Cysteine biosynthesis in *Neisseria* species

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**Abstract**

The principal mechanism of reducing sulfur into organic compounds is via the synthesis of L-cysteine. Cysteine is used for protein and glutathione synthesis, as well as being the primary sulfur source for a variety of other molecules, such as biotin, coenzyme A, lipoic acid and more. Glutathione and other cysteine derivatives are important for protection against the oxidative stress that pathogenic bacteria such as *Neisseria gonorrhoeae* and *Neisseria meningitidis* encounter during infection. With the alarming rise of antibiotic-resistant strains of *N. gonorrhoeae*, the development of inhibitors for the future treatment of this disease is critical, and targeting cysteine biosynthesis enzymes could be a promising approach for this. Little is known about the transport of sulfate and thiosulfate and subsequent sulfate reduction and incorporation into cysteine in *Neisseria* species. In this review we investigate cysteine biosynthesis within *Neisseria* species and examine the differences between species and with other bacteria. *Neisseria* species exhibit different arrangements of cysteine biosynthesis genes and have slight differences in how they assimilate sulfate and synthesize cysteine, while, most interestingly, *N. gonorrhoeae* by virtue of a genome deletion, lacks the ability to reduce sulfate to bisulfide for incorporation into cysteine, and as such uses the thiosulfate uptake pathway for the synthesis of cysteine.

**INTRODUCTION**

Sulfur becomes accessible to animals as bacteria and plants incorporate inorganic sulfur into cysteine. Cysteine biosynthesis is the primary pathway for the incorporation of sulfur into a variety of cellular constituents, including methionine, thiamine, biotin, coenzyme A and more. Cysteine itself plays an important role in protein molecules in Fe–S clusters, catalysis, protein folding and the formation of disulfide bonds. In micro-organisms, cysteine plays an important role in protection from oxidative stress via reducing systems such as thioredoxin and glutathione. In bacteria, cysteine biosynthesis is carried out almost exclusively via the conserved reductive sulfate assimilation pathway, which successively reduces sulfate to bisulfide for the production of cysteine using an activated form of serine.

Due to the important cellular roles of cysteine and the absence of mammalian biosynthetic pathways, the enzymes involved in sulfur metabolism and specifically cysteine biosynthesis are targets for the design of novel antibiotics [1–5]. For example, the inactivation of cysteine and methionine biosynthetic enzymes in *Mycobacterium tuberculosis* significantly reduces persistence and virulence during the chronic stage of infection in mice models [6]. Furthermore, deletion of cysteine synthase in *Salmonella typhimurium* caused a 500-fold increase in sensitivity to ciprofloxacin [7].

The *Neisseria* species include the human pathogens *Neisseria meningitidis* (meningococci) and *Neisseria gonorrhoeae* (gonococci), as well as other non-pathogenic environmental species, such as *Neisseria mucosa*, *Neisseria flava*, *Neisseria sicca*, etc. Meningococci are facultative commensals that colonize the nasopharynx and for reasons that are not fully understood occasionally enter the bloodstream or central nervous system and cause life-threatening septicemia or meningitis, respectively. Despite the availability of antibiotics for prophylactic or therapeutic treatment and the availability of immunization against specific serogroups of meningococcal meningitis, it remains the leading cause of bacterial meningitis worldwide [8]. Meningococcal disease is associated with high morbidity and mortality in children and young people, with effects such as limb loss, hearing loss, cognitive dysfunction, visual impairment, seizure disorders and developmental delays [9]. Gonococci are obligate human pathogens and are the causative agent of the sexually transmitted infection gonorrhoea, which causes an estimated 78 million cases annually worldwide [10]. *N. gonorrhoeae* colonize and invade the epithelium of the urogenital tract to cause localized inflammation, and in rare cases
disseminate and cause more serious disease, such as pelvic inflammatory disease, ectopic pregnancy, infertility and arthritis. Gonorrhoea is now a global health problem due to the increasingly high number of strains that are resistant to all frontline antibiotics. Since the introduction of antibiotic treatment for gonorrhoea, resistance has emerged to sulphonamides, penicillins, tetracyclines, fluoroquinolones, early-generation cephalosporins and, more recently, extended-spectrum cephalosporins, including ceftriaxone, and macrolides, including azithromycin (the main current treatments) [10]. Therefore, the identification of new or alternative effective treatment regimens is critical for future treatment of this disease.

