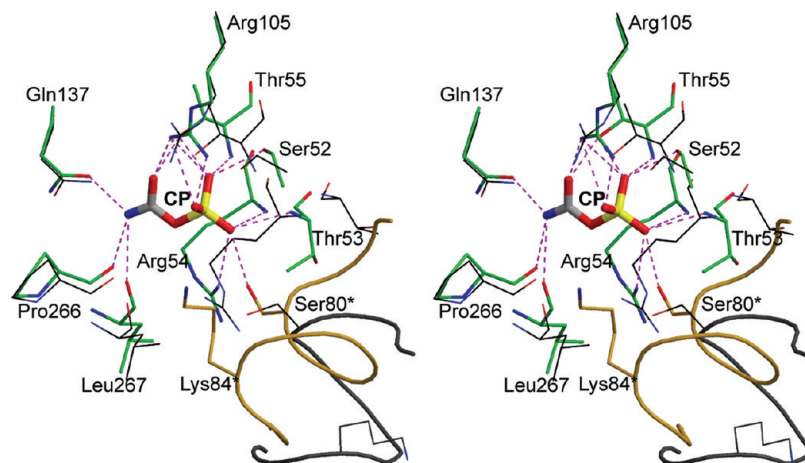


FIGURE 11. Stereoview comparison of the active site of *E. coli* ATCase in the presence of CP (green carbons, thick) and in the absence of CP (black carbons, thin). The 80s loop from the adjacent chain (residue numbers with asterisks) in the presence of CP (gold carbons, thick) and in the absence of CP (gray carbons, thin) is represented as a coil with Ser80* and Lys84* shown. This figure was drawn with MOLSCRIPT.⁴⁹

reorganization of the 80s loop that positions Ser80 and Lys84 into the active site. (Figure 11). In addition, there are some smaller conformational changes involving motions of the 50s and 240s loops. The conformational changes induced by the binding of CP create a binding site for aspartate, not only structurally but electrostatically as well (Figure 12). These conformational changes dramatically alter the electrostatic properties of the active site; they essentially create a positively charged binding pocket for aspartate, the studies of which have provided a mechanism to account for the ordered binding of the substrates.³¹

The three additional interactions observed in the model of the tetrahedral intermediate bound to the enzyme (Figure 9) compared to the structure of the enzyme·PALA

complex involve interactions between the side chain of Gln137 and the backbone carbonyl oxygen of Pro266 to the amino group on the tetrahedral carbon and the side chain of Arg54 with the ester oxygen between the phosphorus and the tetrahedral carbon. When either Gln137 or Arg54 is replaced by Ala, the consequences are extraordinary.³⁷ For the Q137A enzyme, the concentration of CP required to attain one-half of the maximal activity increases by 210-fold. Although Gln137 is not involved directly in binding aspartate, the corresponding value for aspartate increases by 76-fold. The extremely reduced affinity for CP of the Q137A enzyme along with the near abolition of aspartate binding points to the critical role that CP plays in preparing the active site for the binding of Asp. The R54A mutation results in a 17 000-fold loss in activity

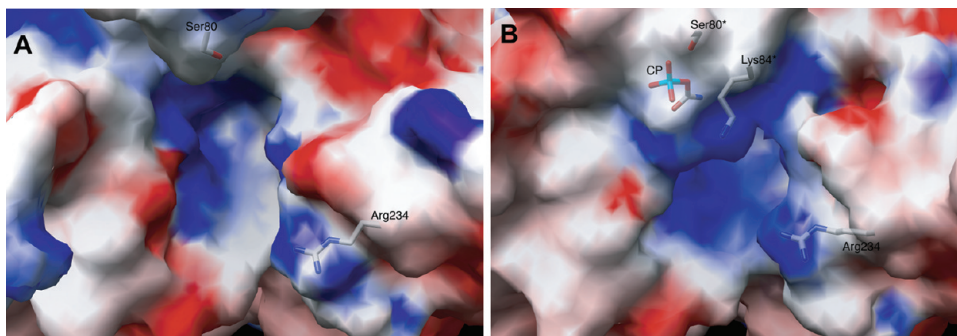


FIGURE 12. (A) Electrostatic potentials mapped onto the surface of ATCase in the absence (A) and the presence (B) of CP. The positions of Ser80*, Lys84*, and Arg234 and CP are overlaid onto the electrostatic map (white carbons, sticks). Reprinted with permission from ref 31. Copyright 2005 National Academy of Sciences.

