Structure of L-methionine γ-lyase 1 from Entamoeba histolytica

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Introduction

Methionine γ-lyase 1 (EhMGL1) contains pyridoxal 5′-phosphate (PLP) as a cofactor, and is categorized to the γ-family of PLP-dependent enzymes. MGL catalyzes α, γ- or α, β-elimination of sulfur-containing amino acids and produces ammonia, α-keto acids and volatile thiols such as hydrogen sulfide or methanethiol. It also catalyzes β- or γ-replacement of cysteine, S-substituted cysteine, methionine, and related compounds. In this study, we determined the crystal structure of EhMGL1.

Results and Discussion

The ligand-free Entamoeba histolytica methionine γ-lyase 1 (EhMGL1) was crystallized (1) and X-ray structure analysis was performed at 1.97 Å resolution. As is true for MGLs from P. putida, T. vaginalis, C. freundii and for other related enzymes, cystathionine β-lyase, cystathionine γ-lyase, cystathionine γ-synthase, EhMGL1 exists as a homotetramer (A: green, B: cyan, C: gray and D: orange), which can be divided into two catalytic dimers (A-D and B-C dimers) (Fig. 1). Each subunit consists of three domains: a small N-terminal domain, the largest PLP-binding domain and a C-terminal domain. The N-terminal domain includes two α-helices (α1 and α2), and a long loop connecting α1 and α2 (α1/α2 loop, residues 13-35) provides most of contacts to neighboring subunits. The PLP-binding domain includes eight α-helices (α3-α10) and seven β-strands (β1-β7). The strands are arranged in the order, β3/β2/β4/β5/β6/β7/β6/β7/β6/β7, respectively, to form a mainly parallel seven-stranded β-sheet. Each strand is connected to a next one by an α-helix except a linker between β6 and β7 (β6/β7 loop, residues 203 - 215). Lys205 Nε in the β6/β7 loop is covalently attached to the PLP aldehyde group to form the internal aldimine (lysine-pyridoxal-5′-phosphate, LLP). Five helices (α3, α6, α7, α8 and α10) flanks on one side of the seven-stranded β-sheet and shield it from solvent, while α4, α5 and α9 located on the other side are involved in the intermolecular interface between the catalytic dimer. The longest α-helix, α10 (residues 241 - 276), is bent at Pro256, after which the polypeptide chain builds the C-terminal domain. The C-terminal domain is composed of five α-helices (α10 - α14) and five β-strands (β8 - β12). The strands are arranged in the order, β8, β9, β12, β11, β10 with directions ↓↑↓↑↑↑↑↑↑, respectively, and one side of the sheet is relatively hydrophobic and shielded from solvent by α12, α14 and the C-terminal half of α10, whereas the other side is studded mainly with hydrophilic amino acid residues and interacts with water and residues from the PLP-binding domain.

A PLP-binding cavity is formed at the interface between the PLP-binding and C-terminal domains. In the catalytic dimer, the cavity of one subunit is veiled with an α2/α3 loop of the adjacent subunit to shield LLP from outside water. The pyrimidine N1 of LLP forms a strong hydrogen bond/salt bridge with Asp180 Oε2 (2.78 (4) Å), and this electrostatic interaction stabilizes the positive charge at N1. The pyrimidine O3 receives hydrogen bonds from the internal aldime Nε (2.54 (2) Å) and Asn155 Nε2 (3.02 (12) Å), and the phosphate group from Gly83 N, Met84 N, Ser202 O, and Ser204 O. In addition, Tyr53 Oε, Arg55 Nε and Arg55 Nε2 located on the α2/α3 loop of the adjacent subunit also contribute to hydrogen bond formation with the LLP phosphate group (Fig. 2). In most of MGLs and related enzymes with known structures, these amino acid residues are conserved and interact with LLP as observed in EhMGL1.

Fig. 1

Fig. 2

References


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