Gonococci and meningococci differ in the way they obtain sulfur for growth. These differences could reflect the different pathogeneses of these two organisms, with gonococci being an obligate human pathogen and meningococci being an 'accidental' pathogen, and their different environmental requirements for growth. Targeting cysteine biosynthesis in these pathogens could be a promising avenue for new antimicrobials to combat gonorrhoea and meningococcal meningitis.

**TRANSPORT OF SULFATE**

There are three sulfate transport systems in bacteria, as described in [11]. Two belong to the ATP-binding cassette (ABC) superfamily and the other belongs to the major facilitator superfamily (MFS) sulfate transporters. *Neisseria* species have a sulfate transport system belonging to one of the ABC superfamilies. This sulfate transporter has a periplasmic sulfate-binding protein (sbp), the permeases CysT (now annotated as CysU to avoid confusion with the tRNA-Cys gene) and CysW, and the ATP-binding subunit CysA [12] (Fig. 1). This system has the ability to take up sulfite and thiosulfate [13]. An additional ABC transporter with the periplasmic binding protein CysP replacing sbp is more specific for thiosulfate uptake. Using sequence similarity searches, we found no homologues of CysP or the MFS sulfate-binding protein CysP replacing sbp is more specific for thiosulfate uptake. Using sequence similarity searches, we found no homologues of CysP or the MFS sulfate-binding protein (sbp) in *Neisseria* genomes. Although there is an annotated *cysP* gene in three recently sequenced *N. gonorrhoeae* strains [14], closer inspection via homology searches revealed that it is actually an inorganic phosphate transporter protein. As such, we hypothesize that the sbp ABC transporter is the only sulfate transport system in *Neisseria* (Fig. 1).

The single ABC transporter identified in *Neisseria* is for the uptake of sulfate and presumably thiosulfate, given the ability of all *Neisseria* species to grow on thiosulfate as a sole source of sulfur [15]. The ABC transporter for the uptake of sulfate consists of the periplasmic binding protein sbp, which is present in all *Neisseria* species, although the protein from *N. gonorrhoeae* is truncated at the N-terminal end compared to the *N. meningitidis* sbp (Fig. 1). The CysU and CysW permeases are present in most *Neisseria*, including *N. gonorrhoeae*, although we could not identify the CysU subunit in the genome of *N. gonorrhoeae* FA 1090, even when using the Basic Local Alignment Search Tool (BLAST) to interrogate the entire genome (Fig. 1). It is, however, present in other *N. gonorrhoeae* strains, such as SK29344, SK22871, ATL_2011_01_17, 1291, MS11 and ATL_2011_01_21. The CysA ATP-binding subunit is present in all *Neisseria*, with the protein from *N. gonorrhoeae* being 97% identical to that from *N. meningitidis*. The cysA and cysW genes appear to be in a bicistronic operon, whereas cysU and the sbp appear individually in *Neisseria* genomes (Fig. 1). Several studies have found an upregulation of the cysAW operon and cysU (cysT) sulfate uptake genes upon the adhesion of meningococci to epithelial cells [16–18].

