

Molecular Recognition by Ornithine and Aspartate Transcarbamylases

NORMA M. ALLEWELL,^{*,†} DASHUANG SHI,[†]
HIROKI MORIZONO,[‡] AND
MENDEL TUCHMAN[‡]

Department of Biochemistry, University of Minnesota,
St. Paul, Minnesota 55108, and Department of Pediatrics,
Medical School, University of Minnesota,
Minneapolis, Minnesota 55455

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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

Norma Allewell received her B.Sc. (Hon.) degree in biochemistry from McMaster University (Hamilton, ON, Canada) in 1965 and her Ph.D. in molecular biophysics from Yale University in 1969. Until January 1999, she was Professor of Biochemistry and Vice Provost for Research and Graduate/Professional Education at the University of Minnesota. She is now Associate Vice President for Sponsored Programs and Technology Transfer at Harvard University.

Dashuang Shi was born in Fujian Province, People's Republic of China in 1964. He received his B.S. (1983) and M.S. (1987) degrees in chemistry from Xiamen University and his Ph.D. in 1997 from the University of Sydney, where his advisor was Professor Hans Freeman. He is currently a postdoctoral associate with Professor Allewell.

Hiroki Morizono received his S.B. degree in life sciences from MIT in 1987, and his Ph.D. degree in biochemistry from the University of Minnesota in 1997 under the direction of Professor Allewell. He is currently a postdoctoral associate in the laboratory of Dr. Tuchman.

Mendel Tuchman received his M.D. (magna cum laude) from the Sackler School of Medicine, Tel Aviv University, in 1979, and joined the faculty in the Department of Pediatrics at the University of Minnesota in 1985. He is currently Professor of Pediatrics and Laboratory Medicine and Pathology and Director of the Biochemical Genetics and Metabolism Laboratories in the Medical School of the University of Minnesota. In August 1999, he became Director of the Clinical Research Center and Chairman of Metabolism at the Children's National Medical Center in Washington, DC.

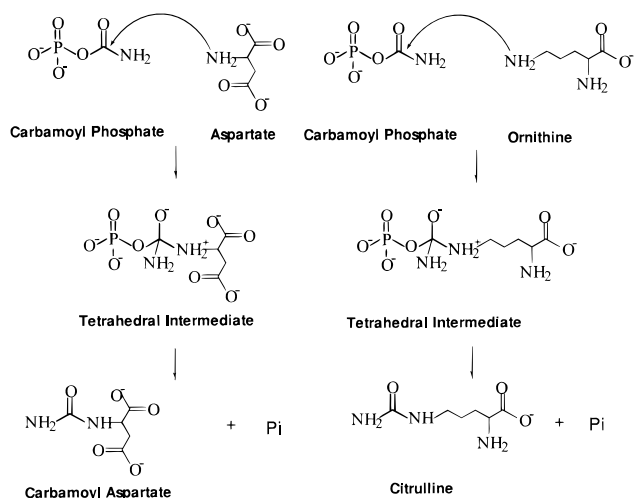


FIGURE 1. Schematic drawings of reactions catalyzed by ATCase and OTCase.

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.

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

* To whom correspondence should be addressed. Current address: Office of Sponsored Research, Harvard University, 4th Floor, Holyoke Center, 1350 Massachusetts Ave., Cambridge MA 02138. E-mail: norma_allewell@harvard.edu.

[†] Department of Biochemistry.

[‡] Department of Pediatrics, Medical School.

