Crystal structure of human glutathione synthetase

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Introduction

Glutathione (GSH) is the most abundant intracellular thiol in aerobic living cells and has been assigned several critical functions. Low GSH levels have been associated with the pathology of a number of diseases. GSH is synthesized from glutamate, cysteine, and glycine by the consecutive action of two enzymes: γ-glutamylcysteine synthetase and GSH synthetase (GS). Genetic studies have revealed mutations in the human GS gene that lead to GS deficiency and suggest that complete loss of function is probably lethal [1, 2]. There are two forms of the disease: a milder form (caused by a GS deficiency limited to erythrocytes) results in chronic hemolytic anemia and neonatal jaundice, and a more severe form, which additionally leads to 5-oxoprolinuria and metabolic acidosis. Human GS (hGS) has received much attention because of its involvement in hereditary disease. We have recently determined the crystal structure of hGS with the aim of understanding at the molecular level how point mutations cause disease [3].

Methods and Materials

Human GS was expressed and purified as described previously [4]. The protein was dialyzed against 10 mM Tris/HCl, pH 7.5, 10 mM $MgSO_4$, 1 mM DTT, and 0.1 mM ATP or ADP, and concentrated to between 3–5 mg ml^{-1} prior to the crystallization. The protein was mixed with an equivolume ratio of a solution containing 100 mM MES, pH 6.0, 10 mM GSH, 5 mM EDTA, 0.1–0.2 M $MgSO_4$, 9–12% PEG 4K and equilibrated over the same solution using the hanging-drop vapor-diffusion technique at 22˚ C. The crystals grew to a size of up to approximately 0.2 mm x 0.2 mm x 0.4 mm within a week. They belonged to the space group $P4_122$ with cell dimensions $a = b = 84.3$ Å, $c = 197.6$ Å and contained one monomer per asymmetric unit with a solvent content of approximately 60%.

The native data set was collected from a single crystal frozen to 100 K using synchrotron radiation at the BioCARS beamline, 14-BM-C. The synchrotron data were collected on both a MARResearch image plate scanner and an ADSC Quantum-4 CCD detector and processed using either the HKL [5] or the MOSFLM package [6]. The locations of heavy atoms for four derivatives were determined and refined using programs in the CCP4 suite [7]. The phases were improved by density modification with DM [7]. The model was constructed with the aid of skeletonized density maps and the program O [8]. Several cycles of refinement with REFMAC [9], interspersed with rounds of model building resulted in a final model of hGS consisting of residues 3–474, one molecule each of ADP and GSH, two Mg^{2+} ions, two sulfate ions, and 234 water molecules.

Results

Human GS is a compact molecule with the shape of a flat, equilateral triangle with the sides of the triangle about 60 Å and a thickness of about 40 Å. The ligands (consisting of ADP, magnesium ions, GSH, and one of the sulfate ions) are bound in a central cavity on one side of the molecule with ADP stacked between two antiparallel beta sheets. The cavity is covered by three loops projecting from three of the main structural units of the structure. The main structural units are an antiparallel beta sheet together with five helices packed on either side of the sheet, a parallel beta sheet together with four helices on both sides, and a domain we call "the lid" because of its role in providing access to the ATP-binding site. The lid domain consists of an antiparallel sheet with three helices packed on one side.

ADP is sandwiched between the strands of the two antiparallel beta sheets. The adenine-binding pocket is largely hydrophobic. The negative charges on the α- and β-phosphates are compensated by lysine residues. Two magnesium ions have been located and both are bound in an octahedral geometry. A sulfate ion, mimicking the position of the γ-phosphate of ATP, forms numerous contacts through its oxygen atoms with both Mg^{2+} ions and the protein. GSH is bound at one edge of the parallel beta sheet and forms extensive interactions with the protein including two salt bridges, 11 hydrogen bonding interactions, and 82 van der Waals contacts.

Discussion

The hGS crystal structure, which represents the enzyme-product complex, is fully consistent with the proposed reaction mechanism for other ligases. It is essential that hGS blocks the active site from the intrusion of solvent during catalysis so as to protect the phosphate intermediate from hydrolytic decomposition. On the other hand, ready access to the active site must be available for the entry of cofactors and substrates and for the exit of products. The enzyme likely accomplishes these tasks through movements of the lid domain and the active site loops.

Several mutations in the GS genes of patients with GS deficiency have been identified, and expression studies on some mutants have been performed [1, 2]. Since these are deficiency alleles with mutations outside the coding sequence and there are individuals with three mutations, it is not

possible to assume that the all coding region mutations are responsible for the disease. To help resolve this question, the mutations detected in GS deficient patients have been mapped onto the GS structure with the aim of understanding the molecular basis of GS deficiency. Many of the mutations appear to affect ligand binding or catalysis, whereas other mutations are likely to affect either dimerization or disrupt folding.

The crystal structure of hGS reveals that it belongs to the ATP-grasp superfamily despite the lack of any significant sequence identity with other members. The most surprising feature of the hGS structure is that its core structural units have been circularly permuted with respect to all the other family members: the ATP-grasp C-terminal domain is split into two in hGS so that approximately half is located at the N-terminus and the other half is located at the C-terminus. This circular permutation most likely arose by tandem duplication of a single ancestral gene encoding all three domains followed by deletions at both ends of the gene to yield the direct ancestor of hGS. There are very few known examples of naturally occurring circular permuted proteins and most of these were identified by sequence comparisons before structures became available [10], which was not possible in this case. A remarkable feature of the genetic permutation is the combination of the permuted secondary structures, irrespective of order, to form a very similar active site located at the domain boundaries. Future sequence-based searches for new members of the ATP-grasp superfamily will need to allow for possible gene permutations.

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