**SULFATE REDUCTION**

Once imported into the cell, sulfate is successively reduced to bisulfide via a series of enzymatic steps, as shown in Fig. 2. The first is catalyzed by ATP sulfurylase (CysD). This enzyme activates sulfate in an ATP-dependent reaction to form adenosine 5'-phosphosulfate (APS) and pyrophosphate. ATP sulfurylase typically consists of two subunits, CysD, the catalytic subunit, and CysN, the GTP regulatory subunit. In *Neisseria* species these subunits are encoded by separate cysD and cysN genes (Fig. 3), but in some bacteria such as *Bacillus subtilis* the two subunits are fused [19]. In most bacteria the next step is catalyzed by APS kinase (CysC) to form phosphoadenosylphosphosulfate (PAPS) in an ATP-dependent reaction. In some bacteria, including the *Mycobacteria* and *Pseudomonads*, CysC, the APS kinase, is fused to CysN. PAPS is subsequently reduced to sulfite and phosphoadenosylphosphosulfate (PAP) by PAP reductase (CysH). However, in *Neisseria* a single enzyme, APS reductase (also termed CysH), directly reduces APS to sulfite [20] (Fig. 2). Curiously, enzymes with both PAPS and APS reductase activities are present in *B. subtilis*, *Pseudomonas aeruginosa* and *M. tuberculosis*, for example, CysH can complement a cysC mutant and vice versa [21–23]. The final step in the reaction is the reduction of sulfite to bisulfide by the multi-subunit enzyme sulfite reductase (CysJ) (Fig. 2). The *Escherichia coli* sulfite reductase is a complex enzyme, composed of two proteins, CysJ and CysL, in a α3β3 structure. The flavoprotein CysJ (αα) contains four FAD and four FMN cofactors and NADPH–cytochrome c reductase activity. CysJ accepts electrons from NADPH and transfers them to CysL, which reduces sulfite to sulfide [13]. CysJ is a hæmoprotein that contains a [4Fe–4S] cluster and a siroheme. An uroporphyrinogen methyltransferase encoded by cysG catalyzes the synthesis of siroheme (for incorporation into CysL) from uroporphyrinogen III [13]. CysJ is present in *Neisseria* species, along with CysG, although in some bacteria such as *B. subtilis* uroporphyrinogen methyltransferase activity is encoded by yhnD and yhnF, which encode the C-terminal and N-terminal domains, respectively [24].

The genes encoding the enzymes for the reduction of sulfate to sulfide are organized differently in different species of bacteria. For example, in *E. coli* there are three transcriptional units: cysDNC for the reduction of sulfate to PAPS,
cysIJH for the reduction of PAPS to bisulfide and cysG the uroporphyrinogen methyltransferase [13]. In B. subtilis a large cysHPylnBCDEF operon is present with the cysJI operon found elsewhere [25, 26]. This led us to investigate the genomic context of genes for the reduction of sulfate in Neisseria species, specifically N. meningitidis and N. gonorrhoeae. N. meningitidis has all the genes necessary for sulfate reduction in the cysGHDNJI operon, whereas N. gonorrhoeae has an unusual cysGNJI operon. In the N. meningitidis serogroup B strain MC58 genome the cysGHDNJI operon appears twice in a ~32 kb region that appears to have been duplicated [27]. In other serogroup B strains such as H44/76 this region occurs only once [28]; we also interrogated other N. meningitidis strains from the same and different clades [29], but could only identify one copy of the cysGHDNJI operon. The N. meningitidis genome shares 90% homology at the nucleotide level to the N. gonorrhoeae genome, and genomic comparisons allowed us to look closely at the unusual cysGNJI operon arrangement and, together with homology searches, determine whether the missing cysH and cysD genes from N. gonorrhoeae are located elsewhere in the genome. We found a deletion of 3500 bp between the cysG and cysN genes that removes cysH and cysD and truncates both the cysG and cysN genes (Fig. 3), as also seen by Rusniok et al. [20]. In addition, a closer inspection of the cysJI genes revealed that they are pseudogenes due to the presence of stop codons within their coding sequences (Fig. 3).

Most N. meningitidis and N. flava strains can grow on sulfate as a source of sulfur, whereas N. gonorrhoeae lacks this ability [15, 30]. The loss of the sulfate reduction pathway in N. gonorrhoeae through gene deletions, truncations and the insertion of stop codons fits with the inability of the bacterium to grow on sulfate as a unique source of sulfur [15]. However, the need for cysteine in the culture media of N. gonorrhoeae can be satisfied by thiosulfate [15], suggesting that the sulfate sbp ABC transporter discussed above is also transporting thiosulfate into the cell. When N. gonorrhoeae is grown in the presence of 2 mM thiosulfate (or more) as the sole source of sulfur, sulfite accumulates in the culture medium [15]. The cleavage of thiosulfate produces sulfur and sulfite, with the sulfur originating from the sulfane moiety being incorporated directly into cysteine. If the bacterium lacks the ability to reduce sulfite to sulfur (Fig. 2), sulfite will accumulate in the culture medium, as seen in N. gonorrhoeae, reaffirming the inability of N. gonorrhoeae to successively reduce sulfate to sulfide.

**CYSTEINE SYNTHASE**

Although the pathway of cysteine biosynthesis has not been studied in Neisseria, the fact that all strains are able to use thiosulfate as a sole source of sulfate suggests that a
functional serine acetyltransferase (SAT, CysE) and O-acetylserine sulfhydrylase (OASS, CysK/CysM) are present.