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 muta-

tions 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

OTC_HUMAN	*KGRDLLTLK	NFTGEEIKVM	LWLSADLKFR	IKQKGEYVPLLQGKS	LGMIFEKRST	RTRLSTETGF	ALLGGHPCFL	TTQDIHLGVN
OTC_MOUSE	*SQVQ	LKGRDLLTLK	NFTGEEIQVM	LWLSADLKFR	IKQKGEYVPLLQGKS	LGMIFEKRST	ALLGGHPSFL	TTQDIHLGVN
OTC_RAT	*SQVQ	LKGRDLLTLK	NFTGEEIQVM	LWLSADLKFR	IKQKGEYVPLLQGKS	LGMIFEKRST	ALLGGHPSFL	TTQDIHLGVN
OTC_RANCA	*TYSQ	LKGRDLLTLK	NYSAEIKYVL	LWVAADLKFR	IKQKGEYVPLLQGKS	LAMIFEKRST	RTRLSTETGF	ALLGGHPSFL
OTC_FACTA	*SSAKMSSQ	KFRHLLVSM	ELSIKELSL	VNRAAYHKQV	...TTQPLSGKT	VSLIFNKRST	RTRVSSBGA	AYLGGQPMFL	GNDDQLGRG
OTC_SCHPO	-----MSFK	KFRHLLSIR	DLSRGEIVKL	IDRSSEIKQA	VQNFQNRRS	VQMSGLSSQN	VAMIFEKRS	RTRVSVESAV	SCLGGNAMFL
OTC_YEAST	---MSSTASTP	SLRHLISIK	DLSDDEFRLL	VQRAQHFQV	FKANKTDFQ	SNHKLKLGRT	IALIFTKRST	RTRISTBGA	TFFGAQMFL
		3	13	23	33	37	47	57	77
OTC1_ECOLI	-----SG	FYHKHFLKLL	DFTPAELNSL	LQLAALKKAD	KKSG.....	KEEAKLTGKN	IALIFEKDT	RTRCSFEVAA	YDQGARVTVL
OTC_HAEIN	-----MAFN	MKNRHLLSLV	HHTEREIKYL	LDLSRDLKRA	KYAG.....	TEQQKLGKGN	IALIFEKTS	RTRCAFEVAA	YDQGAQVTYI
OTCP_PSESH	-----MKITS	LKNRNLLTMN	EFNQSELSHL	IDRAIECKRL	KKDR.....	IFNLGLNHLN	ICGIFLKPST	RTRSTFVVAS	YDGAHFQFT
OTC_ASPTATLKTSP	FAPRHLLSIA	DLTPTEFTTL	VRNASSHKHS	IKSG..SIPT	NLQGSAGKT	VAMMFSKRST	RTRISTBEGAT	VQLGGHPMFL
OTCA_PSEAE	-----MVVS	SVRHFLSPM	DYSPEELIGL	IRRSSELK.D	GLNRGVLVEPLKSRV	LGMVFEKAST	RTRLSFVFRAGM	IQLGGQAIPL
OTC2_BACSU	---MHTVTQTS	LYGRDLLTLK	DLSEEDINAL	LAEGAGEL...	..KQNKIQPIFHGKT	LAMIFEKST	RTRVSVFVAM	AQLGGHALFL
OTC_PYRFU	-----MKVQ	LGRDLLCLQ	DYTAEEIWTI	LETAKMFK.I	WQIKGPHRLLESKT	LAMIFQKPS	RTRVSVFVAM	SAQDLQLRRG
OTCA_MYCBO	-----	MTIRHFLRDD	DLSPABQAEV	LELAELK.K	DEVSRR..PLQGPRG	VAVIFDKNST	RTRFSEFLGI	AQLGGHVVIV
OTCC_PSEAE	-----AFN	MKNRNLLSLM	HHTSTRELRYL	LDLSRDLKRA	KYTG.....	TEQQHLKRRN	IALIFEKTS	RTRCAFEVAA	YDQGANVTYI
OTCC_NEIGO	-----MN	LKNRHFLKLL	DFTPEITAY	LDLAELKDA	KYAG.....	REIQRMKGKN	IALIFEKTS	RTRCAFEVAA	RDQGAQVTYI
OTCC_CLOPE	-----MAVN	LKGRSPLTLK	DFTPAEIRYL	LDLSHDLKAK	KRAG.....	ILGDSLGKGN	VVLLFEKTS	RTRCAFCBGA	AEBGAHVTVL
									..
OTC_HUMAN	ESLTDARVL	SSMADAVLAR	VYKQSDLDL	AKEASIPIN	GLSDLYHPIQ	ILADYLTQE	HYS.....	..SLKGLTL	SWIGDG.NNI
OTC_MOUSE	ESLTDARVL	SSMADAVLAR	VYKQSDLDL	AKEASIPIN	GLSDLYHPIQ	ILADYLTQE	HYS.....	..SLKGLTL	SWIGDG.NNI
OTC_RAT	ESLTDARVL	SSMADAVLAR	VYKQSDLDL	AKEASIPIN	GLSDLYHPIQ	ILADYLTQE	HYS.....	..SLKGLTL	SWIGDG.NNI
OTC_RANCA	ESLTDARVL	SSMADAVLAR	VYKQSDLDL	AKEASIPIN	GLSDLYHPIQ	ILADYLTQE	HYS.....	..SLKGLTL	SWIGDG.NNI
OTC_FACTA	ESLHDTTKI	SSMTSSIFAR	VNKHSDIQEM	CKYSSVPIIN	ALCDTFPHLQ	AITDILTKE	SFGNT....	..TKGLKL	AWIGD.VNVV
OTC_YEAST	ESLYDTSKVI	SSMVSIVAR	VNKSVDVATL	AKHASCVPIN	GLCDTFPHLQ	ALADLLTKE	TF.KS....	..FDGLKG	AWIGD.ANVV
OTC_SCHPO	ESFYDTTKV	SSMVSICIFAR	VNKHEDILAF	CKDSSVPIIN	SCLDTPHPLQ	AICDLLTIE	NFNISLDEVN	KGINSK.LKM	AWIGD.ANVV
	87	97	107	117	127	137	147	153	159
OTC1_ECOLI	ESIKDTRVVL	GRMYDGIQVR	GYGQEIIVETL	AEVASVFPVN	GLTNEFHPQ	LLADLLTQE	HLPGKA....	..FNEMTL	VYAGDARNNM
OTC_HAEIN	ESMKDTRVVL	GRMYDGIQVR	GYGQEIIVETL	AEVASVFPVN	GLTNEFHPQ	LLADLLTQE	HLPGKA....	..FNEMTL	VYAGDARNNM
OTCP_PSESH	ESIKDTRVVL	GRMYDGIQVR	GYGQEIIVETL	AEVASVFPVN	GLTNEFHPQ	LLADLLTQE	HLPGKA....	..FNEMTL	VYAGDARNNM
OTC_ASPTA	ESLYDTAVVV	SSMVSIAVAV	VGHKAEVADL	AKHSTVPVIN	ALCDSPHPLQ	VIADVMTKE	EFG.R....	..IEGVTI	AVYDGRNNM
OTCA_PSEAE	EPIDGSRVVM	SRMLDGMVIR	TFAHATLTFE	AAHSKVPVIN	GLSDLLHPCQ	LLADMOTFE	HRG.....	..SIQKTV	AWIGD.NNV
OTC2_BACSU	ETVADTAKVL	SRVVDAIMIR	TFEHEKVEEL	AKHADIPVIN	GLTDKYVHPQ	ALADLLTKE	IKG.....	..KLGKTV	AVYDGD.NNV
OTC_PYRFU	ETIADTRVVL	SRVVDAIMIR	VYDHDVDEL	AKYATVFPVIN	GLSDFSHPCQ	ALADVMTKE	KKG.....	..TIKGVK	VYVGD.NNV
OTCA_MYCBO	ETLQDTAKVL	SRVVDAIMIR	TFGQERLDM	ASVATVFPVIN	ALSDPEHPQ	VLADLQTI	RKG.....	..ALRGLR	SYFGDANNM
