Structure and Catalytic Mechanism of a Novel N-Succinyl-l-ornithine Transcarbamylase in Arginine Biosynthesis of Bacteroides fragilis*

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A Bacteroides fragilis gene (argFby), the disruption of which renders the bacterium auxotrophic for arginine, was expressed and its recombinant protein purified and studied. The novel protein catalyzes the carbamylation of N-succinyl-l-ornithine but not l-ornithine or N-acetyl-l-ornithine, forming N-succinyl-l-citrulline. Crystal structures of this novel transcarbamylase complexed with carbamyl phosphate and N-succinyl-l-norvaline, as well as sulfate and N-succinyl-l-norvaline have been determined and refined to 2.9 and 2.8 Å resolution, respectively. They provide structural evidence that this protein is a novel N-succinyl-l-ornithine transcarbamylase. The data provided herein suggest that B. fragilis uses N-succinyl-l-ornithine rather than N-acetyl-l-ornithine for de novo arginine biosynthesis and therefore that this pathway in Bacteroides is different from the canonical arginine biosynthetic pathway of most organisms. Comparison of the structures of the new protein with those recently reported for N-acetyl-l-ornithine transcarbamylase indicates that amino acid residue 90 (B. fragilis numbering) plays an important role in conferring substrate specificity for N-succinyl-l-ornithine versus N-acetyl-l-ornithine. Movement of the 120 loop upon substrate binding occurs in N-succinyl-l-ornithine transcarbamylase, while movement of the 80 loop and significant domain closure take place as in other transcarbamylases. These findings provide new information on the putative role of succinylated intermediates in arginine biosynthesis and on the evolution of transcarbamylases.

The canonical arginine biosynthetic pathway in microorganisms and plants involves N-acetylated intermediates in the first five steps. At the fifth step, N-acetyl-l-ornithine is deacetylated either hydrolytically to form ornithine in the linear pathway or by transfer of the acetyl group to glutamate to regenerate N-acetyl-l-glutamate in the cyclic pathway. Subsequently, ornithine is converted to arginine by three enzymes: ornithine transcarbamylase (OTCase),3 argininosuccinate synthase and argininosuccinate lyase (1). We have recently shown that in some eubacteria (e.g. Xanthomonas), N-acetyl-l-ornithine rather than ornithine is carbamylied by a novel transcarbamylase that is essential for arginine biosynthesis (2, 3). In 2002, we reported the crystal structure of a transcarbamylase-like protein from Bacteroides fragilis with phosphate bound at 2.0 Å resolution (4). At the time, we postulated that it is a novel member of the transcarbamylase family, since no activity could be detected with ornithine or other amino acids that are known transcarbamylation substrates, and the crystal structure indicated that it has a unique second substrate binding pocket. We now provide evidence that N-succinyl-l-ornithine is a substrate for this novel transcarbamylase and that this protein is essential for de novo arginine biosynthesis in B. fragilis. These data imply that B. fragilis uses N-succinylated ornithine for arginine biosynthesis and is the first organism known to do so.

EXPERIMENTAL PROCEDURES

Production of B. fragilis Arginine Auxotrophs and Identification of the argFby Gene—Several arginine requiring mutants were isolated after mutagenizing B. fragilis ADB77, a thyA− derivative of TM4000 (5) with transposon Tn4400' (6). Using the “clone out” protocol of Tang and Malamy (6), we were able to determine the chromosomal sequence on both sides of the transposon insertions. Strains JDP3a.3 and JDP3a.4 contained insertions in a gene with high similarity to the argF genes encoding OTCase in eubacteria; we designated this gene as argFby.