SAT catalyzes the first step in the two-step reaction that makes L-cysteine from L-serine. The side-chain carboxyl of L-serine undergoes a CoA-dependent acetylation to form O-acetylserine. The second step is then catalyzed by OASS, which catalyzes the β-replacement of acetate with sulfide to convert O-acetylserine to L-cysteine. SAT and OASS form a bienzyme complex termed cysteine synthase. The SAT–OASS interaction is highly conserved across bacterial species. Most enzyme complexes form to channel substrates from one enzyme to another. However, the cysteine synthase complex is unusual in that the C-terminal tail of SAT inserts into the active site of OASS, competing with the binding of O-acetylserine [31]. In the complex one SAT hexamer binds to two OASS dimers, the formation of which is disrupted by O-acetylserine [31]. In addition to regulation by complex formation, SAT is feedback-inhibited by cysteine. The crystal structure of SAT from *Haemophilus influenzae* in complex with cysteine and also with CoA [32] demonstrates CoA binding in the active site as expected, but cysteine also binds in the active site to compete with serine and inhibit its activity.

Mammals synthesize cysteine from methionine and as such lack OASS, making it a potential target for antimicrobials. OASS is a pyridoxal 5′-phosphate-dependent enzyme belonging to the cysteine synthase superfamily [13]. Most bacteria have two isoforms of O-acetylserine sulfhydrylase, termed A and B. OASS-A, as discussed earlier, forms a tight complex with SAT, whereas OASS-B does not interact with SAT [33, 34]. Proteobacteria such as *E. coli*, *S. typhimurium* and *H. influenzae* have both A and B isoforms of OASS. OASS-A (CysK) is expressed under aerobic conditions, whereas OASS-B (CysM) is expressed under anaerobic conditions [35]. The OASS-A and OASS-B isoforms display subtle but significant structural differences, leading to
differences in their preferred substrates [4], with OASS-B preferring thiosulfate and OASS-A preferring bisulfide. *Neisseria* species appear to have only one OASS isoform, annotated as CysK (OASS-A), and multiple searches of the genomes of species belonging to *Neisseria* found no OASS-B isoforms. We modelled the structures of the SAT (CysE) and OASS (CysK) proteins from *N. gonorrhoeae* using the Pyrex 2 modelling server [36]. The structures of the SAT (Fig. 4a) and OASS-A (Fig. 4d) enzymes were modelled with 100% confidence and 93 and 99% coverage, respectively. We then overlaid the model structures with SAT and OASS-A structures from enzymes not used in the modelling process. There is good conservation of structure for both enzymes, even though there is little sequence homology, as shown by the hidden Markov model (HMM) [37] for each (Fig. 4). Although there is little sequence homology, we see the conservation of key amino acid residues in the SAT and OASS-A proteins from *Neisseria* species (Fig. 4), confirming that these are in fact functional enzymes. As shown in the HMM for OASS, there is conservation of amino acid residues in the active site. These conserved amino acids also interact with a peptide inhibitor based on the C-terminal tail of SAT in *H. influenzae* (Fig. 4f) [5]. *Neisseria* species have the ability to grow on thiosulfate as the sole source of sulfate, which suggests that the OASS-A (CysK) protein is functioning as a dual-function enzyme using both sulfide and thiosulfate to make cysteine. Although this is unusual, as most bacteria have two isoforms of OASS, there is evidence that CysK and CysM are bifunctional in *S. typhimurium*. A cysK/cysM double mutant in *S. typhimurium* required cysteine for growth, whereas strains lacking either cysK or cysM grew in the absence of cysteine [11].

SAT is classified as an essential gene in *N. gonorrhoeae*, whereas OASS-A is not [38]. SAT is the limiting step in the production of cysteine in *N. gonorrhoeae*; it is feedback-inhibited by cysteine and represents the rate-limiting step of the pathway, which presumably defines why it is essential. OASS-A (CysK) is not essential in other bacteria, but when it is knocked out the bacteria show significantly decreased fitness and increased susceptibility to antibiotics [7, 39, 40]. SAT is not classified as essential in *N. meningitidis*, but in a transposon mutagenesis screen exhibited a severe growth defect [41]. OASS-A is also non-essential but has been classified as being important for the meningococcal colonization of epithelial cells [41].

