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Fine tuning of the active site modulates specificity in the interaction of *O*-acetylserine sulfhydrylase isozymes with serine acetyltransferase

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ABSTRACT

O-acetylserine sulfhydrylase (OASS) catalyzes the synthesis of L-cysteine in the last step of the reductive sulfate assimilation pathway in microorganisms. Its activity is inhibited by the interaction with serine acetyltransferase (SAT), the preceding enzyme in the metabolic pathway. Inhibition is exerted by the insertion of SAT C-terminal peptide into the OASS active site. This action is effective only on the A isozyme, the prevalent form in enteric bacteria under aerobic conditions, but not on the B-isozyme, the form expressed under anaerobic conditions. We have investigated the active site determinants that modulate the interaction specificity by comparing the binding affinity of thirteen pentapeptides, derived from the C-terminal sequences of SAT of the closely related species *Haemophilus influenzae* and *Salmonella typhimurium*, towards the corresponding OASS-A, and towards *S. typhimurium* OASS-B. We have found that subtle changes in protein active sites have profound effects on protein–peptide recognition. Furthermore, affinity is strongly dependent on the pentapeptide sequence, signaling the relevance of P3–P4–P5 for the strength of binding, and P1–P2 mainly for specificity. The presence of an aromatic residue at P3 results in high affinity peptides with K_{diss} in the micromolar and submicromolar range, regardless of the species. An acidic residue, like aspartate at P4, further strengthens the interaction and results in the higher affinity ligand of *S. typhimurium* OASS-A described to date. Since OASS knocked-out bacteria exhibit a significantly decreased fitness, this investigation provides key information for the development of selective OASS inhibitors, potentially useful as novel antibiotic agents.

1. Introduction

Sulfur assimilation occurs in many microorganisms and plants via the obligatory formation of cysteine (Scheme 1). Sulfate, taken up by a specific channel, is converted into sulfide in four steps with the consumption of two molecules of ATP and four molecules of NADPH for each molecule of sulfide produced. Concomitantly, L-serine, which originates from the glycolytic intermediate 3-phosphoglycerate, is condensed with CoA to form *O*-acetylserine (OAS) by serine acetyltransferase (SAT) [1,2]. OAS undergoes a β-replacement reaction catalyzed by the pyridoxal 5′-phosphate (PLP)-dependent enzyme *O*-acetylserine sulfhydrylase (OASS) [3]. This reaction takes place via several steps that include: i) the binding of the substrate to the internal aldimine, forming the external aldimine; ii) α-proton abstraction leading to the β-elimination of acetate and the formation of the metastable intermediate α-aminoacrylate Schiff base; iii) the nucleophilic attack of sulfide on the α-aminoacrylate Schiff base generating L-cysteine. This amino acid is a key metabolite for bacteria because it provides sulfur to all sulfur-containing biological molecules, such as methionine, biotin, and iron–sulfur clusters. Moreover, in pathogens, such as *Trichomonas vaginalis*, *Mycobacterium tuberculosis*, and *Salmonella typhimurium*, cysteine contributes directly or as a precursor to the maintenance of the redox state of the cell. This function is critical to facultative intracellular

Abbreviations: OASS, *O*-acetylserine sulfhydrylase; SAT, serine acetyltransferase; OAS, *O*-acetylserine; PLP, pyridoxal 5′-phosphate; CS, cysteine synthase; *Hi*OASS, *Haemophilus influenzae O*-acetylserine sulfhydrylase; *St*OASS, *Salmonella typhimurium O*-acetylserine sulfhydrylase; *Hi*SAT, *Haemophilus influenzae* serine acetyltransferase; *St*SAT, *Salmonella typhimurium* serine acetyltransferase