3 The abbreviations used are: OTCase, ornithine transcarbamylase; AOTCase, N-acetyl-l-ornithine transcarbamylase; CP, carbamyl phosphate; NCS, non-crystalllographic symmetry; PDB, Protein Data Bank; r.m.s., root mean square; SNO, N-succinyl-l-norvaline; SOTCase, N-succinyl-l-ornithine transcarbamylase; LC-MS, liquid chromatography-mass spectrometry; BisTris, 2-[bis(2-hydroxyethyl)amino]-2-(hydroxymethyl)propane-1,3-diol.
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TABLE 1
Data collection and refinement statistics

| Ligands | CP and SNO | SO₄ and SNO |
|---------|------------|-------------|
| Space group | P4₁ | P4₁ |
| Resolution (Å) | 2.9 | 2.8 |
| Unit-cell parameters (Å) | \(a = b = 156.42\) | \(a = b = 157.70\) |
| | \(c = 120.65\) | \(c = 120.55\) |
| Measurements | 354,442 | 316,634 |
| Unique reflections | 64,660 (6420*) | 70,538 (7055) |
| Redundancy | 5.5 (5.1) | 4.5 (4.0) |
| completeness (%) | 99.9 (99.9) | 99.6 (99.9) |
| \((I/\sigma (I))\) | 9.0 (1.7) | 16.2 (2.1) |
| \(R_{	ext{merge}}\) | 15.4 (87.1) | 9.9 (76.4) |
| Wilson B (Å²) | 75.5 | 69.7 |
| Resolution range (Å) | 50-2.9 | 50.0-2.8 |
| No. of protein atoms | 15476 | 15520 |
| No. of water atoms | 265 | 316 |
| No. of hetero-atoms | 148 | 130 |
| r.m.s. deviation of bond lengths (Å) | 0.007 | 0.007 |
| r.m.s. deviation of bond angle (°) | 1.3 | 1.4 |
| \(R_{	ext{work}}\) (%) | 20.8 | 21.6 |
| \(R_{	ext{free}}\) (%) | 22.9 | 26.0 |
| Average B factor (Å²) | 59.1 | 55.1 |

*Values in parentheses apply to the highest resolution shell.

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Expression of the argFᵇf gene and Purification of Its Protein—Cloning and expression of the gene and purification of the protein with a C-terminal His-tag were described previously (4). The pARGFbf plasmid was sequenced to ensure the absence of mutations in the argFᵇf gene before expression of its protein for enzyme assays. For crystallization, protein with an N-terminal His-tag were described previously (4).

Cloning and expression of the gene and purification of the protein with an N-terminal His-tag was prepared. Briefly, the argFᵇf gene was PCR amplified from the plasmid pARGFbf with the primers AGC-GCGGATCCTCATGGTAGATTTTCCAGCAGTC and cloned into a pET28a expression vector (Novagen). The inserted gene was sequenced and was found to have a point mutation causing a single amino acid substitution (T242L) that probably resulted as a PCR artifact.

A mutation changing amino acid 90 from proline to glutamate was introduced into the gene encoding the T242L protein using the "QuickChange" mutagenesis kit (Novagen) according to the manufacturer protocol, and the fidelity was confirmed by DNA sequencing. A single primer TGGACGGGTGACAAAGAGGAAACATCTGCTGAGAAG was used. Both genes were expressed in Escherichia coli BL21(DE3) cells (Invitrogen), and the proteins were purified in two steps using nickel-affinity and DEAE columns (GE Healthcare).

Enzymatic Assays—The carboxylation of a potential second substrate (L-ornithine, N-acetyl-L-ornithine, or N-succinyl-L-ornithine) was assayed using a modified colorimetric method that detects the formation of a ureido group (7). The method has been successfully used to detect carboxylaspartate formation by aspartate transcarbamylase (8), citrulline formation by OTCase (7), and N-acetyl-L-citrulline formation by N-acetyl-L-ornithine transcarbamylase (AOTCase 3)). N-Succinyl-L-ornithine, N-succinyl-L-citrulline, and N-acetyl-L-citrulline were chemically synthesized by Chiral Quest Co. (Monmouth Junction, NJ) Their structures and purity were verified by infrared and NMR analysis. Other chemicals were purchased from Sigma. Unless otherwise indicated, enzymatic assays were performed with the wild-type protein. Reactions were performed in 1 ml of 50 mM Tris buffer (pH 8.3) containing 1 μmol of carbamyl phosphate (CP) with varying amounts of the second substrate and 0.2 μg of the protein. The assay solution was incubated at 298 K for 5 min, and the reaction was stopped by adding 1 ml of fresh colorimetric reagent consisting of a 2,3-butanediol monoxime and antipyrine mixture described previously (7). The assay tubes were protected from light by a tinfoil cover, and incubated overnight at 298 K. Final color development was performed by uncovering the samples for 20 min in a 315 K water bath illuminated by a lamp. Details on optimizing color development are provided in Pastra-Landi and colleagues (8). Color was read at \(\lambda = 466\) nm using a UV-visible spectrophotometer. Standard curves were generated using a range of L-citrulline, N-acetyl-L-citrulline, and N-succinyl-L-citrulline concentrations. Measurements were collected in triplicate.

