Molecular Recognition by Ornithine and Aspartate Transcarbamylases

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The ability of molecules to recognize and interact selectively with other molecules underlies a wide variety of important research areas in both chemistry and biochemistry. Examples include chemical and biochemical catalysis, rational drug design, the development of sensors for environmental pollutants and clinical tests, taste and smell, and insect and weed control. Among biological molecules, proteins have evolved to have the greatest versatility and selectivity in molecular recognition (although RNA may prove a close second), and much of what we understand about the principles that underlie biological recognition has come from studying proteins. The ability of proteins to recognize and discriminate among molecules, both large and small, depends on their complex chemistry and surface properties and their ability to undergo changes in their three-dimensional structure as a result of changes in their environment or molecular interactions that alter both their chemistry and their surface properties. Since the underlying principles are similar for most proteins, these principles can be illustrated with almost any protein or protein family. Here we focus on the transcarbamylases, a family of enzymes that catalyze transfer of the carbamoyl group of carbamoyl phosphate (CP) to an amino group.

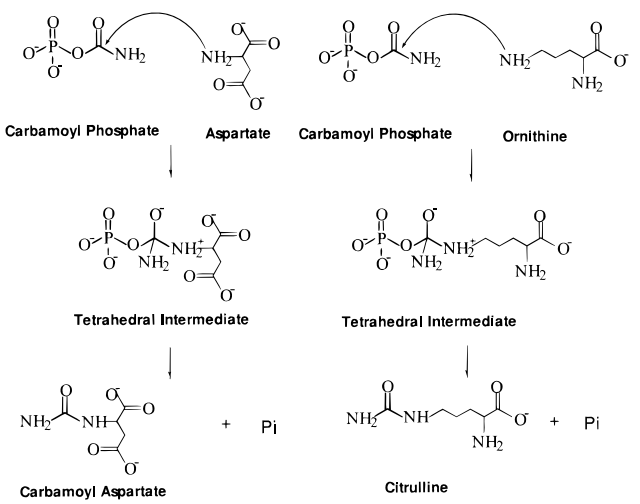

FIGURE 1. Schematic drawings of reactions catalyzed by ATCase and OTCase.

The most widely studied members of this family are the aspartate transcarbamylases (ATCases) and ornithine transcarbamylases (OTCases), enzymes that transfer the carbamoyl group of CP to the α-amino group of L-aspartate (L-Asp) and the δ-amino group of L-ornithine (L-Orn), respectively (Figure 1). ATCases catalyze the first committed step in the biosynthesis of pyrimidines, one of the components of nucleic acids, while OTCases function in the urea cycle, which eliminates excess ammonia, and in the biosynthesis of the amino acid arginine. This laboratory was largely responsible for defining the thermodynamics of protein−protein and protein−ligand interactions in *Escherichia coli* ATCase by reaction and differential scanning microcalorimetry,[1−4] electrostatic modeling,[5,6] hydrogen exchange,[7,8] solvent perturbation,[9,10] and related methods[11−14] and has recently determined high-resolution crystal structures of both *E. coli* and human ornithine transcarbamylase,[15,16] as well as examining the enzymology of the human enzyme.[17,18] This work has provided important new insights into the molecular basis of ornithine transcarbamylase deficiency (OTCD), a relatively common clinical condition in which ammonia levels in the blood are elevated as a result of mutations in the OTCase gene.[19]

E. coli ATCase, the most thoroughly studied transcarbamylase, is a multisubunit protein containing two trimeric catalytic polypeptide chains and three dimeric regulatory chains which undergoes major changes in its three-dimensional structure when substrates bind. It has been

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widely used for more than 30 years as a model system for understanding how protein−protein interactions mediate signal transduction.[20−25] Although structural and mechanistic studies of OTCase have lagged behind ATCase, several OTCase crystal structures have recently been determined,[26−28] including two high-resolution structures from this laboratory,[15,16] generating increased interest and new research opportunities. The new crystal structures indicate that, while OTCases have considerable structural diversity, their fundamental building block is a trimer that is very similar to the catalytic trimers of ATCases, and that they share the conformational flexibility of ATCases. OTCases are important clinically, since OTCD is the most common cause of inherited hyperammonemia (elevated levels of blood ammonia), which in turn produces neurological symptoms, sometimes sufficiently severe to cause death. More than 130 mutations that give rise to OTCD in humans have been identified,[19] many in this laboratory, and this laboratory is actively engaged in developing approaches to gene therapy.

The structural and functional similarities and differences within the transcarbamylase family provide an opportunity to ask a number of questions about molecular recognition. What differences in the L-Asp binding site of ATCase and the L-Orn binding site of OTCase result in ATCase binding L-Asp and OTCase binding L-Orn? Conversely, how similar are the binding sites for CP? Are there similarities between the catalytic mechanisms of the OTCases and ATCases? What are the similarities and differences between subunit interfaces, within the OTCase and ATCase families and between families? What determines whether the basic functional unit, a trimer, forms larger aggregates, or associates with other proteins? What is the relationship between the state of aggregation of the protein and its regulation? This review will focus on these questions.

Sequence Comparisons

More than 30 OTCase genes and more than 15 ATCase genes have been sequenced. These sequences are aligned in Figures 2 and 3, so that similarities and differences can be readily visualized. Similar sequences are likely to give rise to similar three-dimensional structure. Despite their similar folds, *E. coli* OTCase and the catalytic subunit of *E. coli* ATCase have only 32% identical sequence. Homology between OTCase and ATCase is greatest in the N-terminal half of the sequence, the region that binds their common substrate, CP, and much weaker in their C-terminal halves, which bind L-Orn in OTCase and L-Asp in ATCase. Although human OTCase has an even lower level of sequence homology with the catalytic subunit of *E. coli* ATCase (27%) than with that of *E. coli* OTCase, the homology model of human OTCase that we built using the catalytic subunit of *E. coli* ATCase as a model successfully predicted the effects on enzymatic function of many naturally occurring mutations found in patients with OTCD (Figure 5) and was in good agreement with the experimentally determined crystal structure.[29] Most mutations that produce neonatal OTCD are found at the active site, in the interior of the protein where they would interfere with folding, or between subunits where they would interfere with assembly of the trimer, whereas mutations that produce late onset symptoms are more likely to be found in loops on the exterior of the protein.