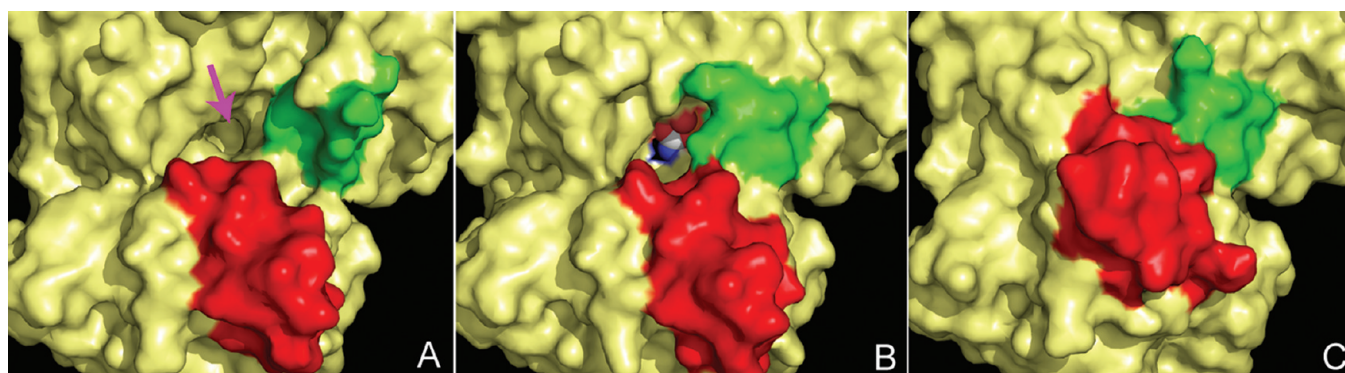


FIGURE 13. Surface of ATCase near the active site detailing the 80s loop (green) and the 240s loop (red) during catalysis. (A) Conformation of the enzyme before substrates are bound; the approximate position of the active site is indicated by the magenta arrow. (B) Conformation of the enzyme after the binding of CP, which is shown in the active site as a CPK model (white carbon). The 80s loop (green) rearranges to help create the binding site for aspartate. (C) Upon the binding of aspartate, the 240s loop (red) undergoes a dramatic conformational change, forcing the substrates toward each other. Solvent is not accessible to the active site in this conformation.

without major influence on the substrate affinity,³⁷ suggesting a role of this residue in transition state stabilization.

The binding of aspartate induces both tertiary and quaternary conformational changes. At the tertiary level, the 240s loop rearranges to position the side chains necessary for catalysis in their high-activity, high-affinity positions (Figure 13). However, due to steric constraints, the movement of the 240s loop cannot occur without the occurrence of a quaternary conformational change (see Figure 5). The repositioning of the 240s loop and the closure of the two domains of each catalytic chain provide an environment favorable for catalysis. The attack of the amino nitrogen of aspartate on the amide carbon of CP, which forms the tetrahedral intermediate, is enhanced by the binding of Arg105, His134, and Thr55³⁷ to the carbonyl oxygen. The carbonyl oxygen of carbamoyl phosphate, as indicated by the schematic of PALA binding (see Figure 7), allows these three residues to influence

substantially the affinity of the enzyme·CP complex for aspartate.

In order for the reaction to occur, the amino group of aspartate must lose two protons starting from $-\text{NH}_3^+$. Because of the electropositive field microenvironment encountered by the $-\text{NH}_3^+$ group, the first proton is lost as aspartate binds. One possibility for the fate of the second proton is that it is captured by the leaving phosphate.³² Another possibility for loss of the second proton involves transfer to either Arg105 or Lys84, the latter being contributed into the active site from the adjacent catalytic chain. It would be expected, based upon the electropositive environment of the active site, that the guanidinium group of Arg105 or the ϵ -amino group of Lys84 would initially be deprotonated, allowing either one to accept a proton. The roles of Arg105 and Lys84 in the function of the enzyme have been probed via mutagenesis. The R105A and K84N mutations result in a 1100-fold³⁷ and 1200-fold²³ reduction in activity, respectively.