NMR of Enzyme Reaction Product—3 mg of purified wild-type enzyme was transferred into a buffer containing 50 mM sodium phosphate, 100 mM NaCl (pH 7.4) using three rounds of centrifugation with a Millipore Microcon YM30 spin filter according to the manufacturer’s directions. 0.44 μg of the protein was reacted with 13.17 μM CP and 5 μM N-succinyl-L-ornithine dissolved in aliquots of the same buffer at pH 8.3. After overnight incubation at 298 K, the enzyme was removed by centrifugation through a Millipore Microcon YM30 filter. Boiled enzyme was used as a negative control. 2 μg of CP, N-succinyl-L-ornithine, and chemically synthesized N-succinyl-L-citrulline standards were dissolved in the phosphate buffer used for the enzyme assay. The enzymatically and chem-
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Samples were centrifuged 10 min at 14,000 × g to remove precipitated protein. 10 μl of the reaction solution was analyzed using an Agilent LC-MS 1100 as described previously (3). Protonated N-succinyl-L-ornithine has an m/z of 233.2, and the m/z of N-succinyl-L-citrulline is 276.1.

Crystallization and Data Collection—Extensive efforts to prepare substrate-bound crystals by soaking and co-crystallization of protein expressed from pARGFbf failed. In retrospect, this is not surprising, since structure determinations of known transcarbamylases indicate that substrate binding induces large conformational changes (2, 9–11). In addition, the C-terminal His-tag that is clearly visible in one monomer of the phosphate-bound structure contributes to favorable crystal packing but only in the absence of substrates (4). To solve this problem, N-terminal His-tagged protein was prepared. The protein (T242L mutant) was concentrated to ~25 mg/ml and supplemented with ~10 mM N-succinyl-L-norvaline (SNO), a non-reactive structural analog of N-succinyl-L-ornithine, or ~10 mM CP plus ~10 mM SNO for crystallization screening using the hanging drop method. The best diffracting crystals of the CP+SNO-bound form were obtained by mixing 2 μl of the protein solution with 2 μl of the well solution containing 56% tacsimate (pH 7.0), while those of the SO4−/SNO-bound protein were obtained from a well solution containing 1.5 M (NH4)2SO4, 0.1 M BisTris (pH 5.5). Protein without a His-tag was also prepared by digestion with thrombin, but crystals of this protein were of poorer quality.

Diffraction data for the CP+SNO- and SO4−/SNO-bound proteins were collected from a single crystal with a rotating-anode x-ray generator (Rikagu). The crystals were cryoprotected by adding 25% ethylene glycerol to the well solution for the CP+SNO-bound form and replacing the well solution with 2.0 mM (NH4)2SO4, 1.0 mM Li2SO4, and 0.1 mM BisTris (pH 5.5) for the SO4−/SNO-bound form. The data were indexed, integrated, and scaled with the software package HKL2000 (12) and reduced using the program TRUNCATE in the CCP4 suite (13). The crystals belonged to tetragonal space group P43 with unit cell parameters: a = b = 156.42, c = 120.65 Å for CP+SNO-bound form. Data collection parameters are listed in Table 1. Reasonable values for the packing density calculation (14) suggested that there are two trimers in the
asymmetric unit and that the solvent content of the crystal was 61%.