OTCC_PSEAE	ESMKDTRVVL	GRMYDGIQVR	GYGQEIIVETL	AEVASVFPVN	GLTNEFHPQ	LLADLLTQE	HLPGKA....	..FNEMTL	VYAGDARNNM
OTCC_NEIGO	ESIKDTRVVL	GRMYDGIQVR	GYGQEIIVETL	AEVASVFPVN	GLTNEFHPQ	LLADLLTQE	HLPGKA....	..FNEMTL	VYAGDARNNM
OTCC_CLOPE	ESIEDTAKVL	GRMYDGIQVR	GYGQEIIVETL	AEVASVFPVN	GLTNEFHPQ	LLADLLTQE	HLPGKA....	..FNEMTL	VYAGDARNNM
									..
OTC_HUMAN	FGMHLQAATP	KGYPEPDSVT	KLAEQYAKEN	G.....T.K	LLTNDPDLA	AHGGNVLITD	TWISMGEQEE	K.KKRLQAFQ	GYQVTMTAK
OTC_MOUSE	FGMHLQAATP	KGYPEPDSVT	KLAEQYAKEN	G.....T.K	LLTNDPDLA	AHGGNVLITD	TWISMGEQEE	K.KKRLQAFQ	GYQVTMTAK
OTC_RAT	FGMHLQAATP	KGYPEPDSVT	KLAEQYAKEN	G.....T.R	LLTNDPDLA	AHGGNVLITD	TWISMGEQEE	K.KKRLQAFQ	GYQVTMTAK
OTC_RANCA	FGMHLQAATP	KGYPEPDSVT	KLAEQYAKEN	G.....T.K	LLTNDPDLA	AHGGNVLITD	TWISMGEQEE	K.KKRLQAFQ	GYQVTMTAK
OTC_FACTA	SGIDVSIAPV	SGLKFPEELIL	SGAKELSAEV	G.....TTLK	I..TNDPDLA	INGANVLITD	TWISMGEQEE	R.LQKQKQFE	GFQITKEMIS
OTC_SCHPO	VGIHTSVAKP	KDVNVRRDIL	SIVNEAANEN	G.....STPE	I..VNDPKVA	VKNADIVVTD	TWISMGEQEE	K.EQRKQFT	GFQVTGEIMK
OTC_YEAST	PGISVSIAPV	PGIEMDSIVT	DEAKVAERN	G.....ATPE	L..THDSLKA	STANILLVTD	TFVSMGEQEE	K.OAKLQKQF	GFQITQELVS
	179	189	194	202	212	222	232	242	262
OTC1_ECOLI	TGLDLRLVAP	QACWP.....	EAALVTEC	RALAQQNGN	ITLTEDVAKG	VGGADFIYTD	VWVSMGEAKE	KWAERIALLR	EYQVNSKMMQ
OTC_HAEIN	LGMVDRICGP	KALLP.....	EANLVTEC	EKFAKESGR	ITVTEIDKLA	VKGVDFIHTD	VWVSMGEAKE	TWGERIKLL	EYQVTEPMK
OTCP_PSESH	FGYLNRIIAP	NALHP.....	TDVAVLAGI	YEQTPEPNSG	IELTFTEVAG	VHQADVIYTD	VWVSMGESVS	V.EERIALLK	PYVTEKEMA
OTC_ASPTA	MGVDLAVATP	KGYPEPDSVT	ELIQRAGKV	A.....NPKG	LQITVTPPEA	VKADILVTD	TWVSMGEQEE	S.LKRMKPE	GFQITSELAK
OTCA_PSEAE	FDQLRVACP	EGYEPKAEFV	ALAGDR....LRVDRPKE	VWAGHVLVTD	VWVSMGEQEE	A.AARIAMFR	PYQVNAALLD
OTC2_BACSU	MGCDISIASP	KGYPELDEAA	EAAKYATQS	G.....S.S	VTLTDDPTEA	VKADVIYSD	VFTSMGEQEE	A.QERLAVFA	PYQVNAALLD
OTC_PYRFU	LGADVVVATP	EGYEDDEKVI	KWAEQNAAES	G.....S.S	FELLHDPVKA	VKADVIYTD	VWVSMGEQEE	A.EERRKIFR	PFQVNAALLD
OTCA_MYCBO	AGIHVTVVAP	EGFLPDPVSR	AAAEARQDT	G.....A.S	VTVTADAHAA	AAAADVLTVD	TWVSMGEQEE	G.LDRVKKFR	PFQVNSRLLA
OTCC_PSEAE	LGMVDRICGP	KALLP.....	HDEFVACQ	KKFABESGAK	ITLTEDPKEA	VKGVDVHTD	VWVSMGEQEE	AWGERIKELL	PYQVNETMK
OTCC_NEIGO	LGMVDRICGP	QSLWP.....	SBGITAAA	HAAAKETGAK	ITLTENAHEA	VKGVDVHTD	VWVSMGEQEE	VWQERIKELL	DYRTVTEIMK
OTCC_CLOPE	MGHVFALGP	DSLKP.....	DEDILKEM	QYESKETGAT	IEFSSNVDEA	VKADVIYTD	IWVSMGEQEE	LYPEVVKLLT	PYKVTREMMN
									..
OTC_HUMAN	LHCLPRKPE	VDDEVFYSR	SLVFPPEAENR	KWTFIMAVMVS	LLTDYSPQLQ	KPKF
OTC_MOUSE	LHCLPRKPE	VDDEVFYSR	SLVFPPEAENR	KWTFIMAVMVS	LLTDYSPVLQ	KPKF
OTC_RAT	LHCLPRKPE	VDDEVFYSR	SLVFPPEAENR	KWTFIMAVMVS	LLTDYSPVLQ	KPKF
OTC_RANCA	LHCLPRKPE	VDDEVFYSR	SLVFPPEAENR	KWTFIMAVMVS	LLTDYSPQLQ	RPTF
OTC_FACTA	MHCLPRHPE	VHDEVFVDEE	RSLVFEEGEN	RLYAATAVLE	GFVNVKGLL
OTC_SCHPO	MHCLPRHPE	VSDEVFYGEN	SLVFPPEAENR	KWTFIVAVLEA	LLVNRGEILP	PASA
OTC_YEAST	MHCLPRHPE	VSDVVFYGEH	SIVFEEAENR	LYAAMSAIDI	FVNNRKGFKD	LK--
	271	281	291	300	310	320	330		
OTC1_ECOLI	LHCLPAFHDD	QITLGGKMAE	EF.GLHGME	VTEVPESAA	SIVFDQAEENR	MHTIKAVMVA	TLSK--
OTC_HAEIN	MHCLPAFHNS	ETKVGRIAE	KYPELANGIE	VTEVPESPM	NIAPFQAEENR	MHTIKAVMVA	SLA--
OTCP_PSESH	MHCLPAFHDL	DTEVARE...	TPDLIVE	VEDEVFEGPQ	SRVFDQGENR	MHTIKALMLE	TVVP--
OTC_ASPTA	MHCLPAHRE	VSEDEVFYSNR	SLVFPPEAENR	LMAAISALEG	FVNVKGLIA
OTCA_PSEAE	MHCLPAHRGE	ISEELLDDPR	SAVWDQAEENR	LHAQKALLEL	LTEHAHYA
OTC2_BACSU	LHCLPAHREE	VTAELIDGN	SAVFDQAEENR	LHVQKALLA	ILYKGESSKN	C----
OTC_PYRFU	MHCLPAHRGE	VTTDDVDSFN	SAVWDQAEENR	LHAQKAVLAL	VMGGTKF
OTCA_MYCBO	LHCLPAHRGD	ITDAVMDGPA	SAVWDQAEENR	LHAQKAVLW	LLERS
OTCC_PSEAE	MHCLPAFHNS	ETKVGRIAE	QYFN..NGIE	VTEVPESPM	NIAPFQAEENR	MHTIKAVMVA	TLADI
OTCC_NEIGO	MHCLPAFHNR	ETKVGRIE	TFG..LNGVE	VTEVPESPA	GIVFDQAEENR	MHTIKAVMVA	ALGD--
OTCC_CLOPE	MHCLPSFHDE	DTEVCKDRWI	DLG..LDIRE	VEDEVFRSNK	SAVFDQAEENR	MHTIKAVMVA	TAGR--
									..