In models of the tetrahedral intermediate bound in the active site,^{23,32} Arg54 is bound to the bridging phosphate oxygen, thus partially reducing the negative charge on the phosphate and serving to make the phosphate a better leaving group. In support of the important role of Arg54, when Arg54 is replaced by Ala enzymatic activity is reduced by a factor of about 17 000-fold.^{37,38}

Cooperativity Induced by Aspartate Binding

As indicated above, the binding of CP induces small but significant tertiary conformational changes which not only creates the binding site for aspartate but also weakens those interactions that stabilize the enzyme in the T state.³¹ The binding of Asp to the enzyme·CP complex, as well, changes the electrostatics of the active site making it more electro-positive. The binding of aspartate induces additional major loop motions, particularly of the 80s and 240s loops. These conformational changes have two consequences: first, they force the substrates closer together, thereby lowering the activation energy of the reaction, and second they further weaken the intersubunit interactions that specifically stabilize the T-state structure. The weakening of these interactions triggers the initiation of the global quaternary conformational change. In fact, the 240s loops cannot attain their final domain-closed conformation without an expansion of the enzyme along the 3-fold axis, which allows the 240s loops from the upper and lower catalytic subunits to slide past each other.³¹ Thus, the binding of Asp to one or so of the active sites is sufficient to convert the enzyme from the T to the R state.³⁹

Heterotropic Effects and Structural Aspects

The allosteric site of *E. coli* ATCase is located in the AI domain of the regulatory chain near the N-terminus and the five-stranded β -sheet (Figure 4). The localization of the N-terminal region of the regulatory chain (residues 1–7) was first modeled in the CTP-bound complex⁴⁰ near the allosteric site.³⁸ Mutagenesis studies⁴¹ support the suggestion that this is the regulatory region of the enzyme. Increased resolution of the X-ray data, 2.1 Å²³ versus 2.5 Å,⁴⁰ yielded electron density for the independent r1 and r6 chains and allowed tracing of the first seven residues and remodeling residues 8r-10r. In this new model, the nucleotide site is somewhat more open than that of a mutated T-state structure.⁴² In addition, the trace of residues 1–7 of the regulatory chain shows some inherent asymmetry between the r1 and r6 chains. The structural asymmetry may contribute to the high- and low-affinity binding constants for the nucleotides as determined by equilibrium dialysis⁴³ and fluorescence studies.⁴⁴ In

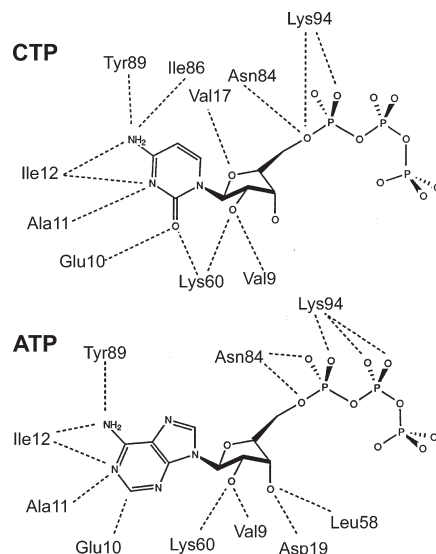


FIGURE 14. Comparison of the interactions between CTP (top) and ATP (bottom) and the allosteric site on the regulatory chains of *E. coli* ATCase. This figure is based on the ATCase·CTP and ATCase·ATP structures in the T state.²²

summary, the N termini of the regulatory chains are involved in both nucleotide binding and heterotropic effects.^{23,45}

The mode by which CTP and ATP bind to the regulatory site⁴⁵ of the enzyme in the T state is shown in Figure 14. This site is about 60 Å away from the active site. Although ATP and CTP bind at this regulatory site in both the T state or R state, there are differences: for example, CTP binds in a more compact form in which its ribose ring is about 1.5 Å deeper in the cleft in the T and R state of the r1 chain and 0.9 Å deeper in the T state of the r6 chain than the ribose ring of ATP.⁴⁵

