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Crystal Structure of the Hexameric Catabolic Ornithine Transcarbamylase from *Lactobacillus hilgardii:* Structural Insights into the Oligomeric Assembly and Metal Binding

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Catabolic ornithine transcarbamylase (cOTC; EC 2.1.3.3) catalyzes the formation of ornithine (ORN) and carbamoyl phosphate from citrulline, which constitutes the second step of the degradation of arginine via the arginine deiminase pathway. Here, we report the crystal structure of cOTC from the lactic acid bacteria *Lactobacillus hilgardii* (Lh-cOTC) refined to 2.1 Å resolution. The structure reveals that Lh-cOTC forms a hexameric assembly, which was also confirmed by gel-filtration chromatography and analytical ultracentrifugation. The homohexamer, with 32 point group symmetry, represents a new oligomeric state within the members of the ornithine transcarbamylase family that are typically homotrimeric or homododecameric. The C-terminal end from each subunit constitutes a key structural element for the stabilization of the hexameric assembly in solution. Additionally, the structure reveals, for the first time in the ornithine transcarbamylase family, a metal-binding site located at the 3-fold molecular symmetry axis of each trimer.

Keywords: crystal structure; metal binding; oligomeric assembly; ornithine transcarbamylase

Introduction

Catabolic ornithine transcarbamylases (cOTC; EC 2.1.3.3) participate in the degradation of the amino acid arginine via the deiminase pathway by catalyzing the formation of ornithine (ORN) and carbamoyl phosphate (CP) from citrulline. Although thermodynamically unfavored,[1] this chemical reaction is exploited by a number of microorganisms that degrade arginine because of the coupling with carbamate kinase, which catalyzes the next downstream step in the catabolic deiminase pathway and, in the case of cOTC from *Pseudomonas aeruginosa*, because of its low affinity toward CP and its strong cooperativity for this substrate.[2–6] Regulation of the enzymatic activity of cOTC from *P. aeruginosa* is highly complex. As well as displaying homotropic cooperativity toward CP, this enzyme is allosterically activated by AMP or inorganic phosphate and

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Abbreviations used: aOTC, anabolic OTC; CP, carbamoyl phosphate; Lh-cOTC, *Lactobacillus hilgardii* catabolic ornithine transcarbamylase; ORN, ornithine; OTC, ornithine transcarbamylase; PALO, *N*--(phosphonacetyl)-L-ornithine.

inhibited by polyamines such as spermidine and putrescine.[5,7] Crystal structures of the native form of cOTC from *P. aeruginosa*[8,9] and a point mutant variant devoid of homotropic interactions[7] represent the only previously determined structures of a cOTC. The latter structure, in which Glu105 is substituted by Gly, reveals that the enzyme is composed of four trimers tetrahedrically arranged according to a 23 point group symmetry. More importantly, the allosteric regulation exhibited by this cOTC is directly correlated to its symmetrical oligomeric structure, since modification of the trimer interfaces renders trimeric and Michaelian variants.[10] Conversely, anabolic OTCs (aOTCs) are cyclic homotrimers that display Michaelis–Menten kinetics.[5] These trimers are generally referred to as "catalytic trimers," as they constitute the basic catalytic structural unit in OTCs and, more generally, amongst the carbamoyltransferases.[11] Representative crystal structures of aOTCs from several sources have been determined: human aOTC complexed with the bisubstrate analog *N*-(phosphonacetyl)-L-ornithine (PALO)[12] and more recently with CP and the inhibitor L-norvaline;[13] unliganded aOTC from *Escherichia coli*[14] as well as complexes with PALO[15] and with N^{δ}-(*N*-sulfodiaminophosphinyl)-L-ornithine;[16] aOTC from *Pyrococcus furiosus*;[17] and, finally, aOTC from *Mycobacterium tuberculosis*, both unliganded and in complex with CP and L-norvaline.[18] Interestingly, aOTC from *P. furiosus*, despite being an anabolic enzyme, is a dodecamer assembly[17] and shows no allosteric regulation. Specifically, the quaternary structure of this OTC has been correlated to its thermostability.[17]

Here we report the crystal structure of the cOTC from *Lactobacillus hilgardii* (Lh-cOTC). The hexameric structure of Lh-cOTC exhibits a new oligomeric state within the ornithine transcarbamylases (OTCs) and reveals the C-terminal end of the polypeptide chains as a key structural element in the association of the subunits. Furthermore, a metal binding site is identified in the structure at the 3-fold molecular symmetry axis, providing the first structural evidence for the association between an OTC and a metal ion.