Tertiary Structure

As shown in Figure 4, the overall topology of the subunits of both OTCases and ATCases is α/β, with 14−16 α-helices and 9−10 β-sheets. Each chain consists of two domains, a carbamoyl phosphate binding domain and a second domain that binds either L-Orn or L-Asp. Each domain consists of a central core made up of a β-sheet surrounded by α-helices. Helices H5 and H11 in *E. coli* OTCase and analogous helices in other transcarbamylases connect the two domains, with a highly conserved hydrophilic cluster holding helices H1, H5, and H11 (H12 in ATCase) together. Active chimeras produced by recombinant DNA methods consisting of the CP domain of *E. coli* ATCase and the L-Orn domain of *E. coli* OTCase, or vice versa, with the substrate specificity of the second domain, were reported a number of years ago.[30] However, more recent efforts to reproduce this result and to exchange secondary structure elements between the two proteins yielded only insoluble proteins, highlighting the importance of specific side-chain packing, even in the context of a shared protein fold.[31]

The individual chains of both the catalytic subunit of *E. coli* ATCase and those of *E. coli* and human OTCases undergo domain closure when substrates and substrate analogues bind. A loop known as the 240s loop in *E. coli* ATCase swings in toward the active site, enabling a number of adjacent residues to interact with bound substrate. A second loop, known as the 80s loop, undergoes a smaller motion. These motions are illustrated in Figure 6, which superimposes the liganded and unliganded catalytic subunits of *E. coli* ATCase and liganded and unliganded subunits of *E. coli* OTCase. In *E. coli* ATCase, movement of these loops propagates to the subunit interfaces between catalytic subunits and between catalytic and regulatory subunits, resulting in a large change in quaternary structure, the T-to-R transition. This conformational change was one of the earliest models of ligand-induced signal transduction and has served as a paradigm for signal transduction in many other systems, including those involved in intercellular signaling in the nervous system and in regulating cellular proliferation of normal cells and neoplastic growth of cancerous cells.

Carbamoyl Phosphate Binding Site

The binding sites for CP in OTCase and ATCase are very similar (Figure 7). Both active sites contain a STRT motif (Ser55-Thr56-Arg57-Thr58 in *E. coli* OTCase), which binds the phosphonate oxygens of CP, and Arg and His residues (Arg 106 and His 133 in *E. coli* OTCase), which bind the carbonyl oxygen of CP. (Sequence numbers throughout are those of *E. coli* OTCase and ATCase, unless otherwise

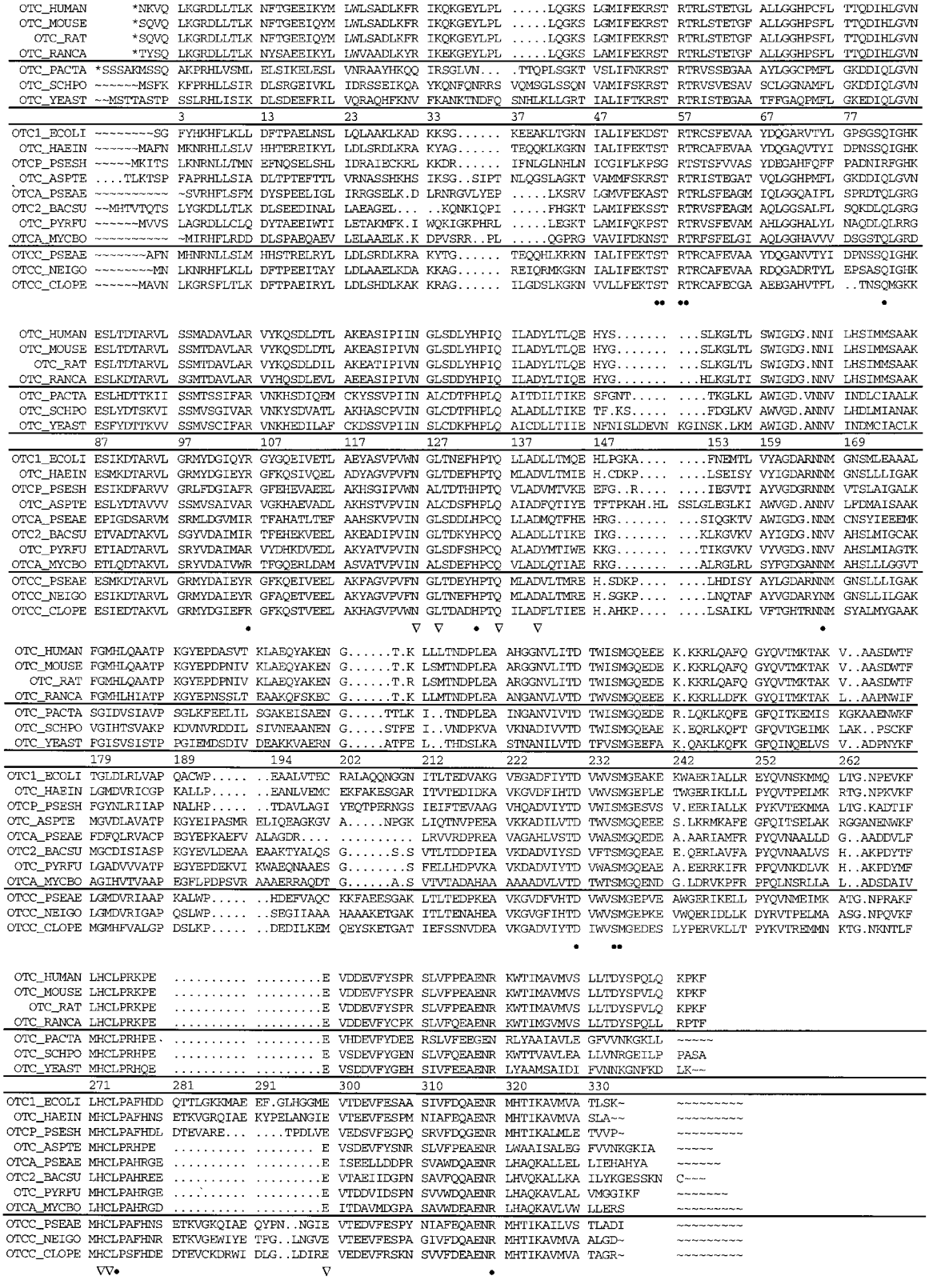

FIGURE 2. Sequence alignment of selected OTCase sequences (four ureotelic OTCases, three yeast OTCases, eight anabolic bacterial OTCases, and three catabolic bacterial OTCases, separated by solid lines). For a more complete list, see ref 19. PALO binding residues are indicated by •, and residues proposed to be catalytically important are indicated by ▽. Sequence numbering is based on the sequence of *E. coli* OTCase. The organisms used in the alignment were *Homo sapiens* (human), *Mus musculus* (mouse), *Rattus norvegicus* (rat), *Rana catesbeiana* (bull frog), *Pachysolen tannophilus, Schizosaccharomyces pombe*, *Saccharomyces cerevisiae*, *Escherichia coli*, *Haemophilus influenzae*, *Pseudomonas syringae*, *Aspergillus terrus*, *Bacillus subtilis*, *Pyrococcus furiosus*, *Mycobacterium bovis*, *Pseudomonas aeruginosa*, *Neisseria gonorrheae,* and *Clostridium perfringens*. * indicates sequences not shown for clarity.