As described above, the domain closure between the CP and the Asp domains of the catalytic chain is caused when aspartate binds to the enzyme·CP complex. A comparison of the active site in the T and R state reveals the change in the position of Arg229 contributes to the affinity of the R state for aspartate compared with the lower affinity for aspartate of the T state. However, the T to R transition is not required for movement of Arg229 into the active site. When unliganded ATCase binds ATP, the guanidinium group of Arg229 moves toward the vacant aspartate cavity, and when CTP binds to the unliganded enzyme, Arg229 moves away from the aspartate cavity. These changes in the T-state structures show that the effects of ATP or CTP binding do not require the quaternary conformational transition. In addition, the binding of CP induces a movement of the side chain of Arg229 toward the aspartate cavity, thus preparing the aspartate region for increased aspartate affinity. The change in the position of Arg229, influenced by the binding of either

ATP or CTP some 60 Å away, occurs in the C4 chain of the lower trimer, but not in the corresponding C1 chain of the upper trimer. This observation serves as a clear example of the effects of molecular asymmetry in the enzyme mechanism.

Conclusion

The regulation of the rates of metabolic pathways by allosteric enzymes is an extremely important mechanism for cellular control. Allosteric enzymes, such as ATCase, play a pivotal role in metabolism because they have three functions: they catalyze a unique metabolic reaction, alter the rate of catalysis in response to cellular conditions, and are responsible for the rate of the larger pathway. Regulation of ATCase involves the binding of signaling molecules to the regulatory sites, and this binding induces an alteration in the rate of catalytic activity. Our studies of ATCase have been directed toward using the three-dimensional structural information to understand, on the molecular level, how ATCase is able to catalyze the reaction between aspartate and carbamoyl phosphate, how the enzyme is able to transition between the T and R conformations, and how the binding of regulatory nucleotides a distance of 60 Å from the active site induces alterations to modulate activity. This understanding of the structure and function in this model system will help us understand the molecular basis of cooperativity and allosteric regulation in other systems as well. In 2002, D. Koshland⁴⁶ emphasized the importance of these types of studies when he said, “this type of cooperativity, which has since been shown in many enzymes, receptors, and ion channels, is of critical importance to both evolution and the field of proteomics because it can serve as a general model for the way in which the networks of interacting enzymes of metabolic pathways are regulated.”

The work on aspartate transcarbamoylase was funded by grants GM26237 (E.R.K.) and GM06920 (W.N.L.) from the National Institutes of Health.

ABBREVIATIONS USED

ATCase, aspartate transcarbamoylase (EC 2.1.3.2, aspartate carbamoyltransferase); c, the catalytic chain of aspartate transcarbamoylase; r, the regulatory chain of aspartate transcarbamoylase; c₃, the catalytic subunit of aspartate transcarbamoylase composed of three catalytic chains; r₂, the regulatory subunit of aspartate transcarbamoylase composed of two regulatory chains; CP, carbamoyl phosphate; Asp, L-aspartate; PALA, N-phosphonacetyl-L-aspartate; PAM, phosphonoacetamide; 80s loop, a loop in the catalytic chain of aspartate

transcarbamoylase comprised of residues 73–88; 240s loop, a loop in the catalytic chain of aspartate transcarbamoylase comprised of residues 230–245;

BIOGRAPHICAL INFORMATION

Evan R. Kantrowitz is Professor of Chemistry at Boston College. His research area focuses on the relationship between the structure and function of allosteric enzymes.

William N. Lipscomb was Emeritus Professor of Chemistry at Harvard University. Professor Lipscomb, or “The Colonel” to those that knew him, investigated a broad array of fields in chemistry, including the inorganic chemistry of boron, crystallography of both small molecules and proteins, the allosteric behavior of enzymes, along with numerous studies in theoretical chemistry. The Colonel was awarded the Chemistry Nobel Prize in 1976 for his work on the structure of boranes that illuminated new aspects of chemical bonding. Before his death on April 15, 2011, at the age of 91, he had finished work on this manuscript.

FOOTNOTES

*To whom correspondence should be addressed. E-mail: evan.kantrowitz@bc.edu.