Structure Determination and Refinement—The structure of the SO\(_4\)+SNO-bound protein was solved by molecular replacement using the phosphate-bound structure as the search model (PDB code 1JS1) with the program Phaser (15, 16). A Z-score of 22.7 in the space group P\(4_3\) confirms the molecular replacement solution. Rigid-body refinement of this model brought the \(R\) factor down to 41.0%. Structure factors were calculated to 6.0 Å resolution and phases were extended to 2.8 Å resolution by solvent flattening and 6-fold non-crystallographic symmetry (NCS) averaging using the program DM in the CCP4 package (13). The calculated electron density was sufficient to allow most residues to be built, including those in the 80 loop. One complete subunit was built using the program O (17). The model was subjected to 1000 steps of simulated annealing refinement with a starting temperature of 2500 K under strict NCS restraints using CNS version 1.1 (18). The refinement process was monitored by the free \(R\) factor for 5% of the data (19). This refinement lowered the \(R\) factor to 30.5%. SO\(_4\) and SNO were clearly visible (Fig. 1) and were built into the model. Further cycles of simulated annealing, individual \(B\) factor refinement, manual model adjustment, and modeling of waters and sulfate ions lowered the crystallographic \(R\) factor to 21.7% (free \(R\) factor 26.1%). The CP/H11001 SNO-bound structure was refined using similar procedures to a final crystallographic \(R\) factor of 20.4% (free \(R\) factor of 22.9%). The bound CP and SNO molecules, as well as the conformation of surrounding active site residues, are all well defined.

Figs. 1 and 3–7 were drawn using programs MOLSCRIPT (20), Raster3D (21), Pymol (22), ALSCRIPT (23), and O (17).

### RESULTS AND DISCUSSION

argF\(_{bf}\) Is Essential for Arginine Biosynthesis—Although the \(E.\ coli\) plasmid containing argF\(_{bf}\) could be induced to produce large amounts of the protein which was purified and crystallized (4), no complementation by this gene of OTCase deficient mutants of \(E.\ coli\) could be detected. By contrast, when the \(B.\ fragilis\) expression vector containing the argF\(_{bf}\) gene was introduced into JDP3a.3 or JDP3a.4, the cells no longer required arginine for growth in the defined medium. These results confirmed that the argF\(_{bf}\) is essential for endogenous production of arginine in \(B.\ fragilis\). The fact that it could not complement an argF (OTCase)-deficient \(E.\ coli\) suggested that it is unlikely to

![Figure 3](image-url)

**FIGURE 3.** *A*, surface representation of the SOTCase hexamer in the asymmetric unit in two different orientations. *B*, ribbon diagram of a monomer. Green arrows indicate segments that are hydrogen-bonded to an adjacent strands, \(\alpha\)-helices are in red, and \(\beta\)-sheets are in green. CP and SNO are represented as ball-and-stick models. *C*, ribbon diagram of a trimer. Monomers are colored red, green, and yellow. Bound CP and SNO are shown as space filling models.

### TABLE 3

**Interactions at the active site**  
Trp\(^{75}\) is the residue from the adjacent monomer. The labels in parentheses are equivalent atoms of sulfamate.