In other organisms, cysteine, as a major reducing agent, plays an important role in survival, mainly due to protection against oxidative stress. *N. gonorrhoeae* lacks any glutathione importers [42] and, as such, we hypothesize that cysteine plays a vital role in overcoming oxidative stress that the bacterium encounters upon infection.

**REGULATION**

The cysteine regulon of other pathogenic bacteria is upregulated *in vitro* in the presence of nitric oxide [43], under oxidative stress [40] and *in vivo* during infection or long-term survival [6, 44]. Upon infection, pathogenic *Neisseria* encounter unique conditions, including low pH, oxidative stress and varying iron and nutrient concentrations. The cysteine regulon is composed of the sulfate/thiosulfate transporter genes, the sulfate reduction pathway genes and the cysteine synthase genes, and is regulated by the transcription factor CysB. The cysteine regulon is important for meningococci host cell colonization [41], and the sulfate reduction operon *cysDGHIJN* is essential for meningococcal growth [45]. Within meningococci, the genes of the cysteine regulon show upregulation upon host cell colonization, yet no expression differences in cysB expression have been observed [41, 45]. However, CysB is highly expressed and classified as an essential gene in *N. gonorrhoeae* [38]. The transcriptome of *N. gonorrhoeae* during infection of the lower genital tract in women (*in vivo*) has been compared to the growth of *N. gonorrhoeae* in chemically defined medium (*in vitro*) [46]. Of significant note was a decrease in the expression of CysB in two of the three subjects sequenced and this encourages further investigation as to the role of CysB in activating the cysteine regulon during infection.

CysB is a transcription factor belonging to the helix-turn-helix family that regulates the cysteine regulon [11, 47–50]. CysB is a homotetramer with an N-terminal DNA-binding motif and has been shown to activate the transcription of cysP, cysJIH and cysK promoters in the presence of N-acetylseryine in *S. typhimurium* [48, 50]. It also binds to its own promoter to negatively regulate its activity. Based on the characterization of the *S. typhimurium* CysB protein, we
identified the inverted repeat sequence CCGTTG-N_{17}-CAACGG 35 nucleotides upstream of the cysB transcriptional start site in both N. gonorrhoeae and N. meningitidis. We suggest that CysB binds to this sequence to regulate its own activity by occluding the RNA polymerase-binding site, thereby repressing transcription. In this situation, binding of N-acetylserine would reduce CysB binding to DNA, which in turn activates transcription [49]. Sulfide and thiosulfate compete with N-acetylserine for binding to CysB and as such act as anti-inducers [48]. We could not identify the putative CysB-binding sequence upstream of other genes in the cysteine regulon, presumably as CysB-binding sites generally show poor sequence homology. CysB positively regulates genes within the cysteine regulon (with the exception

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Fig. 4. Enzymes of the cysteine synthase complex. Model of the N. gonorrhoeae serine acetyltransferase (SAT, CysE) enzyme (a) as predicted with 100% confidence and 93% coverage by Phyre2 using the PDB file 1T3D (SAT from E. coli) as a template (56% sequence identity). Overlay of the model with PDB file 3GVD (SAT from Yersinia pestis) shows the conservation of key structural features (b). The height of the amino acids in the Pfam hidden Markov model (HMM) [37] for the SATase domain shows the conservation of key active site amino acids present in Neisseria SAT enzymes (c) [also shown as sticks in (a)]. Model of the N. gonorrhoeae O-acetylserine sulfhydrylase A isoform (OASS-A, CysK) enzyme (d) as predicted with 100% confidence and 99% coverage by Phyre2 using the PDB file 4AEC (OASS-A from Arabidopsis thaliana) as a template (55% identity). Overlay of the model with PDB file 4H01 (OASS from H. influenzae) shows the conservation of key structural features (e). The active site of OASS-A from H. influenzae (PDB file 3IQG) binds peptides corresponding to the C-terminus of SAT to inhibit its activity (f). The height of the amino acids in the Pfam HMM [37] for OASS (g) shows the conservation of key amino acids [shown as sticks in (d)] in the active site required for activity and also for binding of the peptide inhibitor.
of CysB itself). When N-acetylserine is present a conformational change is thought to occur in CysB that allows the protein to interact with DNA at ‘activation sites’ in the promoter DNA and it is hypothesized that this recruits RNA polymerase.