[†]This paper is dedicated to all our current and former students who worked with us to determine the structures of ATCase and to deduce the mechanisms of catalysis and regulation based on these three-dimensional structures.

REFERENCES

- 1 Yates, R. A.; Pardee, A. B. Control of pyrimidine biosynthesis in *Escherichia coli*. *J. Biol. Chem.* **1956**, *221*, 757–770.
- 2 Umbarger, H. E. Evidence for a Negative-Feedback Mechanism in the Biosynthesis of Isoleucine. *Science* **1956**, *123*, 848–848.
- 3 Shepherdson, M.; Pardee, A. B. Production and Crystallization of Aspartate Transcarbamoylase. *J. Biol. Chem.* **1960**, *235*, 3233–3237.
- 4 Jones, M. E.; Spector, L.; Lipmann, F. Carbamyl phosphate. The carbamyl donor in enzymatic citrulline synthesis. *J. Am. Chem. Soc.* **1955**, *77*, 819–820.
- 5 Lowenstein, J. M.; Cohen, P. P. Studies on the Biosynthesis of carbamylaspartic acid. *J. Biol. Chem.* **1956**, *235*, 57–78.
- 6 Reichard, P.; Hanshoff, G. Aspartate carbamyl transferase from *Escherichia coli*. *Acta Chem. Scand.* **1956**, *10*, 548–560.
- 7 Gerhart, J. C.; Pardee, A. B. Enzymology of control by feedback inhibition. *J. Biol. Chem.* **1962**, *237*, 891–896.
- 8 Gerhart, J. C.; Pardee, A. B. Aspartate transcarbamoylase, an enzyme designed for feedback inhibition. *Fed. Proc.* **1964**, *23*, 727–735.
- 9 Gerhart, J. C.; Pardee, A. B. The effect of the feedback inhibitor CTP, on subunit interactions in aspartate transcarbamoylase. *Cold Spring Harbor Symp. Quant. Biol.* **1963**, *28*, 491–496.
- 10 Gerhart, J. C. Subunits for control and catalysis in aspartate transcarbamoylase. *Brookhaven Symp. Biol.* **1964**, 222–231.
- 11 Gerhart, J. C.; Schachman, H. K. Distinct subunits for the regulation and catalytic activity of aspartate transcarbamoylase. *Biochemistry* **1965**, *4*, 1054–1062.
- 12 Pastra-Landis, S. C.; Evans, D. R.; Lipscomb, W. N. The effect of pH on the cooperative behavior of aspartate transcarbamoylase from *Escherichia coli*. *J. Biol. Chem.* **1978**, *253*, 4624–4630.
- 13 Monod, J.; Wyman, J.; Changeux, J. P. On the Nature of Allosteric Transitions: A Plausible Model. *J. Mol. Biol.* **1965**, *12*, 88–118.
- 14 Fetler, L.; Kantrowitz, E. R.; Vachette, P. Direct observation in solution of pre-existing structure equilibrium for a mutant of allosteric aspartate transcarbamoylase. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 495–500.
- 15 Collins, K. D.; Stark, G. R. Aspartate transcarbamoylase: Interaction with the transition state analogue N-(phosphonacetyl)-L-aspartate. *J. Biol. Chem.* **1971**, *246*, 6599–6605.
- 16 Gerhart, J. C.; Holoubek, H. The purification of aspartate transcarbamoylase of *Escherichia coli* and separation of its protein subunits. *J. Biol. Chem.* **1967**, *242*, 2886–2892.
- 17 Weber, K. K. New Structural Model of *E. coli* Aspartate Transcarbamoylase and the Amino-acid Sequence of the Regulatory Polypeptide Chain. *Nature* **1968**, *218*, 1116–1119.
- 18 Wiley, D. C.; Lipscomb, W. N. Crystallographic Determination of Symmetry of Aspartate Transcarbamoylase. *Nature* **1968**, *218*, 1119–1121.