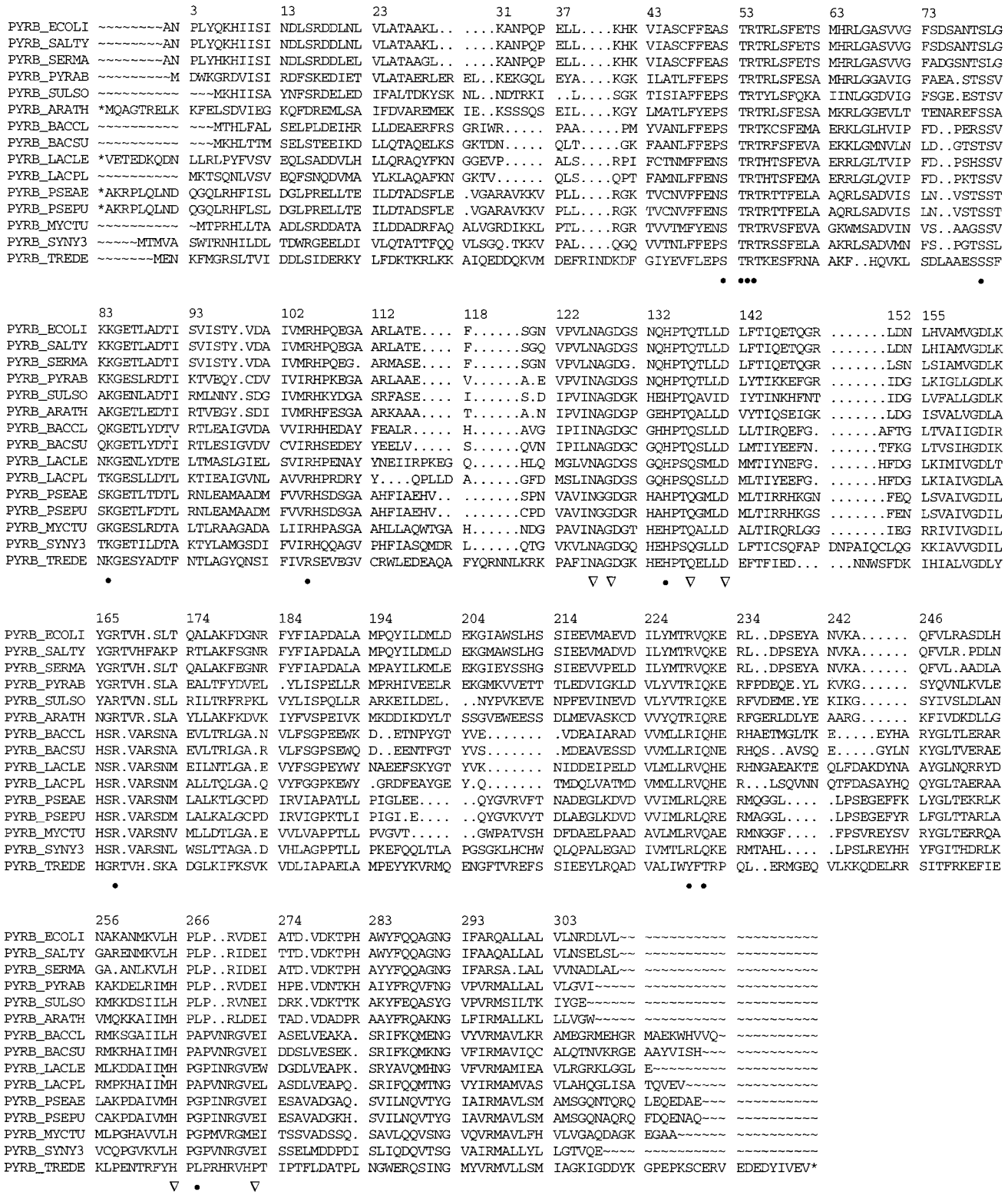

FIGURE 3. Sequence alignment of selected ATCase catalytic subunits. PALA binding residues are indicated by •, and residues proposed to be catalytically important are indicated by ▽. Sequence numbering is based on the sequence of *E. coli* ATCase. The organisms used in the alignment were *Escherichia coli, Salmonella trphimurum, Serratia marcescens*, *Pyrococcus abyssi*, *Sulfolobus solfataricus*, *Arabidopsis thaliana*, *Bacillus caldolyticusd*, *Bacillus subtilis*, *Lactobacillus leichmannii*, *Lactobacillus plantarum*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Mycobacterium tuberculosis*, *Synechocystis* sp., and *Treponema denticola*. * indicates sequences not shown for clarity.

indicated.) The positive charges of the active site complement the negative charge of the ligand. In both families, at least one residue at this binding site is contributed by a second subunit. In *E. coli* ATCase, these residues are Ser 80 and Lys 84. In *E. coli* OTCase, Gln 82 (His 117 in human OTCase), the counterpart of Ser 80 in *E. coli*

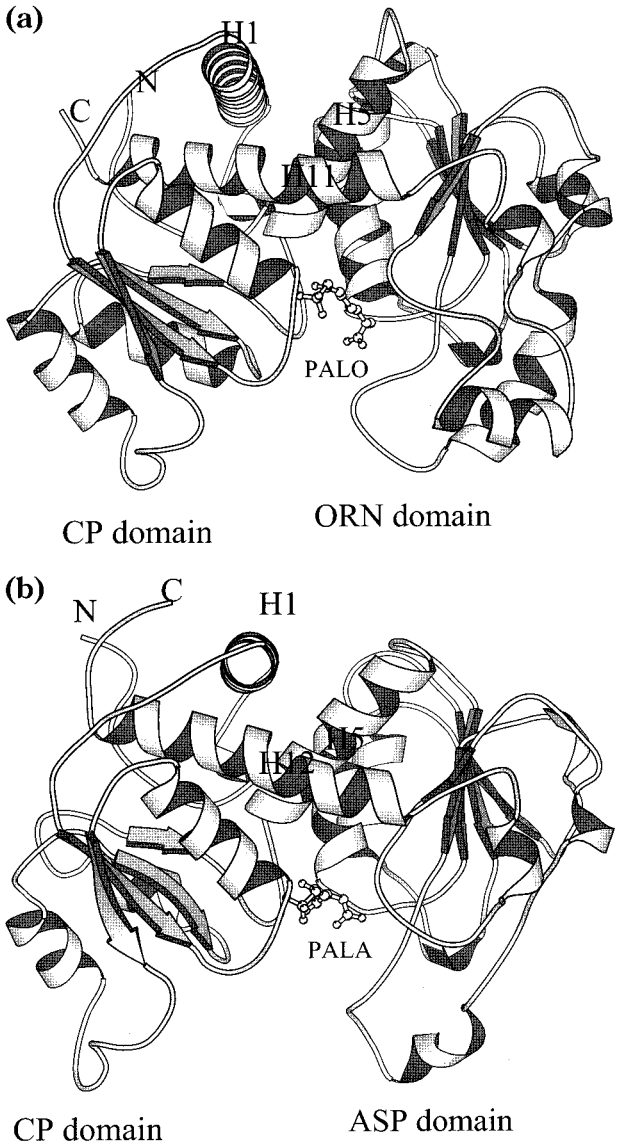

FIGURE 4. Ribbon drawing of subunit of (a) *E. coli* OTCase and (b) ATCase. The bisubstrate analogues PALA or PALO, which are located in the cleft between the CP domain and the L-Asp or L-Orn domain, are shown as ball-and-stick models. The α9a loop, present in *E. coli* OTCase adjacent to the 240s loop, is not present in *E. coli* ATCase and some other OTCases. In general, the tertiary structures of the ATCase catalytic subunit and OTCase subunit are very similar.

