X-ray studies of recombinant rat kidney long-chain hydroxy acid oxidase

L.M. Cunane[a], J.D. Barton[a], Z.-w. Chen[a] , F.S. Mathews[a], A. Belmouden[b], K.H.D. Lê[b], and F. Lederer[b]
[a] *Dept. of Biochemistry and Molecular Biophysics, Washington University School of Medicine, St. Louis, MO 63110 USA*
[b] *Laboratoire d' Enzymologie et Biochimie Structurales, Centre National de la Recherche Scientifique, Gif-sur-Yvette, France*

Introduction

Peroxisomal long-chain hydroxy acid oxidase (LCHAO) is a 4 x 39 kDa homotetramer containing one molecule of FMN per subunit [1]. It catalyzes the oxidation of L-(+)-α-hydroxyacids by dioxygen to yield the keto acid and hydrogen peroxide. The enzyme is specific for substrates with long aliphatic side chains or aromatic acids, such as mandelate or phenyl lactate. LCHAO is similar to the flavin-binding domain of flavocytochrome b2 (37% sequence identity) and to glycolate oxidase (45% identity) [2]. However, it lacks the equivalent active-site residue, Tyr143, that binds the substrate carboxylate, being replaced by Phe instead [3]. LCHAO has been implicated in the production of methylguanidine, a toxic compound found elevated in uremic patients [4]. This compound was reported to be produced from creatol (hydroxycreatinine) by the action of LCHAO. If creatol is indeed a substrate, then knowledge of the LCHAO structure might contribute to the design of therapeutic agents in uremic situations.

Methods and Materials

Crystals of LCHAO were grown by the sitting-drop method. Five μL of protein solution (at 10 mg/ml in 0.1 M Tris buffer, pH = 7.5) were mixed with 5 μL of reservoir solution (0.4 M sodium acetate, 0.2 M sodium citrate, pH = 6.5) and allowed to equilibrate at 4ºC. X-ray data were collected to 2.3 Å resolution from a crystal (soaked for five minutes in 0.4 M sodium acetate, 0.2 M sodium citrate, pH = 6.5, 25% glycerol) at ~100 K at the Structural Biology Center beamline of the Advanced Photon Source, Argonne, Illinois. (Rmerge = 6%, I/σ(I) ~ 3) at 2.3 Å resolution. The crystal was orthorhombic (P21212) with a = 114.8 Å, b = 151.0 Å, c = 111.2 Å and contains one homotetramer of 156 kDa in the asymmetric unit; the data scaled with Rmerge = 5.0%.

Results and Discussion

Molecular replacement analysis using AMORE has led to a clear-cut solution using glycolate oxidase as a search molecule. At the current stage of refinement using CNS [5], R = 0.225 (Rfree = 0.254) with 340 water molecules included. The current model consists of residues 1–170 and 203–349. The gap in the model corresponds approximately to the disordered loop found in FCB2 [6]. However, there is weak electron density connecting residues 170 and 203 that is nearly continuous. Efforts to fit the amino acid sequence to the density are currently underway.

Comparison of the FCB2 structure containing phenylpyruvate bound to the active site (R.C.E. Durley, L.-Y. Chen, and F.S. Mathews, unpublished results) with that of LCHAO shows that F23 (LCHAO) is displaced away from Y143 (FCB2) and both guanidinium nitrogen atoms of Arg164 (LCHAO, equivalent to Arg289 in FCB2) are able to form hydrogen bonds with both carboxylate oxygens of phenylpyruvate. Near the phenyl ring of the phenylpyruvate ligand of FCB2, Leu199 and Leu230 (both in FCB2 and believed to regulate substrate specificity) are replaced by aromatic residues (Phe and Tyr, respectively) that may help modulate the substrate specificity of LCHAO.

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