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 terreus*, *Bacillus subtilis*, *Pyrococcus furiosus*, *Mycobacterium bovis*, *Pseudomonas aeruginosa*, *Neisseria gonorrhoeae*, and *Clostridium perfringens*. * indicates sequences not shown for clarity.

	3	13	23	31	37	43	53	63	73	
PYRB_ECOLI	~~~~~AN	PLVQKHIISI	NDLSRDDLNL	VLATAAKL...	...KANPQP	ELL...KHK	VIASCFFEAS	TRTRLSFETS	MHRLGASVVG	FSDSANTSLSG
PYRB_SALTY	~~~~~AN	PLVQKHIISI	NDLSRDDLNL	VLATAAKL...	...KANPQP	ELL...KHK	VIASCFFEAS	TRTRLSFETS	MHRLGASVVG	FSDSANTSLSG
PYRB_SERMA	~~~~~AN	PLVYKHIISI	NDLSRDDLEL	VLATAAGL...	...KANPQP	ELL...KHK	VIASCFFEAS	TRTRLSFETS	MHRLGASVVG	FADGNTSLSG
PYRB_PYRAB	~~~~~M	DWKGRDIVSI	RDFSKEDIET	VLATAERLER	EL...KEGQL	EYA...KKG	ILATLFFEPS	TRTRLSFESA	MHRLGGAVIG	FAEA.STSSV
PYRB_SULSO	~~~~~	~MKHIIISA	YNFSRDELED	IFALATDKYSK	NL...NDRKI	...L...SGK	TISIAFFEPS	TRTYLSFQKA	IINLGGDVIG	FSGE.ESTSV
PYRB_ARATH	*MQAGTRELK	KFELSDVIBG	KQFDREMLSA	IFDVAREMEK	IE...KSSQS	EIL...KGY	LMATLFFEPS	TRTRLSFESA	MKRLGGVEIT	TENAREFSSA
PYRB_BACCL	~~~~~	~MTHLFAL	SELPLDEIHR	LLDEAERFRS	GRIWR...S	PAA...PM	VVANLFFEPS	TRTKCSFEMA	ERLGLHVIP	FD..PERSSV
PYRB_BACSU	~~~~~	~SELSTEEIKD	SELSTEEIKD	LLQTAQELKS	GKTDN....	QLT...GK	FAANLFFEPS	TRTRFSFEVA	EKKLGMNVLN	LD..GTSTSV
PYRB_LACLE	*VETEDKQDN	LLRLPYFVSV	BQLSADDVLH	LLQRAQYFKN	GGEVP....	ALS...RPI	FCTNMFENS	TRTHTSFEVA	ERLGLTVIP	FD..PSHSSV
PYRB_LACPL	~~~~~	MKTSQNLVSV	BQFSNQDVMA	YLKLAQAFKN	GKTV....S	QLS...RPT	FAMNLFENS	TRTHTSFEVA	ERLGLQVIP	FD..PKTSSV
PYRB_PSEAE	*AKRPLQLND	QGQLRHFLSL	DGLPRELLTE	ILLTADSFLF	.VGARAVKVV	PLL...RGT	TVCNVFFENS	TRTRTFTELA	AQRLSADVIS	LN..VSTSSV
PYRB_PSEFU	*AKRPLQLND	QGQLRHFLSL	DGLPRELLTE	ILLTADSFLF	.VGARAVKVV	PLL...RGT	TVCNVFFENS	TRTRTFTELA	AQRLSADVIS	LN..VSTSSV
PYRB_MYCTU	~~~~~	~MTPRHLLTA	ADLSRDDATA	ILLDADRFAQ	ALVGRDICKL	PTL...RGR	TVVTFMFENS	TRTRVSFEVA	GKMSADVIN	VS..AAGSSV
PYRB_SYNY3	~~~~~MTMVA	SWRNHILLDL	TDRRGEELDI	YLQATHTFQQ	VLGSGQ.TKKV	PAL...QGQ	VVTNLFFFENS	TRTRSSFEVA	AKRLSADVIN	FS..PGTSSV
PYRB_TREDE	~~~~~MEN	KFMGRSLTVI	DDLSIDERKY	LFDKTKRLKK	AIQEDDQKVM	DEFRINDKDF	GIYEVFLEPS	TRTKESFRNA	AKF..HQVKL	SDLAESSSV
							•••			
	83	93	102	112	118	122	132	142	152	155
PYRB_ECOLI	KKGETLADTI	SVLSTY.VDA	IVMRHPQEGA	ARLATE....	F.....SGN	VPVLNAGDGS	NQHPTQTLDD	LFTIQETQGRLDN	LHVAMVGLDK
PYRB_SALTY	KKGETLADTI	SVLSTY.VDA	IVMRHPQEGA	ARLATE....	F.....SGQ	VPVLNAGDGS	NQHPTQTLDD	LFTIQETQGRLDN	LHIAMVGLDK
PYRB_SERMA	KKGETLADTI	SVLSTY.VDA	IVMRHPQEG.	ARMASE....	F.....SGN	VPVLNAGDG.	NQHPTQTLDD	LFTIQETQGRLDN	LSIAMVGLDK
PYRB_PYRAB	KKGESLRDIT	KTVBQY.CDV	IVIRHPKEGA	ARLAAE....	V.....A.E	VPVINAGDGS	NQHPTQTLDD	LYTIRKFEFRIDG	LKIGLLGLDK
PYRB_SULSO	AKGENLADTI	RMLNRY.SDG	IVMRHKYDGA	SRFASE....	I.....S.D	IPVINAGDGK	HEHPTQAVID	TYTINKHFNTIDG	LVFALLGLDK
PYRB_ARATH	AKGETLEDTI	RIVVEGY.SDI	IVMRHPFESGA	ARKAAA....	T.....A.N	IPVINAGDGP	GEHPTQALLD	VYTIQSEIGKLDG	ISVALVGLDA
PYRB_BACCL	QKGETLYDVT	RTLLEAIGVDA	VVIRRHEDAY	FEALR....	H.....AVG	IPINAGDGC	GHHPTQSLDD	LTIIRQEPFGAFG	LTVAIIGDIR
PYRB_BACSU	QKGETLYDIT	RTLLESIGDVL	CVIRHSEDEY	YEELV....	S.....QVN	IPILNAGDGC	GQHPTQSLDD	LMTIYEEFN.TFKG	LTVSIGHDIK
PYRB_LACLE	NKGENLYDTE	LTMASLGIEL	SVIRHPENAY	YNEIIRPEKG	Q.....HLQ	MGLVNAAGDGS	GQHPSQSMLD	MMTIYNEFG.HFDG	LKIMIVGLDLT
PYRB_LACPL	TKGESLDDTL	KTLEAIGVNL	AVVRHPDRDY	Y...QPLLD	A.....GFD	MSLINAGDGS	GQHPSQSMLD	MLTIYEEFG.HFDG	LKIAIVGLDA
PYRB_PSEAE	SKGETLTDTL	RNLEAMAADM	FVVRHSDSGA	AHFIAEHV..SPN	VAVINGGDGR	HAHPTQGMDD	MLTIRRHKGNFEQ	LSVAIVGDIL
PYRB_PSEFU	SKGETLTDTL	RNLEAMAADM	FVVRHSDSGA	AHFIAEHV..CPD	VAVINGGDGR	HAHPTQGMDD	MLTIRRHKGNFEN	LSVAIVGDIL
PYRB_MYCTU	KGESLRDITA	LTLRAGADA	LIIRHPASGA	AHLLAQWTGA	H.....NDG	PAVINAGDGT	HEHPTQALLD	ALTIRQRLGGIEG	RRIVIVGDIL