ATCase, forms a hydrogen bond with one of the phosphonate oxygens of the bound bisubstrate analogue PALO. Lys 86, which corresponds to Lys 84 in *E. coli* ATCase, is adjacent to both the carbonyl O of Gln 82 and the carboxy O of PALO, but not close enough to form direct bonds. However, these interactions appear to be functionally significant, since mutating Lys 86 to Gln lowers the enzyme's maximal velocity by 2 orders of magnitude.[32] These side chains may be bridged by solvent molecules.

Aspartate or Ornithine Binding Site

L-Orn and L-Asp have side chains with different lengths and different charges, and their binding sites have evolved to reflect these differences (Figure 7). Several side chains at the L-Asp binding site of ATCase are positively charged, such as Lys 84, Arg 167, and Arg 229, while others such as Gln 231 are neutral. The binding site for L-Orn in OTCase has a negatively charged side chain, Asp 231, and two long hydrophobic side chains, Met 236 and Leu 125, which form a hydrophobic pocket for the methylene group of L-Orn, as well as two hydrogen-bonding side chains, Ser 235 and Asn 167.

Electrostatic Features of Active Site

Both OTCase and ATCase have a high density of charged residues at their active sites which create electrostatic potentials that can guide incoming substrates to their binding sites and assist in their binding. The electrostatic potentials of the catalytic subunits of *E. coli* ATCase and OTCase are shown in Figure 8. In both ATCase and OTCase, substrate binding and product release are ordered, with CP binding first, L-Asp or L-Orn binding second, carbamoylaspartate or citrulline dissociating first, and phosphate dissociating second.[33,34] When ATCase and OTCase are unliganded, their active sites are dominated by positive electrostatic potentials that will guide their common substrate, CP, with two negative charges at physiological pH, into the CP binding pocket. After CP binds, the electrostatic potentials around the active sites of ATCase and OTCase have the opposite sign. While the active site of ATCase is still dominated by a positive potential, the electrostatic potential of the active site in OTCase has become negative. The positive potential of ATCase will help dock negatively charged L-Asp, while the negative potential of OTCase will play the same role in docking the positively charged L-Orn.

Catalytic Mechanisms

The critical step in the catalytic mechanisms of both ATCases and OTCases is nucleophilic attack of the carbonyl C of CP by an amino group. In both enzymes, the crystal structures indicate that attack is facilitated by interactions between the protein and its substrates, which increase the nucleophilicity of the amino group and the positive charge on the carbonyl C. However, the greater basicity of the δ-amino group of L-Orn requires general acid−base catalysis in OTCase, while L-Asp does not.

The group which appears to function as a general acid−base catalyst in OTCase is the sulfhydryl group of a Cys residue, which is found in a conserved HCLP motif. HCLP is replaced by HPLP in *E. coli* ATCase, which does not require a general acid−base catalyst. The S atom of this Cys in the crystal structure of the complex of the bisubstrate analogue PALO with *E. coli* OTCase is 4.3 Å from the ϵ-N of the L-Orn moiety in PALO. It thus has the potential to act as a general acid−base catalyst and to abstract a proton from the δ-amino group of L-Orn. Its basicity is increased by a hydrogen bond to the side chain of Asp 231. The Cys side chain is also close to the imidazole group of His 272, which in turn interacts with Glu 299. Although this Cys-His-Glu triad is reminiscent

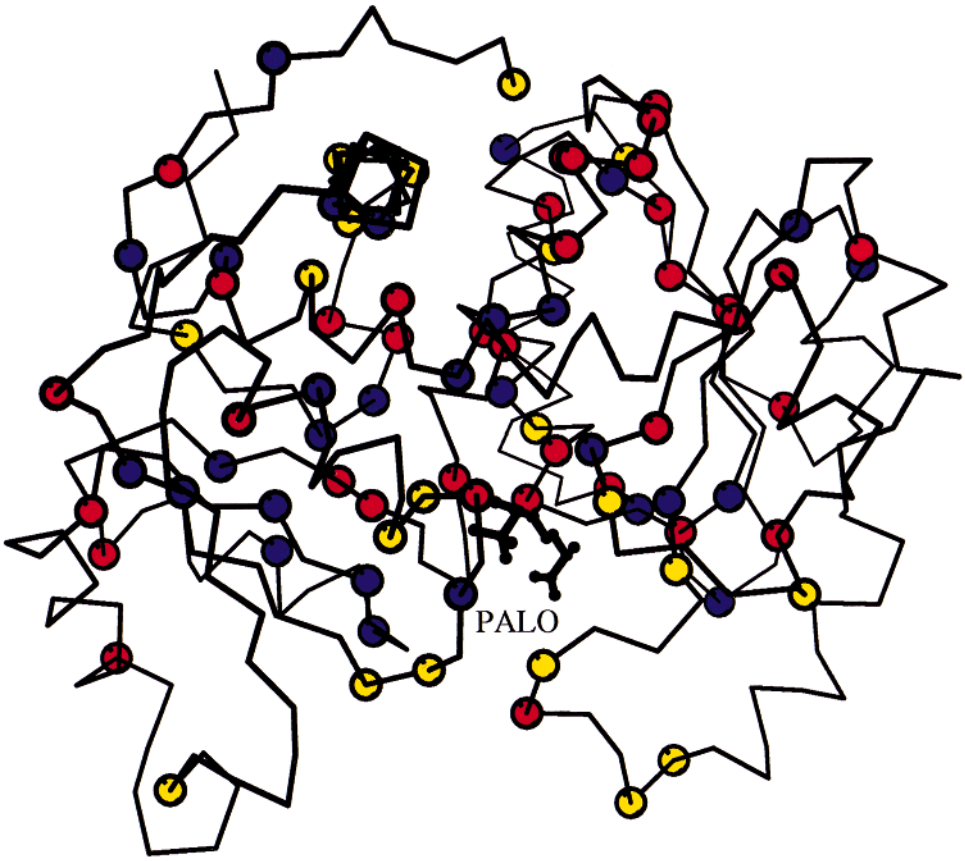

FIGURE 5. Deleterious mutations found in patients with OTCD mapped on the structure of human OTCase. Mutations which produce neonatal OTCD are shown in red, those identified only in females are in blue, and those which produce late onset OTCD are in yellow.

of the catalytic triads in Cys and Ser proteases, the distance between the γ-S of Cys and the imidazole ring is too long for a strong interaction; however, a stronger interaction in some other steps of the catalytic cycle cannot be ruled out at this point.

