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Research Paper

The aldehyde dehydrogenase AldA contributes to the hypochlorite defense and is redox-controlled by protein *S*-bacillithiolation in *Staphylococcus aureus*

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ABSTRACT

Staphylococcus aureus produces bacillithiol (BSH) as major low molecular weight (LMW) thiol which functions in thiol-protection and redox-regulation by protein *S*-bacillithiolation under hypochlorite stress. The aldehyde dehydrogenase AldA was identified as *S*-bacillithiolated at its active site Cys279 under NaOCl stress in *S. aureus*. Here, we have studied the expression, function, redox regulation and structural changes of AldA of *S. aureus*. Transcription of *aldA* was previously shown to be regulated by the alternative sigma factor SigmaB. Northern blot analysis revealed SigmaB-independent induction of *aldA* transcription under formaldehyde, methylglyoxal, diamide and NaOCl stress. Deletion of *aldA* resulted in a NaOCl-sensitive phenotype in survival assays, suggesting an important role of AldA in the NaOCl stress defense. Purified AldA showed broad substrate specificity for oxidation of several aldehydes, including formaldehyde, methylglyoxal, acetaldehyde and glycol aldehyde. Thus, AldA could be involved in detoxification of aldehyde substrates that are elevated under NaOCl stress. Kinetic activity assays revealed that AldA is irreversibly inhibited under H_2O_2 treatment *in vitro* due to over-oxidation of Cys279 in the absence of BSH. Pre-treatment of AldA with BSH prior to H_2O_2 exposure resulted in reversible AldA inactivation due to *S*-bacillithiolation as revealed by activity assays and BSH-specific Western blot analysis. Using molecular docking and molecular dynamic simulation, we further show that BSH occupies two different positions in the AldA active site depending on the AldA activation state. In conclusion, we show here that AldA is an important target for *S*-bacillithiolation in *S. aureus* that is up-regulated under NaOCl stress and functions in protection under hypochlorite stress.

1. Introduction

Staphylococcus aureus is a major human pathogen that causes local wound infections, but also life-threatening systemic and chronic infections, such as septicemia, endocarditis, necrotizing pneumonia and osteomyelitis [1–3]. Moreover, there is an increasing prevalence of hospital- and community-acquired methicillin-resistant *S. aureus* (MRSA) isolates that are often resistant to multiple antibiotics [4]. *S.*

Abbreviations: ADH, aldehyde dehydrogenase; BSH, bacillithiol; BSSB, oxidized bacillithiol disulfide; CFU, colony-forming unit; CD, catalytic domain; Co-BD, coenzyme-binding domain; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; FA, formaldehyde; H_2O_2, hydrogen peroxide; HOCl, hypochloric acid; IPTG, isopropyl-β-D-thiogalactopyranoside; LB, Luria Bertani; LMW thiol, low molecular weight thiol; MD, molecular dynamics; MG, methylglyoxal; MHQ, 2-methylhydroquinone; MPO, myeloperoxidase; MRSA, methicillin-resistant *Staphylococcus aureus*; NADH, nicotinamide adenine dinucleotide; NADPH, nicotinamide adenine dinucleotide phosphate; NaOCl, sodium hypochlorite; NEM, N-ethylmaleimide; OD_{500}, optical density at 500 nm; RCS, reactive chlorine species; RES, reactive electrophilic species; ROS, reactive oxygen species; SCV, small colony variant; SID, subunit interaction domain; X-gal, 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside

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aureus quickly escapes to bactericidal action of new antibiotics and is therefore classified as ESKAPE pathogen by the "European Center of Disease Prevention and Control" [5]. The successful infection of *S. aureus* is mediated by a high diversity of virulence factors, such as toxins, proteases, lipases, superantigens, as well as efficient protection mechanisms against the host immune defense during invasion [6,7]. During infections, *S. aureus* has to cope with the oxidative burst of activated macrophages and neutrophils, including reactive oxygen and chlorine species (ROS, RCS), such as hydrogen peroxide (H_2O_2) and the strong oxidant hypochloric acid (HOCl) [8–11]. HOCl is generated in neutrophils from H_2O_2 and chloride by the myeloperoxidase (MPO) which is the main cause of bacterial killing [12,13].