PYRB_SYNY3	TKGETILDTA	KTYLAMGSDI	FVIRHQQAGV	PHFIASQMDR	L.....QTG	VKVLNAGDQG	HEHPSQSMLD	LFTICSQFAP	DNPAIQCLQG	KKIAVVGDLI
PYRB_TREDE	NKGESYADTF	NTLAGYQNSI	FIVRSEVEGV	CRWLEDEAQA	FYQRNLLKRR	PAFINAGDGG	HEHPTQELDD	EFTFIED..	...NNWSPDK	IHIALVGDLY
							▽▽			
	165	174	184	194	204	214	224	234	242	246
PYRB_ECOLI	YGRTVH.SLT	QALAKFDGNR	FYFIAPDALA	MPQYILDMLD	EKGIAWSLHS	STEEVMAEVD	ILYMTRVQKE	RL..DPSEYA	NVKA.....	QFVLRASDLH
PYRB_SALTY	YGRTVHFAKP	RTLAKFSGNR	FYFIAPDALA	MPQYILDMLD	EKGMAWSLHG	STEEVMAEVD	ILYMTRVQKE	RL..DPSEYA	NVKA.....	QFVLR.PDLN
PYRB_SERMA	YGRTVH.SLT	QALAKFEGNR	FYFIAPDALA	MPAYILKMLE	EKGIEYSSHG	STEEVVEPELD	ILYMTRVQKE	RL..DPSEYA	NVKA.....	QFVL.AADLA
PYRB_PYRAB	YGRTVH.SLA	EALTFYDVLEL	.YLISPELLR	MPRHIVEELR	EKGIMKVVEPT	TLEDVIGKLD	VLVYTRIQKE	RFPDQE.YL	KVKG.....	SYQINLVKLE
PYRB_SULSO	YARTVN.SLL	RLLTRFRPKL	VYLISPELLR	ARKEILDEL.	.NYPVKEVE	NEPVEINEVD	VLVYTRIQKE	RFDVEME.YE	KIKG.....	SYIVSLDLAN
PYRB_ARATH	NGRTVH.SLA	YLLAKFKDVK	IYFVSPETVK	MKDDIKDYL	SSGVVEWESS	DLMEVASKCD	VVYQTRIQRE	RFGERLDLVE	AARG.....	KFIVDKDLG
PYRB_BACCL	YGRTVH.SLA	EALTFYDVLEL	.YLISPELLR	MPRHIVEELR	EKGIMKVVEPT	TLEDVIGKLD	VLVYTRIQKE	RFPDQE.YL	KVKG.....	SYQINLVKLE
PYRB_BACSU	HSR.VARSNA	EVLTRLGA.R	VLFSGPSEWQ	D..EENTPFT	YVS.....	.MDEAVESSD	VMMLLRIQNE	RHQ.S.AVSK	E...GYLN	KYGLTVERAE
PYRB_LACLE	NSR.VARSNM	EILLNLGA.E	VYFSGPEYWY	NAEEFYKGT	YVK.....	NIDDEIPELD	VMMLLRVQHE	RHNGAEAVSE	QLPDAKYNA	AYGLNQRRYD
PYRB_LACPL	HSR.VARSNM	EILLNLGA.Q	VYFSGPKEWY	GRDFPEYGT	YVE.....	.GWPATVSH	VMMLLRVQHE	R...LSQVNN	QTFDASAYHQ	QYGLTARAA
PYRB_PSEAE	HSR.VARSNM	LALKTLGCPD	IRVLABATLL	PIGLBE...	.QYGVVFT	NADEGLKDV	VVIMLRLQRE	RMQGLG...	.LPSEGEFFK	LYGLTEKRLK
PYRB_PSEFU	HSR.VARSNM	LALKALGCPD	IRVIGPKTLI	PIGI.E...	.QYGVVFT	DLAEGKLDV	VVIMLRLQRE	RMAGGL...	.LPSEGEFVR	LYGLTARLA
PYRB_MYCTU	HSR.VARSND	MLLDLTGA.E	VVLVAPPTLL	PVGVV....	.GWPATVSH	DFDAELPAAD	AVLMLRVQAE	RMNGGF...	.PVSREYVS	RYGLTERRQA
PYRB_SYNY3	HSR.VARSNL	WSLTTAGA.D	VHLAGPPTLL	KPEFQQLTIA	PGSGKLHCHW	QLQPALEGAD	IVMTLRLQKE	RMTAHL...	.LPSLREYVH	YFGITHDRLK
PYRB_TREDE	HGRTVH.SKA	DGLKIFKSVK	VDLIAPAEIA	MEPYKVRMQ	ENGTVREFS	SIEBYLRQAD	VALIWFTRP	QL..ERMGEQ	VLKKQDELRR	SITFRKEFIE
	256	266	274	283	293	303				
PYRB_ECOLI	NAKANMKVLH	PLP..RVDEI	ATD.VDKTPH	AWYFQQAGNG	IFARQALLAL	VLNRLDLV~	~~~~~	~~~~~	~~~~~	~~~~~
PYRB_SALTY	GARENMKVLH	PLP..RIDEI	TTD.VDKTPH	AWYFQQAGNG	IFAAQALLAL	VLNSELSL~	~~~~~	~~~~~	~~~~~	~~~~~
PYRB_SERMA	GA.ANLKVLH	PLP..RIDEI	ATD.VDKTPH	AYYFQQAGNG	IFARSA.LAL	VVNADLAL~	~~~~~	~~~~~	~~~~~	~~~~~
PYRB_PYRAB	KAQDELRIH	PLP..RVDEI	HPE.VDNTKH	AIYFRQVFN	VPVRRMALLAL	VLGVI~	~~~~~	~~~~~	~~~~~	~~~~~
PYRB_SULSO	KMKKDSILH	PLP..RVNEI	DRK.VDKTK	AKYFQASYG	VPVRRMILTK	IYGE~	~~~~~	~~~~~	~~~~~	~~~~~
PYRB_ARATH	VMQKAIIMH	PLP..RLDEI	TAD.VDADPR	AAVFRQAKNG	LFIRRMALLKL	LLVGW~	~~~~~	~~~~~	~~~~~	~~~~~
PYRB_BACCL	RMKSGAILH	PAPVNRGVEI	ASELVEAKA.	SRIFFQMENG	VYVRMAVLKR	AMEGRMEHGR	MAEKWHVVQ~	~~~~~	~~~~~	~~~~~
PYRB_BACSU	RMKRHAIMH	PAPVNRGVEI	DDSLVESEK.	SRIFFQMENG	VFIRMAVIQC	ALQTNVVRKE	AAVVISH~	~~~~~	~~~~~	~~~~~
PYRB_LACLE	MLKDDAIMH	PGPINRGVEW	DGDLVEAPK.	SRYAVQMHNG	VFVRMAMIEA	VLRGRKLGGL	E~	~~~~~	~~~~~	~~~~~
PYRB_LACPL	RMPKHAIMH	PAPVNRGVEL	ASDLVEAPQ.	SRIFFQMTNG	VYIRMAVMS	VLAHQGLISA	TQVEV~	~~~~~	~~~~~	~~~~~
PYRB_PSEAE	LAKPDAIVMH	PGPINRGVEI	ESAVADGAQ.	SVILNQVTYG	IATRMAVLSM	AMSGQNTQRQ	LEQEDAE~	~~~~~	~~~~~	~~~~~
PYRB_PSEFU	CAKPDIAIVMH	PGPINRGVEI	ESAVADGKH.	SVILNQVTYG	IAVRMAVLSM	AMSGQNAQRQ	FDQENAQ~	~~~~~	~~~~~	~~~~~
PYRB_MYCTU	MLPGHAVVLH	PGPMVRGMEI	TSSVADSSQ.	SAVLQQVNSG	VQVRMAVLFH	VLVGAQDAGK	EGAA~	~~~~~	~~~~~	~~~~~
PYRB_SYNY3	VCPGKVLH	PGPMVRGVEI	SSELMDPDI	SILIQDQVTS	VAIRMALLVY	LGTVQE~	~~~~~	~~~~~	~~~~~	~~~~~
PYRB_TREDE	KLPENTRFYH	PLPRHRVHPT	IPTFLDATPL	NGWERQSING	MYVRMVLMS	IAGKIGDDYK	GPEPKSCERV	EDEDYIVEV*		

