The Active Site of O-Acetylserine Sulphydrylase Is the Anchor Point for Bienzyme Complex Formation with Serine Acetyltransferase

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The biosynthesis of cysteine in bacteria and plants is carried out by a two-step pathway, catalyzed by serine acetyltransferase (SAT) and O-acetylserine sulphydrylase (OASS; O-acetylserine [thiol] lyase). The aerobic form of OASS forms a tight biienzyme complex with SAT in vivo, termed cysteine synthase. We have determined the crystal structure of OASS in complex with a C-terminal peptide of SAT required for biienzyme complex formation. The binding site of the peptide is at the active site of OASS, and its C-terminal carboxylic group occupies the same anion binding pocket as the α-carboxylate of the O-acetylserine substrate of OASS. These results explain the partial inhibition of OASS by SAT on complex formation as well as the competitive dissociation of the complex by O-acetylserine.

The biosynthesis of cysteine from serine in bacteria and plants is carried out by a two-step pathway beginning with the O-acetylation of serine by serine acetyltransferase (SAT; EC 2.3.1.30), followed by β-replacement of the acetyl group by sulfide by the pyridoxal-5′-phosphate (PLP)-dependent O-acetylserine sulphydrylase (OASS; EC 2.5.1.47) (10, 16, 17, 23) (Fig. 1). The inhibition of SAT by cysteine is the key regulatory feature of sulfur metabolism, as isomerization of the O-acetylserine product of SAT produces the N-acetylserine inducer of the cys regulon of genes responsible for sulfur assimilation (16, 17, 23). The approximately 20-fold abundance of the aerobic form of OASS relative to SAT in vivo is thought to lead to the inclusion of nearly all SAT into a biienzyme complex (1, 16, 18, 23, 32). The role of this complex, termed cysteine synthase (CS), is not well understood and is not thought to confer the advantages of substrate channeling (6), but its formation decreases the cold inactivation of SAT, reduces its proteolytic sensitivity (21), and partially inhibits the OASS component. The complex is reversibly dissociated to its component enzymes by O-acetylserine at concentrations of 0.1 to 1.0 mM (16). The C-terminal portion of SAT is required for both cysteine feedback inhibition of SAT and for the association of SAT and OASS to form the CS complex, based on the feedback insensitivity of mutants in this C-terminal region and the inability of truncation mutants to form the biienzyme complex (16, 17, 20, 22, 23). A 10-residue peptide corresponding to the C-terminal sequence of Escherichia coli SAT (EcSAT) inhibits E. coli OASS (EcOASS) with a Kᵢ of 130 nM (20).

The three-dimensional structures of hexameric Haemophilus influenzae SAT (HiSAT) and EcSAT have recently been determined as apoenzyme and in binary complexes with their cysteine inhibitor and with coenzyme A (8, 25, 28), and a kinetic and chemical mechanism of action for these enzymes has been proposed (11–13, 28). The binary complexes reveal conformational changes of residues 241 to 257 near the C-terminal end of the 267-residue polypeptide (25). These crystal structures, however, never defined a conformation for the disordered C-terminal 10 residues of SAT that are required for biienzyme complex formation. OASS has also been studied from biochemical and structural perspectives (3, 29). The available crystal structures of dimeric Salmonella enterica serovar Typhimurium OASS (StOASS) include the apoenzyme covalently bonded in Schiff base linkage through Lys 41 to its pyridoxal-5′-phosphate (PLP) cofactor as an internal aldimine (3) and the Lys 41 → Ala mutant enzyme in which PLP covalently bonds instead to a methionine residue from the medium in an external aldimine linkage (5). The structure of StOASS covalently bonded to methionine has been taken to be a structural analog of the covalent enzyme-O-acetylserine in-

<p>| TABLE 1. Data measurement and refinement statisticsa |
|---------------------------------|------------------|</p>
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value for space group I4, a = b = 112.6, c = 45.8</th>
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<tr>
<td>Unit cell parameters (Å)</td>
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<tr>
<td>X-ray wavelength (Å)</td>
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<td>Resolution (Å)</td>
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<tr>
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<tr>
<td>Rfactor/Rfree (%)</td>
<td>19.7/20.7 (25.4/26.8)</td>
</tr>
</tbody>
</table>

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Footnotes:

1. Data measurement and phasing
2. Rmerge (%) = Σ |Iᵢ - <I>|/Σ |Iᵢ| × 100, Rfactor (%) = Σ |Fᵢ| - |Eᵢ|/Σ |Fᵢ| × 100 for all available data, but excluding data reserved for the calculation of Rfree. Rfree (%) = Σ |Fᵢ| - |Eᵢ|/Σ |Fᵢ| × 100 for a 5% subset of X-ray diffraction data omitted from refinement calculations. Values in parentheses refer to the corresponding statistic calculated for data in the highest resolution bin.
termediate and has served to identify the location of the amino acid binding site. In addition to the “open” apoenzyme conformation and the “closed” conformation bound to methionine, a form of StOASS bound to chloride and sulfate anions results in an “inhibited” conformation (4).

In order to characterize the binding interactions of SAT to OASS in a homologous system and to address questions concerning the physical organization of enzymes leading to cysteine biosynthesis, we prepared crystals of *H. influenzae* OASS (HiOASS) in complex with the C-terminal 10-residue peptide of HiSAT. The crystal structure reveals the binding location and conformation of the C-terminal four residues of this peptide and provides a structural basis for the binding and partial inhibition of OASS by SAT in the CS complex.

MATERIALS AND METHODS

The *H. influenzae* cysK gene corresponding to the aerobic form of OASS (HiOASS) was inserted into a pET28a vector encoding an N-terminal removable six-histidine tag and used to transform *E. coli* BL21(DE3). The protein was purified from 3 liters of bacterial culture by Ni-nitrilotriacetic acid affinity chromatography, followed by thrombin cleavage of the histidine tag and subsequent Superdex 200 pg gel filtration chromatography. The final yield was 70 mg. The unblocked peptide corresponding to the C-terminal 10 residues of HiSAT (258Gly-Ile-Asp-Asp-Gly-Met-Asn-Leu-Asn-Ile267) was synthesized with a Perkins-Elmer Biosystems 433A peptide synthesizer and subsequently purified by high-pressure liquid chromatography.

Crystals of the HiOASS-peptide complex were prepared under silicon oil by using the sitting drop method by mixing 2 μl of HiOASS at a concentration of 15 mg/ml (0.45 mM of active sites) in 20 mM HEPES (pH 7.5), 50 mM NaCl, and 5 mM peptide with 2 μl of 3.0 M ammonium sulfate, 2% (vol/vol) polyethylene glycol 400, and 100 mM HEPES (pH 7.5). These crystals belonged to space group I41 (a = b = 112.6 Å and c = 45.8 Å). Crystals were passed through solutions containing glycerol (but lacking peptide) before vitrification in liquid nitrogen. These cooled crystals diffracted X rays to a resolution of 1.55 Å by using an in-house Rigaku R-Axis IV+ image plate detector and RU-H3R rotating anode X-ray generator equipped with Osmic Blue optics and operating at 50 kV and 100 mA. The data were reduced with HKL (26).

The structure was solved by molecular replacement with EPMR (15), by using a single subunit of apoenzyme StOASS (1OAS) as the search probe. Model building and atomic parameter refinements were carried out with the programs ARP/wARP (24, 27), O (14), and CNS (2). A Ramachandran plot of main chain torsion angle pairs identified no residues occupying the disallowed or generously allowed regions (19, 30).

The atomic coordinates corresponding to the structure of HiOASS in complex with the C-terminal decapeptide of SAT have been deposited in the Research Collaboratory for Structural Bioinformatics Protein Data Bank (accession code 1Y7L).

RESULTS

The structure of HiOASS in complex with the C-terminal decapeptide of HiSAT was determined to a resolution of 1.55 Å by using the structure of a single subunit of apoenzyme StOASS as the molecular replacement search probe. The

FIG. 1. The reactions catalyzed by SAT and OASS. The feedback inhibition of SAT is also depicted.