- 19 Wiley, D. C.; Evans, D. R.; Warren, S. G.; McMurray, C. H.; Edwards, B. F.; Franks, W. A.; Lipscomb, W. N. The 5.5 Å resolution structure of the regulatory enzyme, aspartate transcarbamoylase. *Cold Spring Harbor Symp. Quant. Biol.* **1972**, *36*, 285–90.
- 20 Wild, J. R.; Loughrey-Chen, S. J.; Corder, T. S. In the presence of CTP, UTP becomes an allosteric inhibitor of aspartate transcarbamoylase. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 46–50.
- 21 Ke, H.-M.; Honzatko, R. B.; Lipscomb, W. N. Structure of unligated aspartate carbamoyltransferase of *Escherichia coli* at 2.6-Å resolution. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 4027–4040.
- 22 Stevens, R. C.; Gouaux, J. E.; Lipscomb, W. N. Structural consequences of effector binding to the T state of aspartate carbamoyltransferase: Crystal structures of the unligated and ATP- and CTP-complexed enzymes at 2.6 Å Resolution. *Biochemistry* **1990**, *29*, 7691–7701.
- 23 Jin, L.; Stec, B.; Lipscomb, W. N.; Kantrowitz, E. R. Insights into the mechanism of catalysis and heterotropic regulation of *E. coli* aspartate transcarbamoylase based upon a structure of enzyme complexed with the bisubstrate analog N-phosphonacetyl-L-aspartate at 2.1 Å. *Proteins: Struct., Funct., Genet.* **1999**, *37*, 729–742.
- 24 Gouaux, J. E.; Stevens, R. C.; Lipscomb, W. N. Crystal structures of aspartate carbamoyltransferase ligated with phosphonoacetamide, malonate and CTP or ATP at 2.8 Å resolution and neutral pH. *Biochemistry* **1990**, *29*, 7702–7715.
- 25 Murzin, A. G.; Brenner, S. E.; Hubbard, T.; Chothia, C. SCOP: a structural classification of proteins database for the investigation of sequences and structures. *J. Mol. Biol.* **1995**, *247*, 536–40.
- 26 Ke, H.-M.; Lipscomb, W. N.; Cho, Y.; Honzatko, R. B. Complex of N-phosphonacetyl-L-aspartate with aspartate carbamoyltransferase: X-ray refinement, analysis of conformational changes and catalytic and allosteric mechanisms. *J. Mol. Biol.* **1988**, *204*, 725–747.
- 27 Krause, K. L.; Voltz, K. W.; Lipscomb, W. N. 2.5 Å structure of aspartate carbamoyltransferase complexed with the bisubstrate analog N-(phosphonacetyl)-L-aspartate. *J. Mol. Biol.* **1987**, *193*, 527–553.
- 28 Macol, C.; Dutta, M.; Stec, B.; Kantrowitz, E. R. The 80s loop of the catalytic chain of *Escherichia coli* aspartate transcarbamoylase is critical for catalysis and homotropic cooperativity. *Protein Sci.* **1999**, *8*, 1305–1313.
- 29 Robey, E. A.; Schachman, H. K. Regeneration of active enzyme by formation of hybrids from inactive derivatives: implications for active sites shared between polypeptide chains of aspartate transcarbamoylase. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 361–365.
- 30 Gouaux, J. E.; Lipscomb, W. N. Three-dimensional structure of carbamyl phosphate and succinate bound to aspartate carbamoyltransferase. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 4205–4208.
- 31 Wang, J.; Stieglitz, K. A.; Cardia, J. P.; Kantrowitz, E. R. Structural basis for ordered substrate binding and cooperativity in aspartate transcarbamoylase. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 8881–8886.
- 32 Gouaux, J. E.; Krause, K. L.; Lipscomb, W. N. The catalytic mechanism of *Escherichia coli* aspartate carbamoyltransferase: A molecular modeling study. *Biochem. Biophys. Res. Commun.* **1987**, *142*, 893–897.
- 33 Gouaux, J. E.; Lipscomb, W. N. Unpublished study, which can be found in Chapter 5 of the Ph.D. Thesis: Gouaux, J. E. Ph.D. Thesis, Harvard University, Department of Chemistry, 12 Oxford Street, Cambridge, MA 02138, 1989.