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 caldolyticus*, *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*

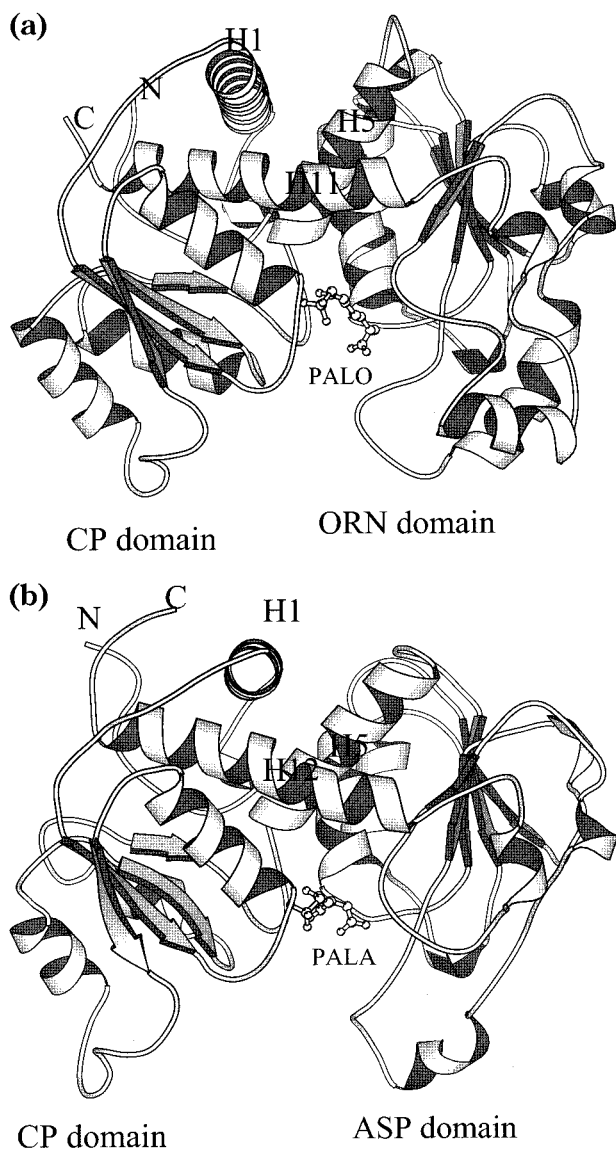


FIGURE 4. Ribbon drawing of subunit of (a) *E. coli* OTCase and (b) ATCase. The bisubstrate analogues PALA or PALO, which are located in the cleft between the CP domain and the L-Asp or L-Orn domain, are shown as ball-and-stick models. The $\alpha 9a$ loop, present in *E. coli* OTCase adjacent to the 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 phosphate 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