The catalytic mechanisms of both ATCase and OTCase involve a tetrahedral intermediate (Figure 1). Gln 136 in *E. coli* OTCase, which is 4.1 Å from the carbonyl C of the CP moiety, is positioned so as to be able to stabilize this intermediate, while Gln 137 plays the same role in *E. coli* ATCase. The sequence motif NxLxxxxHxxQxxD around Gln 136 is strongly conserved in OTCases, as is the corresponding motif NxGDGxxxHxxQxxD in ATCases. The similarity between these motifs suggests that both OTCase and ATCase may use a similar mechanism to stabilize the tetrahedral intermediate. However, replacement of the GDG motif in ATCase by L in OTCase reduces backbone flexibility and enables the long side chain of L-Orn to be held in place by the Leu side chain of the protein.

Subunit Interfaces

The interface between subunits in the trimers of both OTCase and ATCase is formed primarily by residues from the CP binding domains. In *E. coli* OTCase, these residues are 57−68, 72−98, and 278−317; the residues at the subunit interfaces of ATCase are analogous. Binding of substrate analogues to the active site triggers localized conformational changes in the vicinity of residues 52 and 84 which strengthen subunit interactions; the restructuring is greater in ATCase, where it contributes to the T → R quaternary transition,[35] than it is in OTCase (Figure 6). Although most residues involved in intersubunit interactions are variable, the salt bridge between Arg 57 and Glu 87 is conserved in both OTCase and ATCase, as is the interaction between Arg 59 and the carbonyl O of residue 75. These interactions are likely to be important in positioning Gln 82 in OTCase and Ser 80, Lys 84 in ATCase to interact with bound CP.

Other Protein−Protein Interfaces

Although both *E. coli* and human OTCase are trimeric in vitro, the OTCases of *Pseudomonas aeruginosa* and *Pyrococcus furiosus*, a thermophilic archaeabacterium, are dodecameric, with 23-point group symmetry. The trimers are organized tetrahedrally, with their convex surfaces in contact. However, a 6° rotation of the trimers of the *Pseudomonas* and *Pyrococcus* OTCases with respect to each other results in the intertrimer interfaces being quite different in the two proteins. In *P. aeruginosa*, a cluster of charged residues rich in Arg forms a 3-fold channel, which may bind negatively charged ions such as sulfate or phosphate.[26] Contacts between monomers from adjacent catalytic trimers around the 2-fold symmetry axes appear to play a role in the allosteric behavior of this

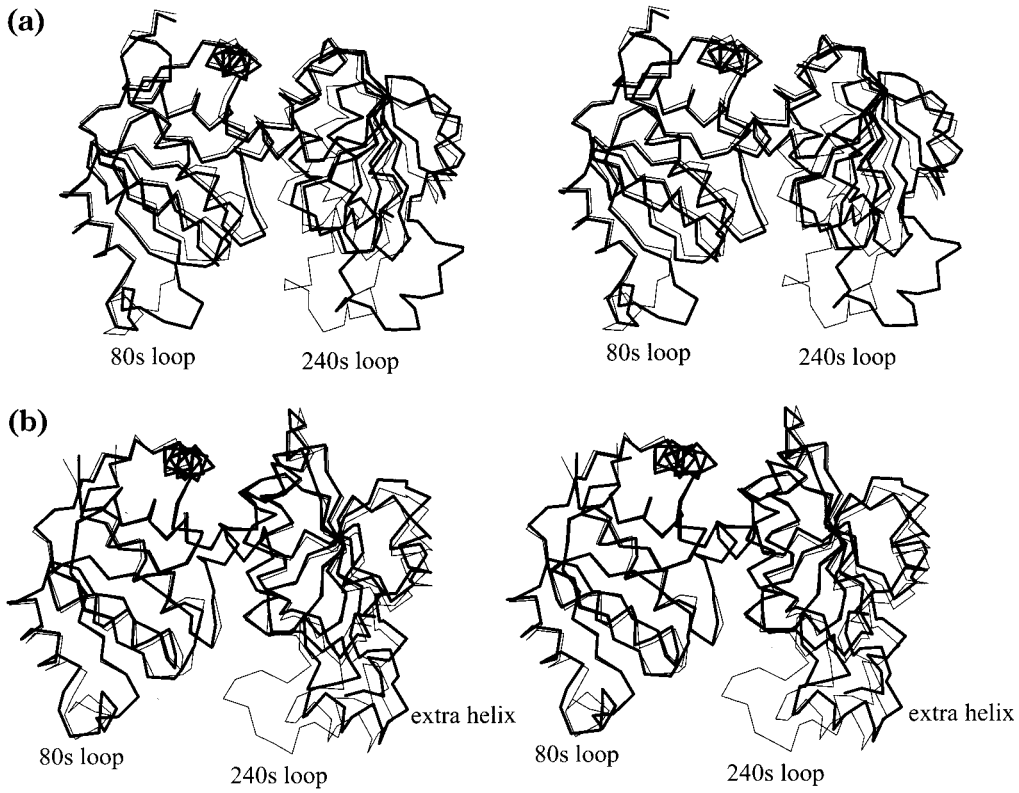

FIGURE 6. Superposition of liganded (thin line) and unliganded (thick line) catalytic subunits of (a) *E. coli* ATCase and (b) OTCase. The Protein Data Bank references for the structures are 8ATC (liganded *E. coli* ATCase), 1RAI (unliganded *E. coli* ATCase), 2ORT (liganded *E. coli* OTCase), and 1AKM (unliganded *E. coli* OTCase).