Apart from ROS and RCS, *S. aureus* is frequently exposed to reactive electrophile species (RES), such as quinones and aldehydes that originate from cellular metabolism, as secondary oxidation products from ROS and RCS as well as from external sources, such as antibiotics and host-defense components [11,14–17]. RES are α,β-unsaturated dicarbonyl compounds that have electron-deficient centers and can react with protein thiols *via* oxidation or thiol-S-alkylation chemistries [16,17]. Methylglyoxal is an example for a highly toxic and reactive aldehyde produced as by-product from triose-phosphate intermediates during glycolysis [14,15]. Methylglyoxal detoxification pathways and their regulatory mechanisms have been widely studied in *E. coli* and *B. subtilis*. *E. coli* utilises a glutathione (GSH)-dependent glyoxalase pathway and a GSH-independent pathway for methylglyoxal detoxification. In the glyoxalase pathway, methylglyoxal reacts spontaneously with GSH to form hemithioacetal which is converted by glyoxalase-I to *S*-lactoylglutathione. *S*-lactoylglutathione is the substrate for glyoxalase-II leading to lactate production [14,18]. The glyoxalase *gloA* and the *nemRA* operon are induced by quinones, aldehydes and HOCl and regulated by the TetR-family NemR repressor in *E. coli* [19–22]. GloA functions as glyoxalase in methylglyoxal detoxification and NemA is an FMN-dependent oxidoreductase involved in detoxification of quinones and aldehydes. Moreover, it was shown that methylglyoxal is produced as consequence of hypochlorite stress and that NemR confers protection to methylglyoxal and HOCl *via* control of the *gloA-nemRA* operon [20].

Gram-positive Firmicutes, such as *Bacillus subtilis* and *S. aureus* produce bacillithiol (BSH) as GSH surrogate which functions as protection mechanism against redox-active compounds and co-factor for thiol-dependent enzymes [23,24]. Methylglyoxal detoxification in *B. subtilis* involves BSH-dependent and BSH-independent pathways [23,25]. In the BSH-dependent glyoxalase pathway, BSH reacts with methylglyoxal to form BS-hemithioacetal which is converted to *S*-lactoyl-BSH by Glx-I and further by Glx-II to lactate [23,25]. In addition, the thiol-dependent formaldehyde dehydrogenase AdhA confers protection under formaldehyde and methylglyoxal stress in *B. subtilis* which is controlled by the MerR/NmlR-like regulator AdhR [35]. However, the enzymatic pathways involved in detoxification of reactive aldehydes are unknown in *S. aureus*.

Recently, we identified the glycolytic glyceraldehyde-3-phosphate dehydrogenase GapDH as major *S*-bacillithiolated protein in *S. aureus* under NaOCl stress [26]. Apart from GapDH, the aldehyde dehydrogenase AldA was *S*-bacillithiolated at its active site Cys279 under NaOCl stress, which could function in detoxification of methylglyoxal or other aldehyde substrates. Here, we have studied the expression and function of AldA of *S. aureus* under formaldehyde, methylglyoxal and NaOCl stress. Transcriptional studies revealed an increased *aldA* transcription under aldehyde, NaOCl and diamide stress in *S. aureus*. In survival phenotype assays, the *aldA* mutant was more sensitive to NaOCl stress. Using biochemical activity assays, we provide evidence that *S*-bacillithiolation functions in redox-regulation of AldA activity. All-atom molecular dynamics (MD) simulations suggest that the location of BSH in the AldA active site depends on the Cys activation state in the apo- and holoenzyme structures. In conclusion, our results indicate that AldA plays an important role in the NaOCl stress defense and is redox-regulated by *S*-bacillithiolation in *S. aureus*.