**INHIBITORS OF CYSTEINE BIOSYNTHESIS**

In the last 6 years, microbial cysteine biosynthesis has been investigated as a potential drug target, as the repression of cysteine synthesis leads to a reduction in the fitness of the bacterium and reduced pathogenesis [2–4, 51]. Amino acid biosynthesis is an exploitable target for new antimicrobials, as most pathogens spend a part of their life cycle in extremely challenging environments, such as macrophages in the case of *M. tuberculosis* or the gastric mucosa in the case of *S. typhimurium* [52–54]. Survival and growth depend on the ability of the bacteria to metabolize in this extreme environment. Pathogenic *Neisseria* inhabit mucosal surfaces and, as such, are subjected to oxidative stress. Glutathione is one of the first-line defences against oxidative stress [42]; it scavenges free radicals and restores oxidized molecules by donating hydrogen. High concentrations of glutathione (>15 mM) are present in *N. gonorrhoeae* [42], presumably for defence against oxidative stress. Cysteine or cystine are required as a precursor for the biosynthesis of glutathione. In accordance with the role of cysteine in combating oxidative stress, upon cysteine depletion the gene expression profile in *N. meningitidis* resembles that encountered during oxidative stress [55]. Designing inhibitors to cysteine biosynthetic genes from *N. gonorrhoeae* or *N. meningitidis* could be a promising avenue for future antimicrobials. In addition, mammals synthesize cysteine from methionine and, as such, lack SAT and OASS.

Cysteine biosynthesis has also been implicated in the development of antibiotic resistance [7, 40]. Antibiotic-induced oxidative stress is a well-known phenomenon and interestingly bacteria with impaired cysteine biosynthesis exhibit reduced antibiotic resistance. Altogether, this suggests that inhibitors of cysteine biosynthesis could provide new antimicrobials for inhibiting growth or the ability of pathogenic *Neisseria* to infect and invade, or enhance the efficacy of existing antibiotics and help to combat the increase in resistance.

SAT is a key enzyme in cysteine biosynthesis and, given that it is essential in *N. gonorrhoeae*, represents a promising drug target. There have been few attempts to discover inhibitors of SAT, with the main attempt being virtual screening using the crystal structure of *E. coli* SAT and the National Cancer Institute chemical database [40]. Of the 11 molecules with the highest scores, 3 compounds were evaluated biologically, and the best had an IC$_{50}$ value of 72 µM and could serve as a platform for the development of a more potent inhibitor.

OASS is the other key enzyme in cysteine biosynthesis and is the last enzyme in the pathway (Fig. 2). In many pathogenic organisms there are two or more OASS isoforms, which activated under different conditions. As *Neisseria* appear to only have one OASS isoform, efficient inhibition of this enzyme would drastically reduce cysteine biosynthesis and the fitness of the organism, leading to an increased susceptibility to antibiotics [7, 39, 40, 56].

As discussed earlier, the C-terminus of SAT interacts with OASS-A to regulate its activity. Peptide inhibitors were designed based on this sequence and screened *in silico* for binding to OASS [5] (Fig. 4f). These

**Fig. 5.** The cystine and cysteine ABC transporter operons from *N. meningitidis* and *N. gonorrhoeae*. The complete cystine ABC transporter operon is present in both *Neisseria* species (top). The cysteine ABC transporter operon is present in *N. gonorrhoeae* but absent from the *N. meningitidis* genome (bottom).
studies determined the important structural requirements for the design of pentapeptide inhibitors of OASS, in that the three C-terminal amino acids determine the affinity of the peptide for OASS and the first two positions are important for dictating binding to OASS.

Synthetic peptides and docking studies found peptides that bound tighter than the native peptide and their derivatives. A carboxylic acid, a small hydrophobic chain at C2 and a cyclopropane linker grant tight packing of the structure and are essential for synthetic peptides to inhibit OASS by locking the enzyme in a closed conformation [57].