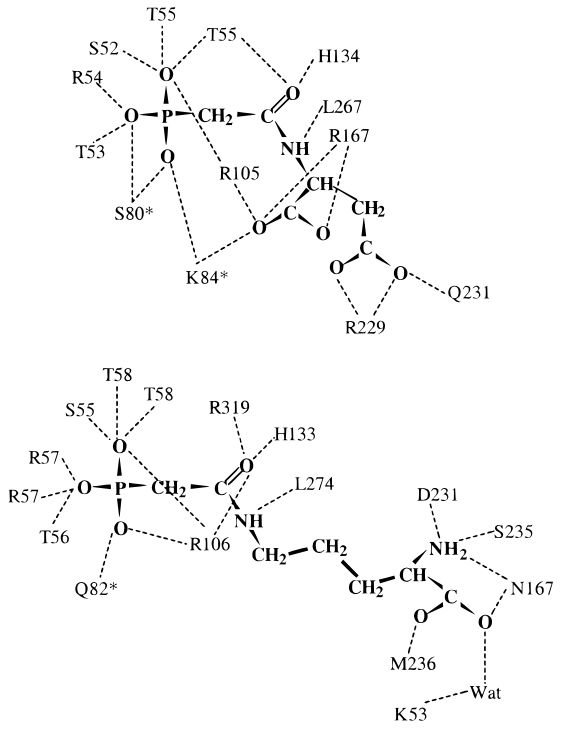

FIGURE 7. Schematic drawing showing the interaction of (a) the bisubstrate analogue PALA with active-site residues in *E. coli* ATCase and (b) the bisubstrate analogue PALO with active-site residues in *E. coli* OTCase. The residue indicated with * is from an adjacent subunit.

enzyme, since eliminating these residues eliminates allosteric regulation. In *Py. furiosus* OTCase, the Arg residues at this interface are replaced by Trp, giving it a hydrophobic character.[28] Since hydrophobic interactions strengthen as temperature increases, this feature of *Py. furiosus* OTCase, together with an increase in the number of electrostatic interactions within each monomer, probably accounts for its ability to retain 50% activity, even after being heated at 100 °C for as long as 1 h.[36]

E. coli ATCase also exists as a dodecamer, however, composed of six catalytic chains and three regulatory chains, organized as two catalytic subunits and three regulatory subunits with 32-point group symmetry. This complex organization creates three unique subunit interfaces for each catalytic chain, one between catalytic subunits, and two types of interfaces between catalytic and regulatory subunits. In contrast to the prokaryotic OTCases discussed above, contacts between the catalytic trimers of *E. coli* ATCase in the T state involve the concave surfaces of the trimers, with residues in the 240s loops interacting, primarily through ionic or polar interactions. All subunit interfaces undergo major changes in the T → R allosteric transition. When the transition occurs, the contact between the catalytic subunits and one of the contacts between catalytic and regulatory subunits are eliminated.

The homology between *E. coli* OTCase and ATCase raises the question of why OTCase does not associate with the regulatory subunit of ATCase, since both coexist in the cytoplasm. This interaction may be prevented by an extra helix (α9a) present in both *E. coli* and *P. aeruginosa* catabolic OTCase which interacts with the 240s loop,

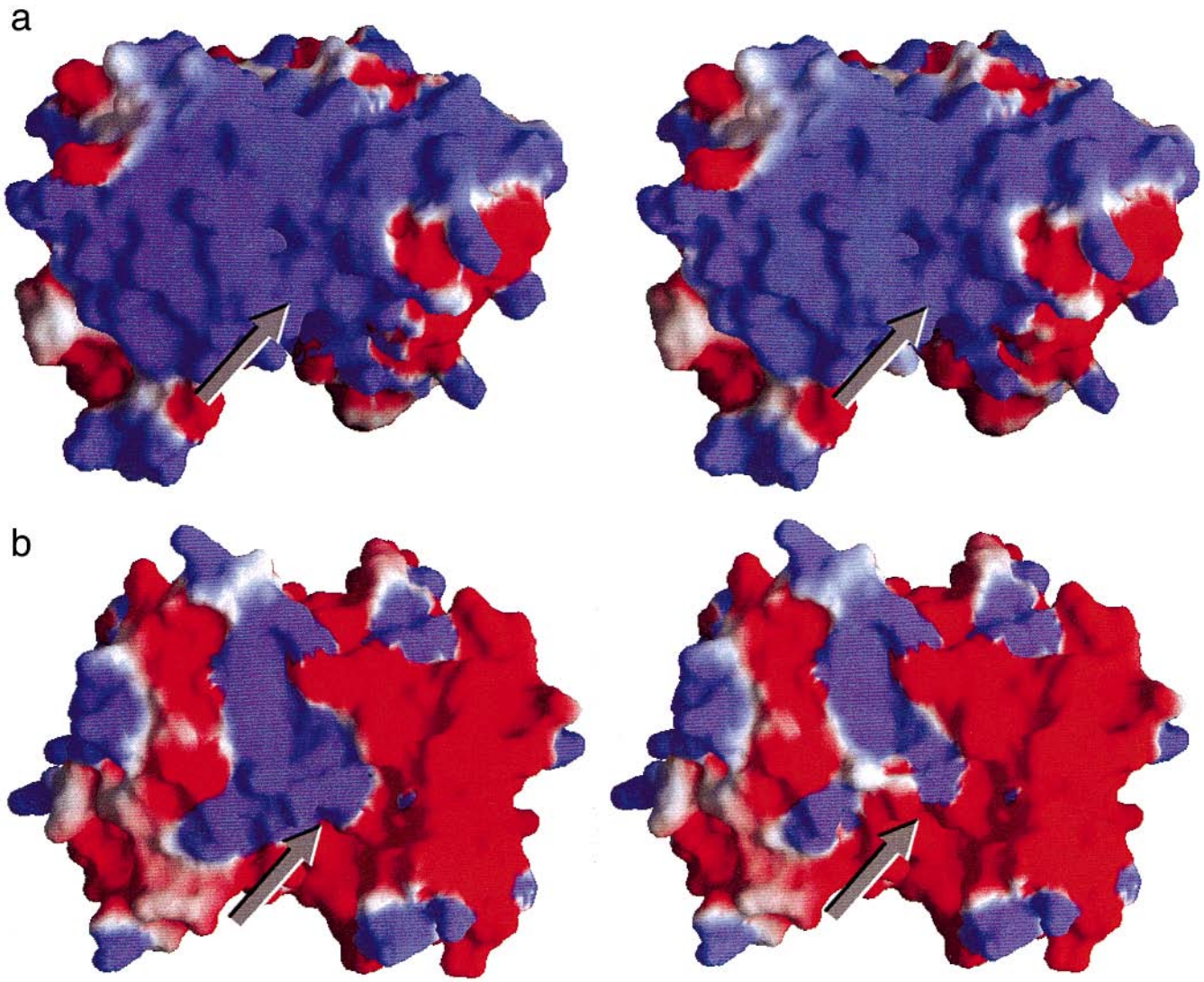

FIGURE 8. Electrostatic potential of (a) *E. coli* ATCase and (b) OTCase catalytic subunit before and after binding the first substrate CP. Potentials greater than +1 kT are shown in blue, and those less than −1 kT in are shown in red, mapped onto the molecular surface. The subunit is viewed in the same orientation as that in Figure 5. The active site is in the cleft between two domains, as indicated by the arrow. This figure was produced with GRASP.[41]

mainly through hydrophobic bonds, and with the 80s loop. The position of this helix, combined with sequence differences in a number of residues, would disrupt the potential interface between *E. coli* OTCase and the regulatory subunit of *E. coli* ATCase.[15]

Ureotelic OTCases have a C-terminus extension which folds back on the L-Orn domain and forms a ridge on the convex face of the trimer. This extension has a surprising homology with several membrane associated proteins, including two yeast mitochondrial inner membrane carrier proteins.[16] In models of these carrier proteins, this sequence occurs in a loop between two transmembrane helices in a region of the molecule which has been shown to be important in carrier-specific transport. Since OTCase has been shown to be associated with the inner mitochondrial membrane,[37] the sequence of this extension may be a protein recognition motif which enables OTCase and other membrane-associated proteins to interact with other proteins with related functions.