2. Materials and methods

2.1. Bacterial strains, growth and survival assays

Bacterial strains, plasmids and primers are listed in Tables S1 and S2. For cloning and genetic manipulation, *E. coli* was cultivated in Luria Bertani (LB) medium. *S. aureus* COL was cultivated either in LB or RPMI medium as described previously [26]. For survival phenotype assays, *S. aureus* COL was grown in RPMI medium until an OD_{500} of 0.5, exposed to 2 mM formaldehyde, 4 mM methylglyoxal and 3.5 mM NaOCl stress and 10 µl of serial dilutions were spotted onto LB agar plates for 24 h to observe colonies. All complemented *aldA* deletion mutants with plasmid pRB473 were grown in the presence of 1% xylose and 10 µg/ml chloramphenicol. Sodium hypochlorite, diamide, dithiothreitol (DTT), hydrogen peroxide (H_2O_2, 35% w/v), formaldehyde, methylglyoxal and 2-methylhydroquinone (MHQ) were purchased from Sigma Aldrich.

2.2. RNA isolation and Northern blot analysis

For RNA isolation, *S. aureus* COL was cultivated in RPMI medium and treated with sub-lethal doses of 1 mM NaOCl, 0.75 mM formaldehyde (FA), 0.5 mM methylglyoxal (MG), 10 mM H_2O_2 and 50 µM MHQ for different times as described previously [26]. *S. aureus* COL cells were harvested before and after stress exposure and disrupted in lysis buffer [10 mM Tris-HCl, pH 8.0; 200 mM sodium chloride (NaCl); 3 mM ethylene diamine tetra acetic acid (EDTA)] with a Precellys24 ribolyzer. RNA was isolated using acid phenol extraction as described [26] and RNA quality was assessed using the Nanodrop. Northern blot hybridizations were performed with the digoxigenin-labelled *aldA*-specific antisense RNA probe synthesized *in vitro* using T7 RNA polymerase and the primer pairs aldA-for and aldA-rev (Table S2) as described [26,27].

2.3. Cloning, expression and purification of His-tagged AldA and AldC279S mutant proteins in E. coli

The *aldA* gene was amplified from chromosomal DNA of *S. aureus* COL by PCR using primers aldA-for-*Nhe*I and aldA-rev-*Bam*HI (Table S2), digested with *Nhe*I and *Bam*HI and inserted into plasmid pET11b (Novagen) that was digested using the same enzymes to generate plasmid pET11b-*aldA*. For construction of pET11b expressing AldAC279S mutant protein, Cys279 was replaced by serine using PCR mutagenesis. Two first-round PCR reactions were performed using primer pairs aldA-for-*Nhe*I and aldA-C279S-Rev as well as primer pairs aldA-C279S-for and aldA-rev-*Bam*HI (Table S2). The two first round PCR products were hybridized and subsequently amplified by a second round of PCR using primers aldA-for-*Nhe*I and aldA-rev-*Bam*HI. The second-round PCR products were digested with *Nhe*I and *Bam*HI and inserted into plasmid pET11b digested with the same enzymes to generate plasmid pET11b-*aldAC279S*. The correct *aldA* and *aldAC279S* sequences of the plasmids were confirmed by DNA sequencing. Plasmid pET11b-*aldAC279S* was also used for construction of the *aldAC279S* mutant *in vivo* and subcloned into the *E. coli/S. aureus* shuttle vector pRB473 as described below.

For expression and purification of His-tagged AldA and AldAC279S mutant protein, *E. coli* BL21(DE3) p*lysS* was used expressing plasmids pET11b-*aldA* and pET11b-*aldAC279S*, respectively. Cultivation was performed in 1 l LB medium until the exponential growth phase at OD_{600} of 0.8 followed by addition of 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) for 3.5 h at 37 °C. Recombinant His-AldA and His-AldAC279S mutant proteins were purified after sonication of the *E. coli* cells in binding buffer (20 mM NaH_2PO_4, 500 mM NaCl, 20 mM imidazole, pH 7.4). Lysates were cleared from cell debris by repeated centrifugation and purification of the His-AldA and His-AldAC279S