FIG. 2. (A) Stereoview superposition of HiOASS with peptide (yellow), StOASS with methionine in external aldimine linkage (cyan), and StOASS apoenzyme (gray). The atoms corresponding to PLP at the active site of HiOASS are depicted. (B) Space-filling diagram of dimeric HiOASS in complex with the peptide (yellow) and PLP (green) viewed parallel to the dimeric twofold axis. Figures were prepared with PyMOL (7).
FIG. 3. (A) Unbiased \( F_o - F_c \) electron density corresponding to the last four residues of the C-terminal peptide of SAT bound to the active site of HiOASS. The map was calculated before fitting the peptide to the electron density. The contour level is 3.0 rms units. (B) Superposition of the active sites of HiOASS in complex with the C-terminal four residues of HiSAT (yellow bonds) and StOASS in external aldimine linkage with methionine (cyan bonds). Hydrophilic interactions are indicated by dotted lines and illustrate the shared interactions of OASS with the C-terminal carboxylate of the peptide and the \( \alpha \)-carboxylate group of the methionine ligand bound to PLP. The locations of PLP, the methionine of StOASS linked to PLP, and the C-terminal residue of the peptide (Ile-P267) are labeled. (C) Stereoview of the active site of the HiOASS peptide complex. The peptide (pink), PLP (green), and water molecules (cyan) are depicted as are the amino-terminal residues of helix \( \alpha_2 \) that interact with the peptide carboxyl.
atomic model was refined to a crystallographic R-factor of 19.7% (R_free = 20.7%) (Table 1). The asymmetric unit of the atomic model included a single subunit of the dimeric enzyme (residues 2 to 311 of the 316-residue polypeptide), a single molecule of its PLP cofactor covalently bound to Lys 42 (equivalent to StOASS Lys 41), the C-terminal four residues of the 10-residue peptide, one sulfate ion, and 247 water molecules. The overall conformation of HiOASS near the active site is most similar to the apoenzyme, or open and anion-inhibited forms of StOASS (root mean square [rms] discrepancies of 0.5 and 0.4 Å, respectively) (Fig. 2A).

The peptide binds at the active site of OASS near the PLP cofactor (Fig. 2B). The distance between the two peptides bound to the OASS dimer is 36 Å. Interpretable electron density for the peptide was observed for only its C-terminal four residues (Fig. 3A). According to a residue numbering scheme from the numbering of the full-length 267-residue HiOASS, the visible residues of the peptide correspond to P264 to P267 (264Asn-Leu-Asn-Ile267). The observed peptide-OASS interactions are all formed by the last two residues (Fig. 3B and C). The side chain Oε2 (residues 71 to 84) as do their side chains.

**DISCUSSION**

The structure of HiOASS in complex with the C-terminal decapeptide of HiSAT reveals the conformation and interactions of the last four residues of SAT with the active site of OASS. A superposition of the StOASS-methionine and HiOASS-peptide structures revealed that the carboxyl groups of the methionine and Ile P-267 superimpose near the N-terminal end of a helix α2 (residues 71 to 84) as their side chains.

Asn-P266 interact with Ser 70 N and O/H9253, respectively. One peptide-OASS interactions are all formed by the last two residues (Fig. 3B and C). The side chain Oε2 (residues 71 to 84) as do their side chains.

The question of why the CS complex would remain catalytically competent for cysteine biosynthesis in vivo if its formation required its OASS active sites to be tethered (and inhibited) in the manner described here may be answered by noting that a dimer of OASS may be tethered to SAT in the CS complex by only one of its active sites. This is consistent with the measured turnover number for OASS in the purified CS complex of *S. enterica* serovar Typhimurium, which is 55% of that measured for the resolved enzyme (16). The observed mass of the CS complex derived from equilibrium ultracentrifugation studies is 309 kDa for the *S. enterica* serovar Typhimurium complex (16) and 293 kDa for the complex from *E. coli* (21). These observations are best explained by a stoichiometry for the complex of one molecule of hexameric SAT and two dimers of OASS (calculated mass, 314 kDa for both organisms) (9, 16). Such a complex could be formed with each dimer of OASS contributing one active site as an anchor point for biennial complex formation, leaving the other active site unblocked.

These results add to the diverse set of roles played by the C-terminal region of SAT, which now include its participation in the high-affinity cysteine binding site of SAT (25, 26), its competitive inhibition of OASS activity with respect to O-acetylserine, and its role in tethering OASS to form the CS complex. Remaining unanswered, however, are questions concerning the biological advantages conferred by complex formation since substrates are not thought to channel between the active sites of SAT and OASS (6). However, EcOASS stimulates the activity of *E. coli* ATP sulfurylase (31), an enzyme that catalyzes the initial step of sulfur assimilation into activated organic molecules, suggesting that OASS may form additional bienzyme or higher order enzyme complexes.

**ACKNOWLEDGMENTS**

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**REFERENCES**