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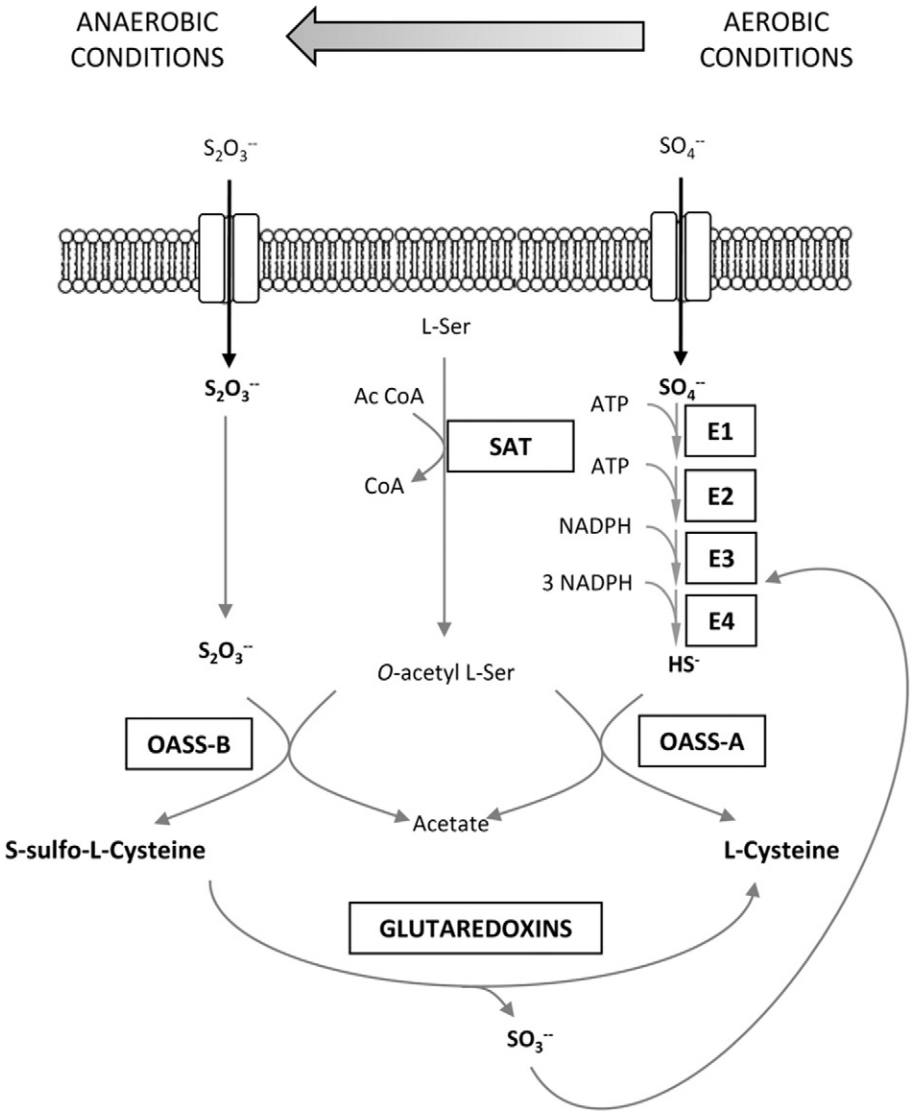

Scheme 1. Cysteine biosynthesis in Proteobacteria. Enzymes are boxed. E1, ATP sulfurylase; E2, APS kinase; E3, PAPS sulfotransferase; E4, NADPH sulfite reductase. OASS-A is the predominant form under aerobic conditions [61] and requires HS^- produced by the reductive assimilation of sulfate. It has been suggested that OASS-B is induced under anaerobic conditions [12,62] and is, in fact, capable of directly incorporating inorganic thiosulfate into S-sulfocysteine that is subsequently reduced to cysteine by glutaredoxins [63].

microorganisms, such as *Brucella*, *Salmonella* and *Mycobacterium* that spend part of their life-cycle in the highly oxidizing environment of macrophages, thus supporting long-term infections [4,5]. Therefore, it is not surprising that enzymes involved in reductive sulfate assimilation are potential drug targets [4,6–9]. It was demonstrated that strains of swarming *S. typhimurium*, knocked out for OASS exhibit an impaired ability to develop antibiotic resistance, likely linked to induced oxidative stress defense, and a 500-fold increased vulnerability to ciprofloxacin [10,11].

In the proteobacteria *Escherichia coli*, *S. typhimurium* and *Haemophilus influenzae*, two isozymes, OASS-A and OASS-B, catalyze the biosynthesis of L-cysteine. OASS-A, the product of *cysK*, is the predominant isozyme, while OASS-B, the product of *cysM*, is expressed under anaerobic conditions [12]. However, the relative role of the two isozymes in infection and long-term survival has never been studied in detail. Furthermore, OASS-B accepts a wider variety of substrates and bulkier ligands, suggesting an active site volume greater than OASS-A [12,13]. The two isozymes share a 43% identity and exhibit very similar three-dimensional structures [13]. A key difference between OASS-A and OASS-B is their distinct ability to interact with SAT and to form the cysteine synthase complex (CS), since only OASS-A is endowed with this property. Formation of CS leads to an inhibition of OASS catalysis, thus reducing cysteine synthesis [14]. The three-dimensional structure of CS is not available, but structures of OASS-A, both in the absence and presence of peptides derived from the C-termini of SAT have been crystallographically characterized [9,15–18]. Three dimensional structures of OASS-B [13,19] and SAT [20–22] have also been solved. Biochemical studies were instrumental in proposing models for CS from bacteria [23–27] and plants [28–33]. The interaction between OASS-A and SAT is mediated by the insertion of the SAT C-terminal peptide into the OASS active site [18,26]. In *H. influenzae* this process occurs in two steps, a very fast formation of the encounter complex, followed by a slower conformational change [14]. A second isomerization step was detected while investigating *E. coli* CS formation [34]. Computational analysis, carried out on *Arabidopsis thaliana* OASS, suggests the presence of two gates that control active site accessibility [35].

In a previous study aimed at developing high affinity pentapeptides for the identification of potential antibiotic compounds, the interaction of OASS-A from *H. influenzae* with a virtual library of 400 pentapeptides, MNXXI, was characterized [9]. Pentapeptides were docked in the OASS-A active site and their predicted free energies of binding were found well correlated with the binding affinities for 14 pentapeptides experimentally determined via a direct fluorescence method [23]. In the present investigation, 13 pentapeptides were exploited as a tool to understand: i) the fine-tuning of SAT