

Abstract No. shi25

Crystal Structure of a new Transcarbamylase from the Anaerobic Bacterium *Bacteroides fragilis* at 2.0 Å Resolution

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Beamline(s): X12C

Introduction: In the course of characterizing the arginine biosynthetic genes in *Bacteroides fragilis* (*B. fragilis*), an anaerobic bacterium, which is a major component of the intestinal flora, we identified a distinctive transcarbamylase-like gene. Transposon mutagenesis of this gene demonstrated that the corresponding protein to be essential for arginine biosynthesis. However, although the sequence suggests the presence of a carbamoyl phosphate binding motif seen in all other transcarbamylases, the sequence of the second substrate binding domain has a number of insertions or deletions in regions of the structure that are essential for substrate binding and catalysis of known transcarbamylases: ATCase and OTCase [1]. To gain insight into the function of this protein and the evolution of transcarbamylases, we determined its three dimensional structure at 2.0 Å resolution.

Methods and Materials: Plasmid pARGFBF-his containing the *argF*-like gene of *B. fragilis* under the control of the pTRC hybrid promoter was constructed. The protein was overexpressed in *E. coli* HMS174(DE3) cells and purified by Ni-NTA affinity and DEAE fast-flow columns. The crystals were grown at pH 8.0 from 1.9 M ammonium sulfate solution by the hanging drop method and were approximately 0.3 × 0.3 × 0.4 mm in size. Native data to 2.0 Å were recorded at beam line x12c. A heavy atom derivative was prepared by soaking a native crystal in a mother liquor containing 1 mM HgCl₂. The structure was solved by a single isomorphous HgCl₂ derivative with anomalous scattering (SIRAS) using program SOLVE. The structure was refined at 2.0 Å resolution to an *R*-factor of 20.6% (*R*-free = 25.2%).

Results: The final model consisted of three polypeptides (324 amino acid residues for chain X, 318 residues for chain Y and Z; Figure 1), three phosphate ions and 422 water molecules. The general fold of the subunit structure is similar to the other known OTCase and ATCase structures (Figure 1). However, there are several striking differences in important regions of the active site. The 80's loop (residues 70 to 92), which contributes residues from an adjacent subunit to the CP binding sites of ATCases and OTCases, is 12 amino acid residues longer than in OTCase and ATCase and contains two short parallel strands (residue 77 to 78, residue 91 to 92) that are unique to this protein. The 120's loop (residue 111 to 124), which links B4 and H4 near the active site, is 10 residues longer than the respective loop in OTCase or ATCase. As a result, this loop extends further along the active site and is able to interact with the 240's loop (residue 236 to 259). The 240's loop (residue 236 to 259), which is involved in recognizing the second substrate in OTCase and ATCase and in triggering conformational changes when the second substrate binds, lacks the characteristic motifs of OTCases (DxxxSMG motif) and ATCases (RxQxER motif) and has a different three-dimensional structure (Figure 1). Even more surprising is the presence of a proline-rich loop (residue 173-183) that links B6 to H6 and located between the active site and 240's loop. Because of the presence of four prolines, this loop is not mobile, and would be expected to prevent movement of the 240's loop towards the active site when the second substrate binds. In OTCase and ATCase, the equivalent 240's loop is disordered in the unligated structure [2-4] but well defined in the ligated structure [5-8]. Finally, H7 and β -strands B8 are 11 residues shorter than *E. coli* OTCase, increasing the tilt of B8 towards other β -strands.