- 34 Tsuruta, H.; Vachette, P.; Sano, T.; Moody, M. F.; Tauc, P.; Amemiya, Y.; Wakabayashi, K.; Kihara, H. Kinetics of the quaternary structure change of aspartate transcarbamoylase triggered by succinate, a competitive inhibitor. *Biochemistry* **1994**, *33*, 10007–10012.
- 35 Wedler, F. C. Mechanisms of substrate binding with glutamine synthetase: Equilibrium isotope exchanges with the ovine brain, pea seed, and *Escherichia coli* enzymes. *J. Biol. Chem.* **1974**, *249*, 5080–5087.
- 36 Gouaux, J. E.; Lipscomb, W. N. Crystal structures of phosphonoacetamide ligated T and phosphonoacetamide and malonate ligated R states of aspartate carbamoyltransferase at 2.8 Å resolution and neutral pH. *Biochemistry* **1990**, *29*, 389–402.
- 37 Stebbins, J. W.; Xu, W.; Kantrowitz, E. R. Three residues involved in binding and catalysis in the carbamyl phosphate binding site of *Escherichia coli* aspartate transcarbamoylase. *Biochemistry* **1989**, *28*, 2592–2600.
- 38 Stebbins, J. W.; Robertson, D. E.; Roberts, M. F.; Stevens, R. C.; Lipscomb, W. N.; Kantrowitz, E. R. Arginine 54 in the active site of *Escherichia coli* aspartate transcarbamoylase is critical for catalysis: A site-specific mutagenesis, NMR and X-ray crystallographic study. *Protein Sci.* **1992**, *1*, 1435–1446.
- 39 Macol, C. P.; Tsuruta, H.; Stec, B.; Kantrowitz, E. R. Direct structural evidence for a concerted allosteric transition in *Escherichia coli* aspartate transcarbamoylase. *Nat. Struct. Biol.* **2001**, *8*, 423–426.
- 40 Kosman, R. P.; Gouaux, J. E.; Lipscomb, W. N. Crystal structure of CTP-ligated T state aspartate transcarbamoylase at 2.5 Å resolution: Implications for aspartate transcarbamoylase mutants and the mechanism of negative cooperativity. *Proteins: Struct., Funct., Genet.* **1993**, *15*, 147–176.
- 41 Dembowski, N. J.; Kantrowitz, E. R. The use of alanine scanning mutagenesis to determine the role of the amino terminus of the regulatory chain in the heterotropic mechanism of *Escherichia coli* aspartate transcarbamoylase. *Protein Eng.* **1994**, *7*, 673–679.
- 42 Sakash, J. B.; Kantrowitz, E. R. The N-terminus of the regulatory chain of *Escherichia coli* aspartate transcarbamoylase is important for nucleotide binding and heterotropic effects. *Biochemistry* **1998**, *37*, 281–288.
- 43 Winlund, C. C.; Chamberlin, M. J. Binding of cytidine triphosphate to aspartate transcarbamoylase. *Biochem. Biophys. Res. Commun.* **1970**, *40*, 43–49.
- 44 Mendes, K. R.; Martinez, J. A.; Kantrowitz, E. R. Asymmetric allosteric signaling in aspartate transcarbamoylase. *ACS Chem. Biol.* **2010**, *5*, 499–506.
- 45 Lipscomb, W. N. Aspartate Transcarbamoylase from *Escherichia coli*: Activity and Regulation. *Adv. Enzymol.* **1994**, *68*, 67–151.
- 46 Koshland, D. E.; Hamadani, K. Proteomics and models for enzyme cooperativity. *J. Biol. Chem.* **2002**, *277*, 46841–46844.
- 47 Reprinted from *Trends in Biochemical Science*, Vol. 5, E. R. Kantrowitz & W. N. Lipscomb, "E. coli aspartate transcarbamoylase: Part I: Catalytic and regulatory functions", 125, 1980, with permission of Elsevier.
- 48 Pettersen, E. F.; Goddard, T. D.; Huang, C. C.; Couch, G. S.; Greenblatt, D. M.; Meng, E. C.; Ferrin, T. E. UCSF Chimera - A visualization system for exploratory research and analysis. *J. Comput. Chem.* **2004**, *25*, 1605–1612.
- 49 Kraulis, P. J. MOLSCRIPT: A Program to Produce Both Detailed and Schematic Plots of Protein Structures. *J. Appl. Crystallogr.* **1991**, *24*, 946–950.