Relationship between State of Assembly and Regulation

Although trimeric OTCases undergo domain closure when substrates bind, wild-type trimeric OTCases appear to be unregulated, although allostery can be induced through site-directed mutagenesis. Higher order assemblies of OTCase may or may not be regulated. Catabolic *P. aeruginosa* OTCase is regulated by the binding of negatively charged allosteric activators such as phosphate or nucleoside monophosphates at the interfaces between trimers in the dodecamer, while anabolic *Py. furiosus* OTCase, also a dodecamer, is not.

In yeast, a regulatory complex consisting of one molecule of OTCase and one molecule of arginase forms to inactivate OTCase, effectively uncoupling the biosynthetic and catabolic pathways in arginine metabolism.[38,39] The conformational changes which promote association of

OTCase with arginase have been proposed to be linked to binding of substrates at the active site.

ATCases comprised of both catalytic and regulatory subunits are highly regulated, although the details of the regulation vary between species. Chimeric enzymes have provided considerable insight into the elements of secondary structure that determine the nature of the regulation. For example, CTP stimulates the catalytic activity of *Serratia marcescens* ATCase, while CTP and UTP synergistically inhibit *E. coli* ATCase. When five divergent residues in the S5′ β-strand at the junction of the allosteric and zinc domains of the regulatory chain of *S. marcescens* ATCase were replaced by the corresponding residues in *E. coli* ATCase, the chimeric ATCase acquired the allosteric properties of *E. coli* ATCase.[40]

Conclusions

Structural and functional comparisons between the OTCases and ATCases provide an unusual opportunity to compare how two families of enzymes recognize their substrates and assemble to form large aggregates. The primary and tertiary structures of the domains that bind the common substrate CP are similar, while those of the domains that bind the second substrate, L-Asp or L-Orn, are different. The binding pockets for the second substrate are complementary to the substrates and therefore differ between OTCases and ATCases, despite some common features. The similarity in the active sites of ATCase and OTCase implies that they use similar mechanisms to stabilize the tetrahedral intermediate in the reaction mechanism. Differences in the active site, particularly replacement of the HPLP motif in ATCase by HCLP in OTCase, compensate for the difference in basicity of the α-amino group of L-Asp and the δ-amino group of L-Orn. The electrostatic properties of the active site are consistent with the ordered reaction mechanism for both enzymes, with the change of electrostatic potential in OTCase after binding the first substrate, CP, reflecting the different net charge of the second substrate, L-Asp or L-Orn. Variability in primary structure within and between the two enzyme families determines their ability to associate with other enzymes or to self-assemble to form larger aggregates. Association and aggregation of the enzymes create new properties, such as allosteric regulation, thermal stabilization, or inactivation.

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References

(1) Knier, B.; Allewell, N. M. Calorimetric analysis of aspartate transcarbamylase from *Escherichia coli*: Binding of substrates and substrate analogs to the native enzyme and catalytic subunit. *Biochemistry* **1978**, *17*, 784−790.

(2) Edge, V.; Allewell, N. M.; Sturtevant, J. M. Differential Scanning calorimetric study of the thermal denaturation of aspartate transcarbamylase of *Escherichia coli*. *Biochemistry* **1988**, *27*, 8081−8087.

(3) Burz, D. S.; Allewell, N. M. Interactions of ionizable group in *Escherichia coli* aspartate transcarbamylase with adenosine and cytosine 5′-triphosphates. *Biochemistry* **1982**, *21*, 6647−6655.

(4) McCarthy, M. P.; Allewell, N. M. Thermodynamics of assembly of *Escherichia coli* aspartate transcarbamylase. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 6824−6828.

(5) Oberoi, H.; Trika, J.; Yuan, X.; Allewell, N. M. Identification and analysis of long-range electrostatic effects in proteins by computer modeling: Aspartate transcarbamylase. *Proteins: Struct., Funct. Genet.* **1996**, *25*, 300−314.

(6) Hariharan, M.; Allewell, N. M. Effects of the T→R transition on the electrostatic properties of *Escherichia coli* aspartate transcarbamylase. *Proteins: Struct., Funct. Genet.* **1998**, *32*, 200−210.

(7) Lennick, M.; Allewell, N. M. Changes in the hydrogen exchange kinetics of *Escherichia coli* aspartate transcarbamylase produced by effector binding and subunit association. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *21*, 6759−6763.

(8) Mallikarachchi, D.; Burz, D. S.; Allewell, N. M. Effects of CTP and ATP on the conformation of the regulatory subunit of *Escherichia coli* aspartate transcarbamylase: A medium resolution hydrogen exchange study. *Biochemistry* **1989**, *28*, 5386−5391.

(9) LiCata, V. J.; Allewell, N. M. Functionally linked hydration changes in *Escherichia coli* aspartate transcarbamylase. *Biochemistry* **1997**, *36*, 10161−10167.

(10) LiCata, V. J.; Burz, D. S.; Moerke, N. J.; Allewell, N. M. The magnitude of the allosteric conformational transition of aspartate transcarbamylase is altered by mutations. *Biochemistry* **1998**, *37*, 17381−17385.

(11) Allewell, N. M.; Hofmann, G. E.; Zaug, A.; Lennick, M. The Bohr effect in *Escherichia coli* aspartate transcarbamylase: Linkages between proton binding, ligand binding, and conformational transitions. *Biochemistry* **1979**, *18*, 3008−3015.

(12) Bromberg, S.; LiCata, V. J.; Mallikarachchi, D.; Allewell, N. M. Ligation alters the pathway of urea-induced denaturation of the catalytic trimer of *Escherichia coli* aspartate transcarbamylase. *Protein Sci.* **1994**, *3*, 1236−1244.

(13) Allewell, N. M.; LiCata, V. J. Thermodynamic approaches to understanding aspartate transcarbamylase. *Methods Enzymol.* **1995**, *259*, 608−628.

(14) Ha, Y.; Allewell, N. Intersubunit hydrogen bond acts as a global molecular switch in *Escherichia coli* aspartate transcarbamoylase. *Proteins: Struct., Funct. Genet.* **1998**, *33*, 430−443.