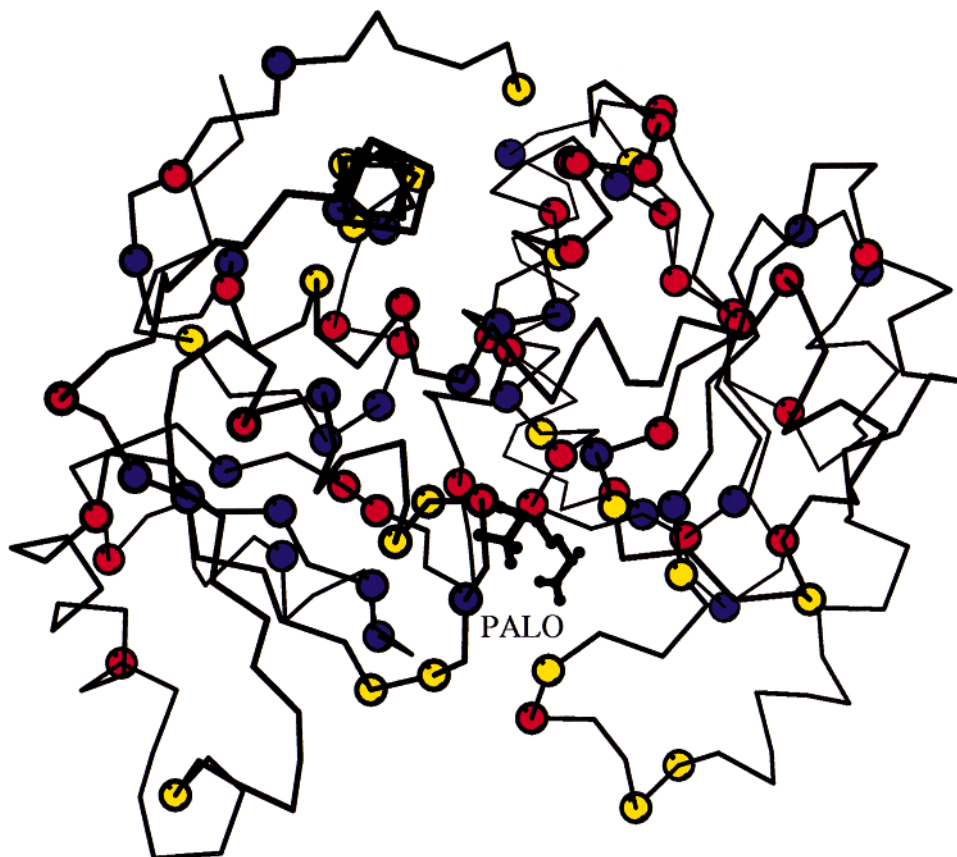


FIGURE 5. Deleterious mutations found in patients with OTCD mapped on the structure of human OTCase. Mutations which produce neonatal OTCD are shown in red, those identified only in females are in blue, and those which produce late onset OTCD are in yellow.

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 \rightarrow 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 archaeobacterium, 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

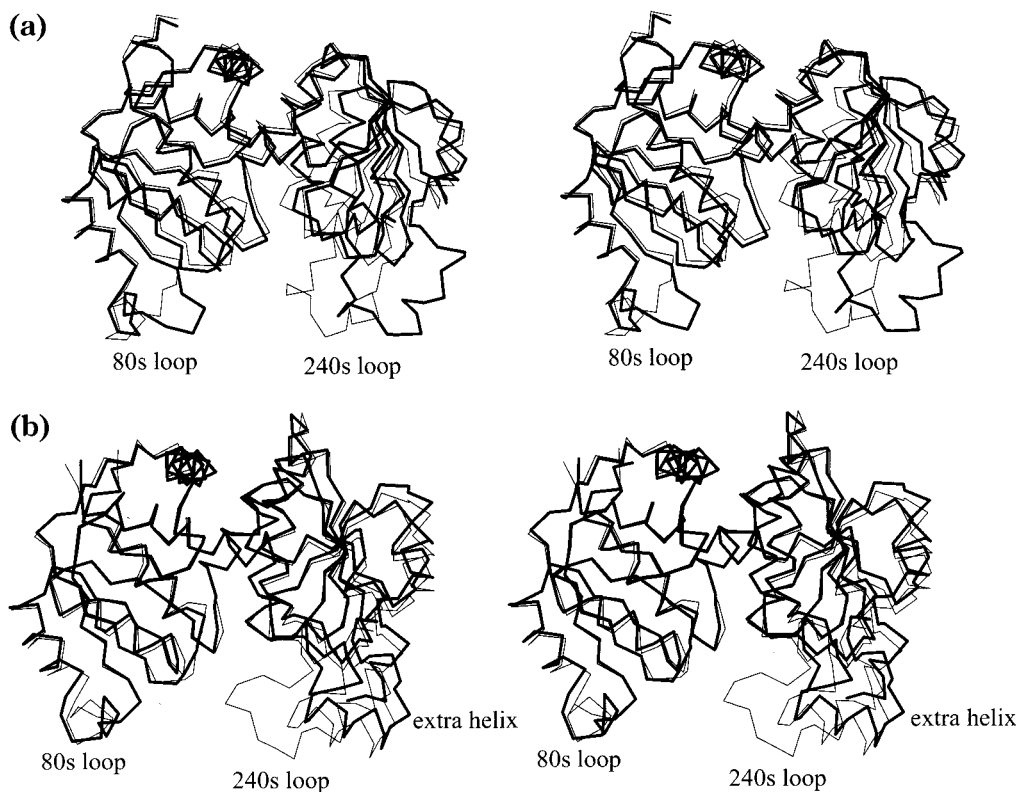


FIGURE 6. Superposition of liganded (thin line) and unliganded (thick line) catalytic subunits of (a) *E. coli* ATCase and (b) OTCase. The Protein Data Bank references for the structures are 8ATC (liganded *E. coli* ATCase), 1RAI (unliganded *E. coli* ATCase), 2ORT (liganded *E. coli* OTCase), and 1AKM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

