

The Crystal Structure of Maleylacetoacetate Isomerase

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Introduction

The glutathione dependent *cis-trans* isomerization of maleylacetoacetate (MAA) to fumarylacetoacetate is the penultimate step in the catabolism of phenylalanine and tyrosine. Deficiencies of most enzymes in this pathway lead to a range of disorders, including alkaptonuria phenylketonuria and several forms of tyrosinemia. Inhibitors of maleylacetoacetate isomerase (MAAI) may be useful in the clinical management of hereditary tyrosinemia type I patients. The recent cloning of a cDNA encoding human MAAI revealed that it was identical to the previously described zeta class glutathione transferase, GST Z1-1.¹

Methods and Materials

MAAI was expressed, crystallized and purified as described previously.² Native data were collected from a single crystal frozen to 100 K on an ADSC Quantum-4 CCD detector using synchrotron radiation at the BioCARS beamline 14-BM-C. The two heavy-atom derivatives, *cis*-Pt(NH₃)₂Cl₂ and Pt(ethylenediamine)Cl₂, were collected. A data set for the former was collected in-house off a single crystal frozen to 100 K, whereas multiple anomalous dispersion (MAD) data were collected from the latter derivative on the BioCARS beamline 14-BM-D. The structure of MAAI was determined by a combination of single isomorphous replacement and MAD data. The final model of MAAI (Rfactor = 22.8%, Rfree = 27.7% to a resolution of 1.9 Å) consists of residues 5 to 212, 1 molecule of glutathione (GSH), 1 sulfate ion, 1 DTT molecule and 109 water molecules.²

Results

The molecule adopts the canonical GST fold with an N-terminal thioredoxin-like domain and a C-terminal all α -helical domain. GSH and the sulfate ion are located in a very deep crevice of about 25 Å between the two domains. The depth of the crevice provides an explanation as to why the enzyme has a relatively weak affinity for GSH purification columns. GSH binds to MAAI in an extended conformation with the γ -glutamyl moiety pointing towards the protein core. It forms extensive interactions with the protein, including 1 salt bridge and 15 hydrogen bonding interactions. The sulfate ion forms numerous contacts with the protein and the entrance to the sulfate binding site is bordered by a rim of residues with two-thirds of the rim consisting of hydrophobic residues and the rest contributed from the two positively charged residues, Arg 13 and Arg 175.

Discussion

The crystal structure of MAAI reveals that it belongs to the GST superfamily despite exhibiting either little or no activity with standard substrates and the lack of any significant sequence identity with other members for which there are crystal structures. The most significant difference is the small, polar active site in MAAI compared with the much larger, hydrophobic active sites typical

of other members of the GST family.² MAAI was originally characterized based on its ability to catalyze the isomerization of a *cis* double bond in MAA to the *trans* configuration during the catabolism of phenylalanine and tyrosine. Although details of the reaction mechanism are unknown, it is thought that the catalysis proceeds through a series of steps. In the first step, MAAI catalyses the attack of GSH on the α -carbon of MAA; upon GSH conjugation there would be freedom to rotate about the resulting single bond; following elimination of GSH the product would be generated. There is a pronounced electropositive surface surrounding the active site that would attract negatively charged compounds such as MAA. We have modeled MAA into the active site of MAAI and energy minimized the complex. The only assumption in the modeling process was that the acetate carboxylate would most likely be located close to the position of the bound sulfate ion. This orientation ensures that the α carbon is close to the GSH thiol so as to ensure a productive complex. The modeling led to a very convincing fit. The acetate carboxyl is within hydrogen bonding distance of a number of side-chains. The hydroxyl and keto substituents are within hydrogen bonding distance of Arg 13 and Arg 175 and some of the carbon backbone makes van der Waals interactions with the protein. The other carboxylate could possibly interact with the Arg 13 and hydroxyl of Tyr 11. Thus, the crystal structure provides a molecular basis to explain the specificity of MAAI for the substrate and shows how the active site orientates the substrate for optimal attack by GSH.²

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