There is conservation of key amino acids in the active site of the OASS enzyme from Neisseria species (Fig. 4d) and, although the sequence identity is low, the structural conservation of these enzymes is high (Fig. 4e). The five C-terminal amino acids of SAT from N. gonorrhoea form a tail (Fig. 4d) and have the sequence IDFMI. The conserved isoleucine at the end of the tail is important for peptide binding and inhibition. We therefore suggest that what is known about peptide inhibitors of OASS from other organisms can be applied to the homologues in Neisseria species. To date, no cysteine biosynthesis enzymes from any Neisseria species have been characterized, yet inhibitor design relies on the characterization of the key enzymes in this pathway. Inhibition of cysteine biosynthesis could be a promising avenue for the design of new antimicrobials for the treatment of gonorrhoea and meningococcal disease. This is especially promising for N. gonorrhoeae, given that it has a limited cysteine biosynthesis pathway, as the ability to reduce sulfate has been lost and the SAT appears to be essential.

TRANSPORT OF CYSTEINE AND CYSTINE

Under oxidizing conditions, two cysteine molecules dimerize by the formation of a disulphide bond to form cystine. In most bacteria cystine is imported as a source of cysteine. In N. gonorrhoeae two ABC transporter operons for the uptake of cystine and cysteine (ngo0372-0374 and ngo2011-2014, respectively) have been identified [58]. The extracellular solute receptors (NGO0372 and NGO2014) for each transporter were crystallized in an ‘open’ ligand-free form and a ‘closed’ form bound to L-cystine and L-cysteine, respectively. N. meningitidis has an operon with 97% identity to the ngo0372-0374 cystine ABC transporter operon from N. gonorrhoea, so they presumably transport cystine in the same manner. Interestingly, the ngo2011-2014 operon encoding the ABC transporter for the import of cystine is absent from N. meningitidis but present in most other Neisseria species (Fig. 5). This suggests the operon has only been deleted in the N. meningitidis species and, as such, meningococci do not require the ability to import cysteine or have an alternative transporter for the import of cysteine. The extracellular concentration of cysteine that these organisms encounter is unknown, but presumably in the intracellular environments concentrations are low. In addition, the Neisseria pathogens N. gonorrhoeae and N. meningitidis often encounter oxidative stress, and in these oxidizing environments cysteine is most likely in the cystine form, which both species have the transporters for.

CONCLUSION

Sulfur is found in many important biomolecules, such as the amino acids cysteine and methionine, the cofactors CoA, Fe–S clusters and biotin, and the reducing agents cysteine, glutathione and thioredoxin. Plants and bacteria incorporate inorganic sulfur into cysteine, which then becomes available to higher organisms. Cysteine is essential to all organisms, but cysteine biosynthesis is especially important to pathogenic bacteria to overcome the oxidative stress encountered upon infection. The pathogenic Neisseria N. meningitidis and N. gonorrhoeae have the ability to synthesize cysteine, although as outlined in this review, N. gonorrhoeae has lost the ability to reduce sulfate to sulfide and, as such, relies on thiosulfate as its sole source of sulfate for cysteine biosynthesis. N. gonorrhoeae colonize mucosal surfaces in the urogenital tract and, as such, is exposed to oxidative stress throughout most of its in vivo life cycle. It encounters oxidative stress from the host immune system and from commensal lactobacilli that colonize the female urogenital tract and generate oxidants. To protect itself against oxidative stress, N. gonorrhoeae has evolved unique defence strategies [42], one of which is elevated levels of glutathione [59]. Glutathione synthesis relies on cysteine as a precursor. Disruption of the cysteine biosynthesis pathway in N. gonorrhoeae could be an effective mechanism for reducing virulence, reducing the incidence of persistent bacteria and increasing susceptibility to antibiotics. Neisseria species appear to only have a single OASS gene, OASS-A (CysK), and not the two isoforms (OASS-A/CysK and OASS-B/CysM) seen in other bacteria. OASS-A from other organisms is inhibited by peptides that mimic the C-terminus of SAT (CysE), which binds to OASS-A, reducing its activity. This is a promising therapeutic strategy for Neisseria species, as in other bacteria, such as S. typhimurium and M. tuberculosis, impaired cysteine biosynthesis has been linked to reduced antibiotic resistance and an increase in susceptibility to a broader range of antibiotics [40, 60, 61]. In addition, SAT (CysE) is essential in N. gonorrhoeae [38] and is a requirement for growth in N. meningitidis [41] and, as such, also represents an attractive target for inhibitor design. Targeting cysteine biosynthesis in the Neisseria species is a promising avenue for the design of antimicrobial drugs against gonococcal and meningococcal disease.

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