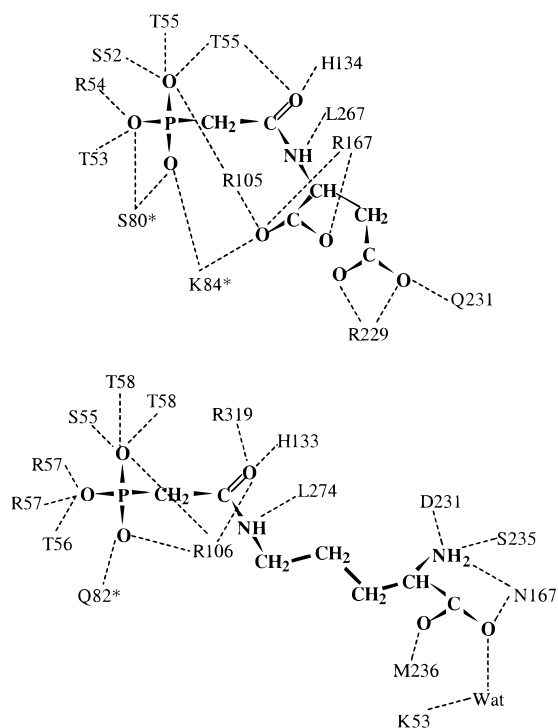


FIGURE 7. Schematic drawing showing the interaction of (a) the bisubstrate analogue PALA with active-site residues in *E. coli* ATCase and (b) the bisubstrate analogue PALO with active-site residues in *E. coli* OTCase. The residue indicated with * is from an adjacent subunit.

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 (α 9a) present in both *E. coli* and *P. aeruginosa* catabolic OTCase which interacts with the 240s loop,

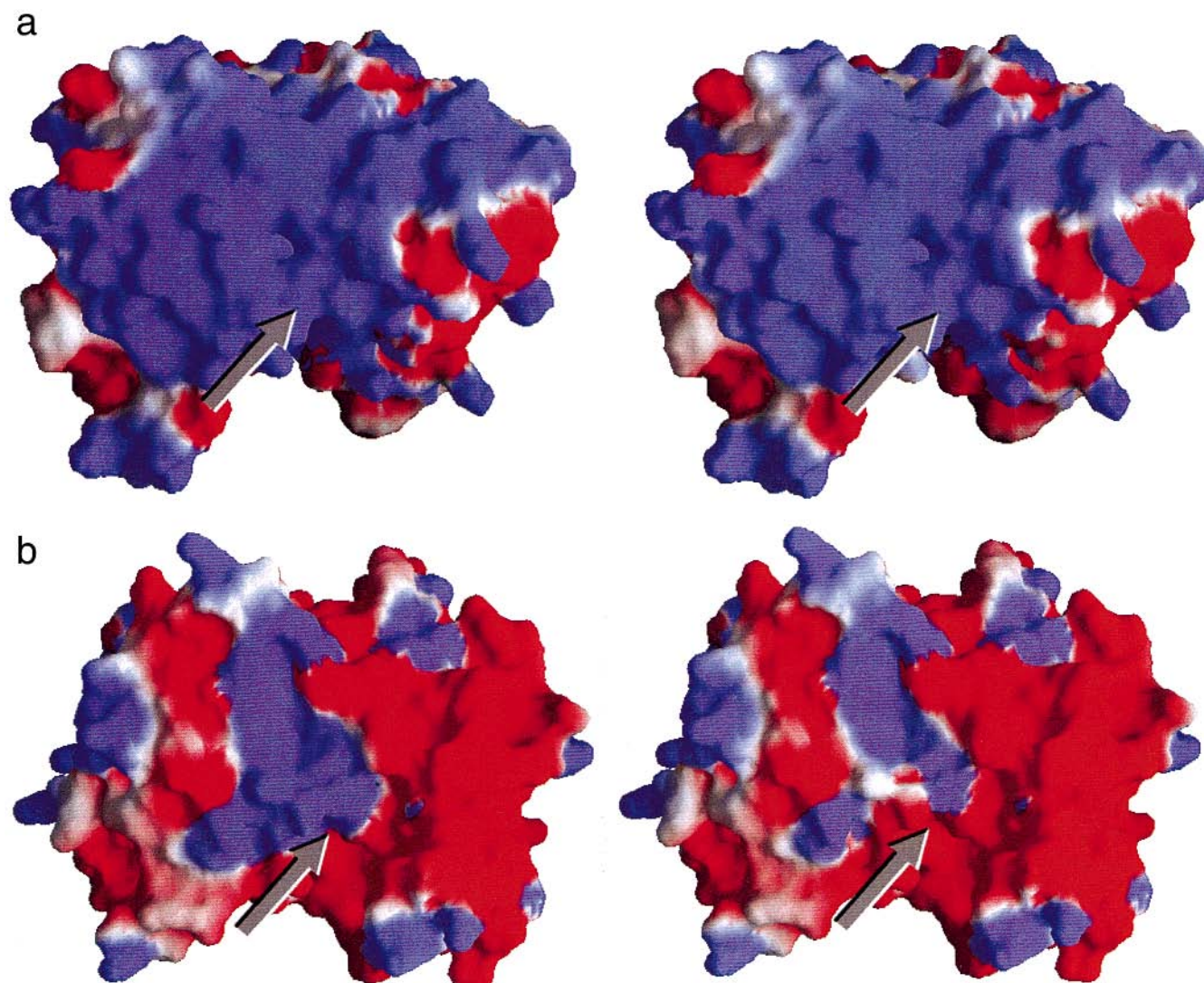


FIGURE 8. Electrostatic potential of (a) *E. coli* ATCase and (b) OTCase catalytic subunit before and after binding the first substrate CP. Potentials greater than +1 kT are shown in blue, and those less than -1 kT in are shown in red, mapped onto the molecular surface. The subunit is viewed in the same 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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