| Hetero-atoms | Protein atoms | Distance \(\AA\) |
|-------------|---------------|----------------|
|             | CP or SO\(_4\)| SNO and SO\(_4\) |
| O1P (O1)    | Arg\(^{110}\)NH1 | 2.61 2.94 |
| O2P (O2)    | Trp\(^{75}\)NE1 | 3.34 3.14 |
| O3P (O3)    | Ser\(^{47}\)O     | 2.58 2.57 |
|             | Arg\(^{110}\)N    | 3.17 3.22 |
|             | Thr\(^{25}\)N     | 2.57 3.24 |
|             | Thr\(^{25}\)OG1   | 3.06 3.49 |
| O4P (O4)    | Leu\(^{48}\)N    | 2.75 2.82 |
|             | Arg\(^{110}\)H2   | 3.06 3.08 |
|             | Trp\(^{75}\)NE1   | 2.58 3.09 |
|             | CP or SO\(_4\)    | SNO and SO\(_4\) |
| O1P (O1)    | Arg\(^{110}\)NE   | 2.88 2.93 |
| O2P (O2)    | Gln\(^{150}\)OE2  | 3.00 3.17 |
|             | Cys\(^{27}\)O     | 3.17 3.03 |
|             | Arg\(^{110}\)H1   | 3.19 3.19 |
| O3P (O3)    | Thr\(^{25}\)OG1   | 2.66 2.74 |
|             | Arg\(^{110}\)NH2  | 2.74 3.29 |
|             | His\(^{47}\)NE2   | 3.29 3.19 |
|             | Arg\(^{110}\)H1   | 3.19 3.19 |
| SNO         | Glu\(^{14}\)OE2   | 2.66 2.82 |
| OXT         | Lys\(^{28}\)NZ    | 2.82 2.68 |
| OD1         | His\(^{70}\)NE2   | 2.91 3.01 |
| OD2         | Arg\(^{110}\)NE   | 2.94 2.93 |
|             | Arg\(^{110}\)NH2  | 2.89 3.09 |
|             |Arg\(^{110}\)NH2   | 2.82 3.23 |
FIGURE 4. **Active site of SOTCase.** The final refined positions of the ligands are represented as colored sticks. A, stereo view showing the active site of B. fragilis CP+ SNO-bound SOTCase. Interactions of active site residues with bound CP and SNO (yellow stick) are illustrated in pink dashed lines. Residue Trp**90** indicated by * is from an adjacent monomer. B, comparison of the active sites of SOTCase (left) and AOTCase (right) near the succinyl group. SNO and N-acetyl-L-citrulline are shown as thick ball-and-stick models, while the surrounding residues are shown as thin ball-and-stick models. Residue Pro**90** in SOTCase from the adjacent subunit (equivalent to Glu**92** in AOTCase) shown in red is the residue that distinguishes SOTCase from AOTCase. Coordinates of AOTCase are taken from PDB 1YH1.

encode an OTCase and that B. fragilis may possess a different pathway for arginine biosynthesis.

**Specificity of Transcarbamylation Activity**—The substrate specificity for the formation of a ureido-containing product by the purified argF* _by_ protein is shown in Table 2. Both the wild-type protein and T242L mutant protein (which resulted from a cloning artifact) carbamylate N-succinyl-L-ornithine with similar efficiency but do not carbamylate L-ornithine or N-acetyl-L-ornithine. Both enzymes showed similar catalytic properties, including substrate inhibition at 20 mM N-succinyl-L-ornithine, half-maximal activities for the same substrate at 3.2 and 2.9 mM, respectively, and similar catalytic velocities as shown in Table 2. By contrast, introduction of a single P90E amino acid substitution generated an enzyme capable of carbamylation of N-acetyl-L-ornithine at a rate ~7-times greater than N-succinyl-L-ornithine, thus practically converting it from a N-succinyl-L-ornithine transcarbamylase (SOTCase) to an OTCase.

**N-Succinyl-L-citrulline Is the Reaction Product**—The peaks seen in the proton NMR spectra of the enzymatically generated product have chemical shifts that are identical to those of the chemically synthesized N-succinyl-L-citrulline (Fig. 2A). Similarly, in the analysis by LC-MS, the retention time and mass ion of the enzymatically synthesized product are identical to those of the chemically synthesized N-succinyl-L-citrulline (Fig. 2B). These two different methods confirm that upon incubation of the new protein with CP and N-succinyl-L-ornithine, N-succinyl-L-citrulline is formed.