Results and Discussion

Description of the monomer structure

Each monomer of Lh-cOTC is composed of 343 amino acid residues and, as with previously described OTCs, comprises two structurally and functionally distinct domains: the N-terminal CP-binding domain and the C-terminal ORN-binding domain. Each domain is formed by a parallel β-sheet (four- and five-stranded for the N-terminal and C-terminal sheets, respectively), which is flanked by α helices (Fig. 1). The secondary structural elements of Lh-cOTC together with sequence alignments against OTCs of known three-dimensional (3D) structure are shown in Fig. 2. Helices α5 (residues 141–155) and α13 (residues 316–335) constitute the interface between the domains of the Lh-cOTC monomers. Residues participating in main stabilizing interactions involving these two helices are indicated in Fig. 2. Helices α5 and α13 are key structural elements, as they constitute the inner core of the monomer framework and are involved in extensive interactions both between themselves and with helices α1 (residues 23–42), α2 (residues 63–75), and α6 (residues 172–184). Furthermore, α5 and α13, together with helices α2 and α6 and the connecting loop between β9 and α11 (residues 278–287) make up the active site, which is located within a cleft between the lobes of the monomer. The β9–α11 loop contains the conserved HCLP motif that participates in ORN binding.

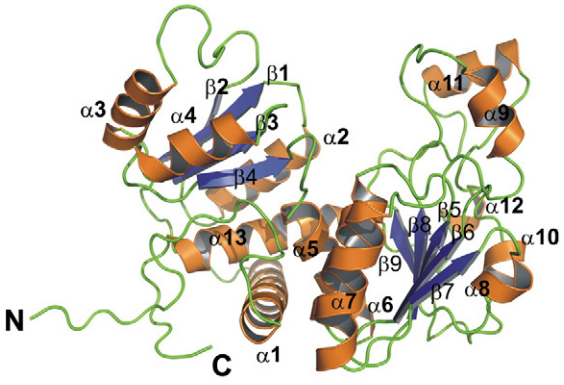

Fig. 1. 3D structure of the monomeric subunit of Lh-cOTC. Ribbon model of the crystal structure with secondary structural elements colored blue for β-strands, orange for α-helix, and green for regions with no regular secondary structure. The secondary structural assignments as well as the N- and C-termini of the model are shown. The figure was prepared with PyMol.[19]

The three independent monomers of the asymmetric unit of the Lh-cOTC trigonal crystal superimpose almost perfectly, showing a maximum r.m.s.d. value of 0.3 Å for 343 aligned C^{α} atoms. Comparison of the overall topologies of the OTCs reveals that the monomeric architecture is highly conserved with r.m.s.d. values ranging from 1.1 to 1.5 Å for ~290 C^{α} atoms. Interestingly, the most significant structural variations are found at the N- and C-terminal segments of the proteins, which in the case of Lh-cOTC participate in hexamer formation. Finally, Lh-cOTC possesses a sequence motif characteristic of prokaryotic OTCs (residues 283–301), which has been implicated in potential protein–protein interactions.[12]

Interactions between monomers within trimers

The threefold cyclical arrangement of the Lh-cOTC subunits results in the formation of an indented triangular homotrimer with an approximate side length of 90 Å and a thickness of 55 Å. The individual monomers mainly interact not only through their N-terminal CP-binding domains, which form the inner part of the assembly, but also through the loop containing helix α11 (residues 283–301). The average