(15) Ha, Y.; McCann, M. T.; Tuchman, M.; Allewell, N. M. Substrate-induced conformational change in a trimeric ornithine transcarbamoylase. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 9550−9555.

(16) Shi, D.; Morizono, H.; Ha, Y.; Aoyagi, M.; Tuchman, M.; Allewell, N. M. 1.85 Å resolution crystal structure of human ornithine transcarbamoylase complexed with *N*-phosphonacetyl-L-ornithine. *J. Biol. Chem.* **1998**, *273*, 34247−34254.

(17) Morizono, H.; Tuchman, M.; Rajagopal, B. S.; McCann, M. T.; Listrom, C. D.; Yuan, X.; Venugopal, D.; Barany, G.; Allewell, N. M. Expression, purification and kinetic characterization of wild-type human ornithine transcarbamylase and a recurrent mutant that produces 'late onset' hyperammonaemia. *Biochem. J.* **1997**, *322*, 625−631.

(18) Morizono, H.; Listrom, C. D.; Rajagopal, B. S.; Aoyagi, M.; McCann, M. T.; Allewell, N. M.; Tuchman, M. 'Late onset" ornithine transcarbamylase deficiency function of three purified recombinant mutant enzymes. *Human Mol. Genet.* **1997**, *6*, 963−968.
(19) Tuchman, M.; Morizono, H.; Rajagopal, B. S., Plante, R. J.; Allewell, N. M. Biochemical and molecular spectrum of ornithine transcarbamylase deficiency. *J. Inh. Met. Dis.* **1998**, *21*, 40−58.
(20) Schachman, H. K. From allostery to mutagenesis: 20 years with aspartate transcarbamoylase. *Biochem. Soc. Trans.* **1987**, *15*, 772−775.
(21) Allewell, N. M. *Escherichia coli* aspartate transcarbamoylase: structure, energetics, and catalytic and regulatory mechanisms. *Annu. Rev. Biophys. Chem.* **1989**, *18*, 71−92.
(22) Hervé, G. Aspartate transcarbamylase; In *Allosteric Enzymes*; CRC Press: Boca Raton, FL, 1989; pp 61−79.
(23) Kantrowitz, E. R.; Lipscomb, W. N. *Escherichia coli* aspartate transcarbamoylase: the molecular basis for a concerted allosteric. *Trends Biochem. Sci.* **1990**, *15*, 53−59.
(24) Wild, J. R.; Wales, M. E. Molecular evolution and genetic engineering of protein domains involving aspartate transcarbamoylase. *Annu. Rev. Microbiol.* **1990**, *44*, 193−218.
(25) Lipscomb, W. N. Aspartate transcarbamylase from *Escherichia coli*.: activity and regulation. *Adv. Enzyme Relat. Areas Mol. Biol.* **1994**, *68*, 67−151.
(26) Vileret, V.; Tricot, C.; Stalon, V.; Dideberg, Crystal structure of *Pseudomonas aeruginosa* catabolic ornithine transcarbamoylase at 3.0-Å resolution: a different oligomeric organization in the transcarbamoylase family. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 10762−10766.
(27) Jin, L.; Seaton, B. A.; Head, J. F. Crystal structure at 2.8 Å resolution of anabolic ornithine transcarbamylase from *Escherichia coli*. *Nature Structural Biology* **1997**, *4*, 622−625.
(28) Villeret, V.; Clantin, B.; Tricot, C.; Legrain, C.; Roovers, M.; Stalon, V.; Glansdorff, N.; Jozef van Beeumen. The crystal structure of *Pyrococcus furiosus* ornithine carbamoyltransferase reveals a key role for oligomerization in enzyme stability at extreme high temperature. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 2801−2806.
(29) Tuchman, M.; Morizono. H.; Reish, O.; Yuan, X.; Allewell, N. M. The molecular basis of ornithine transcarbamylase deficiency: Modeling the human enzyme and the effects of mutations. *J. Med. Genet.* **1995**, *32*, 680−688.
(30) Houghton, J. E.; O'Donovan, G. A.; Wild, J. R. Reconstruction of an enzyme by domain substitution effectively switches substrate specificity. *Nature* **1989**, *338*, 172−174.
(31) Murata, L. B.; Schachman, H. K. Structural similarity between ornithine and aspartate transcarbamoylases of *Escherichia coli*: Characterization of the active site and evidence for an interdomain carboxy-terminal helix in ornithine transcarbamoylase. *Protein Sci.* **1996**, *5*, 709−718.
(32) Murata, L. B.; Schachman, H. K. Structural similarity between ornithine and aspartate transcarbamoylase of *Escherichia coli*: Implications for domain switching. *Protein Sci.* **1996**, *5*, 719−728.
(33) Porter, R. W.; Modebe, M. O.; Stark, G. R. Aspartate transcarbamylase: kinetic studies of the catalytic subunit. *J. Biol. Chem.* **1969**, *244*, 1846−1859.
(34) Legrain, C.; Stalon, V. Ornithine carbamoyltransferase from *Escherichia coli*: purification, structure and steady-state kinetic analysis. *Eur. J. Biochem.* **1976**, *63*, 289−301.
(35) Ke, H.; Lipscomb, W. N.; Cho, Y.; Honzatko, R. B. Complex of *N*-phosphonacetyl-L-aspartate with aspartate carbamoyltransferase. *J. Mol. Biol.* **1988**, *204*, 725−747.
(36) Legrain, C.; Demarez, M.; Glansdorff, N.; Piérard, A. Ammonia-dependent synthesis and metabolic channelling of carbamoyl phosphate in the hyperthermophilic archaeon *Pyrococcus furiosus*. *Microbiology* **1995**, *141*, 1093−1099.
(37) Powers-Lee, S. G.; Mastico, R. A.; Bendayan, M. The interaction of rat liver carbamoyl phosphate synthetase and ornithine transcarbamoylase with inner mitochondrial membranes. *J. Biol. Chem.* **1987**, *262*, 15683−15688.
(38) Eisenstein, E.; Hensley, P. Ligand binding-promoted conformational changes in yeast ornithine transcarbamoylase. *J. Biol. Chem.* **1986**, *261*, 6192−6200.
(39) Hensley, P. Ligand binding and multienzyme complex formation between ornithine carbamoyltransferase and arginase from *Saccharomyces cerevisiae*. *Curr. Top. Cell Regul.* **1988**, *29*, 35−75.
(40) Liu, L.; Wales, M. E.; Wild, J. R. Conversion of the allosteric regulatory patters of aspartate transcarbamoylase by exchange of a single β-strand between diverged regulatory chains. *Biochemistry* **1997**, *36*, 3126−32.
(41) Nichols, A. *GRASP: Graphical Representation and Analysis of Surface Properties*; Columbia University: New York, 1993.

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