**Structural Model of SOTCase**—The structures of the B. fragilis SOTCase T242L mutant complexed with CP+SNO, and SO₄+SNO have been solved at 2.9 and 2.8 Å resolution, respectively. The electron densities of the ligands are shown in Fig. 1. We used SNO as a non-reactive ligand since it has a structure similar to N-succinyl-L-ornithine but without the reactive ε-amino group. Norvaline and N-acetyl-L-norvaline, structural analogs of ornithine and N-acetyl-L-ornithine, respectively, have been successfully used in a similar fashion for OTCase and AOTCase structural studies (24, 25). The final structural model of the CP+SNO-bound enzyme consists of six monomers with a total of 1922 amino acids, 6 CP, 6 SNO, 2 SO₄, and 177 water molecules. The two trimers in the asymmetric unit are related by a 2-fold NCS axis in the crystal. The concave surfaces of the two trimers are packed face to face, with their respective 3-fold NCS axes tilted at an angle of ~10° relative to each other, so that the two trimers interact only through two subunits (Fig. 3A). The structures of the monomers are very similar with a RMS difference <0.17 Å. Since the recombinant protein has an N-terminal His-tag with a thrombin cleavage site, the electron density map shows three additional N-terminal residues (Gly-Ser-His) in chains X and Y and two residues (Ser-His) in chain Z, C, D, and E before the authentic initial methionine. The overall g value of 0.26 indicates good geometry. All the main-chain, side-chain, and planar group parameters assessed with PROCHECK (26) are comparable with those in structures at similar resolution in the PDB. Residues Glu**142** and Leu**275** are in energetically unfavorable conformations, as we have observed in the phosphate-bound structure (4). There is one cis peptide bond between Leu**275** and Pro**276** in each chain that maintains the His**273**, Cys**274**, Leu**275**, Pro**276** (HCLP) motif in a conformation characteristic of all known transcarbamylases. The substituted residue 242 is located within the 240 loop, and its side chain is on the protein surface, suggesting that it does not affect the normal structure of the protein. Since the
chains of His\textsuperscript{147}, Gln\textsuperscript{150}, Arg\textsuperscript{302}, and the carbonyl oxygen atoms of Arg\textsuperscript{110}, Arg\textsuperscript{178}, and Arg\textsuperscript{278} in SOTCase are required specifically to anchor the succinyl group. Interestingly, the residue equivalent to Pro\textsuperscript{90} of SOTCase is Glu\textsuperscript{92} in AOTCase. In the AOTCase structure, this glutamate interacts with His\textsuperscript{180} and Arg\textsuperscript{298} (Xanthomonas campestris numbering) occupying the space that in SOTCase accommodates the succinyl moiety and thus precluding N-succinyl-l-ornithine binding to AOTCase (Fig. 4B). Therefore, this residue (90 in SOTCase and the corresponding residue 92 in AOTCase) appears critical for conferring specificity toward the second substrate. The ability of the P90E mutant of \textit{B. fragilis} SOTCase to carbamylate N-acetyl-l-ornithine more rapidly than N-succinyl-l-ornithine supports the above mechanism.

Loop Movement and Domain Closure—The electron density indicates that significant conformational changes occur in the 80 loop, relative to the phosphate-bound structure. Significant loop movement and domain closure occur upon substrate binding to SOTCase. Shown in Fig. 5 is a superimposition of the phosphate-bound and CP+SNO-bound structures, with the $\beta$-core (residues 38–44, 64–68, 106–110, and 138–140) of the CP domain as the reference. The movements involve the 80 loop (residue 70–91), 120 loop (residue 113–120), and 50 loop (residue 42–50). The 80 loop contributes one or two residues from the adjacent subunit that interact with the bound substrate in all known transcarbamylases. However, uniquely to SOTCase, the CP+SNO- and SO\textsubscript{4}\textsuperscript{2-}+SNO-bound structures show that Trp\textsuperscript{75} from the adjacent chain stabilizes the negative charge of the phosphate moiety of CP, and this group is within 0.19 Å of the sulfate group in the SO\textsubscript{4}\textsuperscript{2-}+SNO-bound structure. The side chains of His\textsuperscript{147}, Gln\textsuperscript{150}, Arg\textsuperscript{302}, and the carbonyl oxygen atoms of Cys\textsuperscript{274} and Leu\textsuperscript{275} are involved in binding the carbamyl group. The N-succinyl-l-ornithine binding site involves the side chains of Glu\textsuperscript{142} and Lys\textsuperscript{236}. A number of hydrophobic interactions involving Phe\textsuperscript{112}, Leu\textsuperscript{180}, Pro\textsuperscript{181}, Pro\textsuperscript{276}, and the phenyl ring of Trp\textsuperscript{75} from the neighboring subunit are also important for binding the second substrate. In particular, the $\chi_1$ angle of Phe\textsuperscript{112} in the CP+SNO-bound structure is rotated $\sim 140^\circ$ relative to the phosphate-bound structure (4) to position its side chain toward the active site upon substrate binding. Although several interactions between the substrate and protein in SOTCase are similar to those in AOTCase, additional residues (His\textsuperscript{176}, Arg\textsuperscript{178}, and Arg\textsuperscript{278}) in SOTCase are required specifically to anchor the succinyl group.
hydrogen bonds with the phosphate oxygen (10–11, 24, 34).