One phosphate and two water molecules were found at putative CP binding site that is very similar to that of human OTCase. Similarly positioned residues include Ser 47 (Ser 90 in human OTCase sequence), Arg 49 (Arg 92), Thr 50 (Thr 93), Arg 110 (Arg 141), His 147 (His 168), Gln 150 (Gln 171) and Arg 302 (Arg 330). Main chain atoms, the backbone nitrogen of Leu 48 (Thr 91) and carbonyl oxygen atoms of Cys 274 (Cys 303), Leu 275 (Leu 304), involved in binding CP also have similar conformations. The *cis* peptide bond between Leu 275 and Pro 276, which is conserved in OTCase and ATCase and which is required for CP binding, is also found in the *B. fragilis* structure. In contrast to the CP binding site that is similar to OTCase and ATCase, the putative binding site for the second substrate is strikingly different from other transcarbamylases. None of the residues involved in binding L-ornithine to OTCase are present here. Asn 199 in human OTCase is replaced by Ala 183, Asp 263 by Lys 236, Leu 163 by Glu 142 and Lys 88, which interacts to ornithine *via* a water molecule, is replaced by Asn 45. As results of the presence of proline-rich loop, the side chains of residues His 176, Leu 180, Pro 181, Ala 183 and Val 184, are close to active site. If we assume the ornithine binds to the present protein in a similar way as human OTCase, unfavorable interactions will occur between the α -amino group and Lys 236, and between the side-chain of ornithine and Glu 142. There is also no similarity between this site and the aspartate binding site of ATCase. Arg167 in the *E. coli* ATCase sequence is replaced by Ala 183, Arg 229 changes to Asn 237 and Gln 231 is replaced by Tyr 241.

Acknowledgments: This work was supported by Public Health Service grant DK-47870 from the National Institute of Diabetes, Digestive and Kidney Diseases and HD 32652 from the National Institute of Child Health and

Human Development. We thank Dr. David Davies for facilitating our use of the diffraction equipment in the Molecular Structure Section of the National Institute of Health and Dr. Fred Dyda for help in data collection and processing. We also thank Drs. Bob Sweet and Anand Saxena for their assistance during data collection at beamline X12C at the National Synchrotron Light Source at Brookhaven National Laboratory. This facility is supported by the United States Department of Energy under contract No. DE-AC02-76CH00016.

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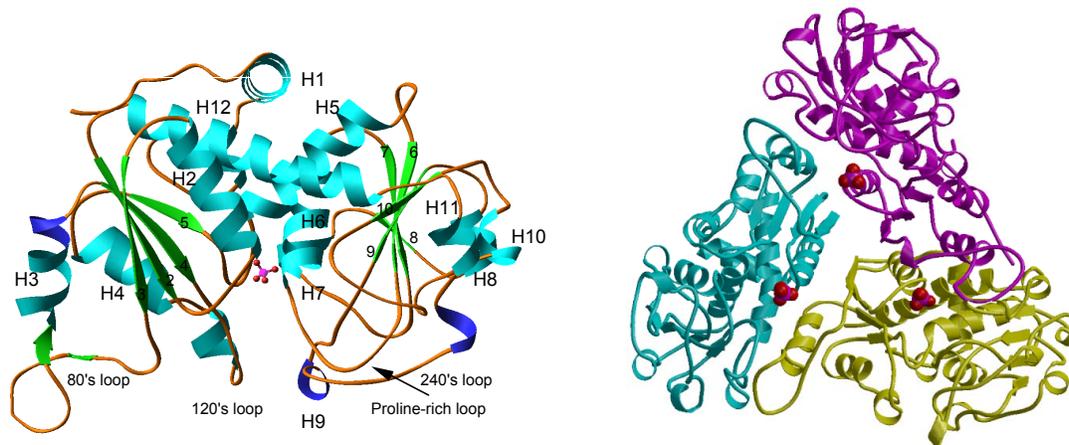


Figure 1. Ribbon diagram of the monomer (left) and trimer (right). Left: Green arrows indicate segments that are hydrogen bonded to an adjacent strand as required by the Kabsch & Sander definition of β character. α -helices are light blue and 3_{10} helices are dark blue. The phosphate group is represented as a pink ball-and-stick model. Right: The subunits are colored pink, blue and yellow. The bound phosphate molecules, shown in red, are located in the cleft between the two domains at a site shared by two adjacent monomers. The extended structure at the C-terminal of the pink subunit corresponds to the six histidine tag. The view angle is parallel to the molecular 3-fold axis facing the concave face of the molecule.