The movement of the 80 loop in X. campestris AOTCase upon substrate binding is much smaller than in SOTCase probably because hydrogen bonding interactions of Glu92 from the adjacent chain with His180 and Arg298 pre-anchor the 80 loop near the active site (Fig. 4B). The 120 loops of SOTCase also move significantly when substrate analogs bind, with the C/H9251 atom of Glu116 undergoing the greatest change in position (2.6 Å), but this movement seems to be the result of domain closure. The 50 loop moves 0.7–1.6 Å toward the active site when SNO binds, enabling Ser47, Arg49, and Thr50 to interact with the phosphate group of CP. In E. coli and human OTCase, the domains of the monomer move 4–12° upon substrate binding (10–11). In E. coli ATCase, the corresponding movement is 8.0° (9) while an even larger domain movement (8.4–12.3°) was observed in the isolated catalytic trimer of E. coli ATCase (35–36). However, a much smaller (<2.2°) domain movement was observed in X. campestris AOTCase (2). The domain movement observed in B. fragilis SOTCase when CP+SNO or

FIGURE 7. Selected sequence alignment of SOTCase and AOTCase from B. fragilis, C. hutchinsonii, M. xanthus, T. forsythensis, X. campestris, and X. fastidiosa. Loops discussed under “Results and Discussion” are boxed. Residues that are involved in hydrogen bonding with substrates are colored red. Residues that are involved in hydrophobic interactions are colored blue. The residue that is proposed to distinguish SOTCase from AOTCase is colored green.
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SO₄⁺-SNO bind is ~5.6°, comparable with OTCase but larger than in AOTCase.

Catalytic Mechanism—Replacing SNO in the CP+SNO-bound structure with N-succinyl-L-ornithine, and in the SO₄⁺+SNO-bound structure with N-succinyl-L-citrulline, provides useful models for the initial and final stages of catalysis. The similarity between the CP+SNO- and SO₄⁺+SNO-bound structures suggests that the conformational change and domain movement occur largely when substrates bind rather than during the reaction. Since the binding site of the second substrate in SOTCase is similar to that in AOTCase, and the N-acetyl-L-ornithine- and N-acetyl-L-citrulline-bound AOTCase structures have been determined at high resolution (PDB code: 1ZQ6 and 1YH1, respectively), the model of the reaction catalyzed by SOTCase can be reliably derived, as shown in Fig. 6. In this model, the δ-amino nitrogen of N-succinyl-L-ornithine is 2.5 Å from the carbon atom of the carbamyl group, in a position poised to attack the carbamyl group and to form the S-configured tetrahedral intermediate. The distance between the O1 atom of the phosphate group of CP and the δ-amino nitrogen of N-succinyl-L-ornithine is 2.6 Å, within hydrogen bonding distance, and therefore capable of transferring a proton during the reaction. The product binding mode of SOTCase should be very similar to that of AOTCase (2) and would predict that the distance between the O4 atom of the phosphate group and CZ atom of N-succinyl-L-citrulline would be only 2.2 Å, allowing the reverse reaction to occur. Whether SOTCase can play a catabolic role as certain OTCases do (37) needs to be specifically studied.

Other Bacterial SOTCase-like Genes—argF genes with sequences homologous to AOTCases from X. campestris and SOTCase from B. fragilis have been identified in the genomes of other bacteria including Bacteroides thetaiotaomicron, Cytophaga hutchinsonii, Myxococcus xanthus, Prevotella ruminicola, Tamerella forsythensis, Xanthomonas axonopodis, Xanthomonas campestris, Xanthomonas oryzae, and Xylella fastidiosa. The sequence alignment is shown in Fig. 7. The structures of AOTCase and SOTCase indicate that these transcarbamylases are characterized by extended 80 and 120 loops within hydrogen bonding distance, and therefore capable of catalyzing the phosphate group and CZ atom of CP—configured tetrahedral intermediate. The distance between the O1 atom of the phosphate group of CP and the δ-amino nitrogen atom of CP is 2.6 Å, comparable with OTCase but

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