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Methionine aminopeptidase as a novel target for antibiotic therapy against *Staphylococcus aureus:* a proteomic approach

Key Messages

1. Methionine aminopeptidase (MetAP) is an essential enzyme in *Staphylococcus aureus* and a potential target for novel antibiotics.
2. Two-dimensional electrophoresis gel identified more than 100 differences in protein expression between wild type and MetAP-deficient strains of *S aureus*.
3. Using mass spectroscopic techniques, 63 differentially expressed proteins were identified, of which some were related to purine biosynthesis and methionine metabolism.

Introduction

Staphylococcus aureus is a common aetiologic agent of pneumonia in the hospital setting, often as a consequence of influenza, with a mortality of 15 to 20%.[1] Surgical site infections constitute approximately 15% of the infections reported to the National Nosocomial Infections Surveillance System.[2] These infections increase the economic burden of the hospital system, partly owing to the prolonged length of hospital stay. Thus, research in the pathogenesis of *S aureus* and identification of novel targets for antibacterial therapy against this pathogen are necessary.

Co- and post-translational modifications are essential steps for the maturation of proteins for proper folding, regulation, function, targeting and eventually degradation.[2] Translation of proteins is initiated with methionine in eukaryotes and N-formyl methionine in prokaryotes, mitochondria, and chloroplasts. The N-formyl group is removed from proteins in prokaryotes and eukaryotic organelles by a deformylase, leaving a methionine residue at the amino terminus. However, most mature proteins do not retain the initiator residue. Methionine aminopeptidase (MetAP) is the enzyme responsible for the catalytic cleavage of the N-terminal methionine from proteins, when the penultimate residue has a small and uncharged side-chain. MetAPs is essential in Gram-negative bacteria (such as *Escherichia coli* and *Salmonella typhimurium*) and in *Saccharomyces cerevisiae*. Its physiological importance guided researchers to design antimicrobial inhibitors that specifically target this enzyme. However, the precise roles of MetAP in cellular functions are still elusive. By applying proteomic techniques, the wild type and MetAP-deficient strains of *S aureus* showed similar protein profiles. Of more than 600 proteins detected on the two-dimensional electrophoresis (2-DE) gel, more than 100 showed differences in the expression level. We quantified and compared 63 differentially expressed proteins. Using mass spectrometric techniques, the identities of these selected proteins were confirmed and some were identified to be those related to purine biosynthesis and methionine metabolism.

Methods

This study was conducted from February 2006 to January 2008. Whole cell lysates of *S aureus* were prepared using a modified protocol.[3] Protein (600 μg) was applied onto an IPG Strip (18 cm, pH 4-7) by overnight passive rehydration. Isoelectric focusing was performed using Ettan IPGphor II (GE Healthcare, USA). The strip was then equilibrated for 15 minutes in 10 mL SDS equilibration buffer with 100 mg dithiothreitol (DTT), followed by an equilibration step for 15 minutes in 10 mL SDS equilibration buffer with 250 mg iodoacetamide. The second dimension electrophoresis was carried out on 1.5 mm thick 12.5% self cast polyacrylamide gel in a Ettan DALT*six* electrophoresis apparatus (GE Healthcare) at 17 W/gel with a maximum voltage of 300 V at 10ºC until the dye front reached the bottom of the gel. Three separated batches of cell lysates were analysed by three independent 2-DE experiments. Gels were stained using Coomassie brilliant blue G250, as described previously.[4]

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Stained gels were digitalised using an ImageScanner (GE Healthcare). Intensity calibration was carried out by using the Kodak Step Tablet number 2 (Kodak, USA) before image acquisition. Comparative analysis of the gel images was carried out using the ImageMaster 2D Platinum version 5.0 software (GE Healthcare) according to the manufacturer's instructions.

Protein spots were picked manually and de-stained in 1:1 solution of 100 mM ammonium bicarbonate and acetonitile. After de-staining, enzymatic digestions were carried out as previously described.[5]

Results

Using 2-DE, we were able to compare the proteomes of RKC261 (MetAP-sufficient *S arueus*) and RKC264 (MetAP-deficient *S aureus*). As the MetAP-deficient *S aureus* was barely growing in extreme pH conditions and elevated temperatures, proteomes were compared using brain-heart infusion broths. Among more than 600 protein spots detectable in the 2-DE, we were able to quantify and compare the expression levels of differentially expressed proteins between RKC261 and RKC264 (data not shown). We further classified the identified proteins into related biological processes (data not shown). In the MetAP-deficient state, 80% of the differentially expressed proteins detected by 2-DE were down-regulated. Apart from recognising more general biological processes such as protein synthesis, energy generation, etc, proteins involved in methionine biosynthesis/metabolism were identified. In addition, a number of proteins involved in purine biosynthesis/metabolism were identified.

Discussion

In this study, the 2-DE gel results indicated a down-regulation of particular proteins, which are required and implicated in the *de novo* synthesis of inositol-monophosphate IMP,[6] the precursor of ATP and GTP, for example, phosphoribosylaminoimidazole-succinocarboxamide synthase (purC) and phosphoribosylamine-glycine ligase (purD). The enzymes involved in the salvage and interconversion of purine nucleosides and nucleotides, for example, adenylosuccinate synthase (purA), and purine nucleoside phosphorylase (deoD) were also noted to be down-regulated. These purC and purD genes belong to the putative purine biosynthesis operon purEKCSQLFMNHD in *S aureus*, but purA and deoD also have their independent promoters. Yet they are all involved in the biosynthesis of IMP and are linked in this manner. At the same time the gene for inositol-monophosphate dehydrogenase (guaB) was found to be up-regulated, implying that the cell uses more IMP in order to synthesise GMP.

In addition, MetAP deficiency in *S aureus* may lead to impaired methionine recycling and consequently down-regulation in the production of S-adenosyl-L-methionine. Deficiency in S-adenosyl-L-methionine will lead to a decrease in the production of polyamines. This may lead to a slower production of methylthioadenosine (MTA), which is believed to lead to the formation of the purine adenine. Methionine has been involved in purine salvage and purine recycling pathways and therefore a sufficient decrease in the production of this amino acid may lead to a decrease in the formation of purines downstream.[7]

Another very important protein that has been detected by the 2-DE gel analysis was the branched-chain amino acid aminotransferase. This enzyme is important in the conversion of ketomethiobutyrate (KMTB) to methionine.[8] According to the 2-DE gel results, its production is severely down-regulated in the MetAP-deficient state. This enzyme is also found to be linked with MTA recycling to methionine. As methionine production is costly in terms of energy requirements, the existence of this unique pathway, which recycles methionine from MTA, serves to conserve energy. The final step in methionine regeneration is the transamination of KMTB by the branched-chain amino acid aminotransferase. Our study suggests that the impact of MetAP deficiency in *S aureus* (and possibly in other organisms too), is profound and multidimensional. The expression profile of a wide range of proteins, spanning nucleotide biosynthesis, energy production, protein synthesis, glycolysis, electron transport, vitamins biosynthesis and metabolism, transcription regulation, amino acid production, and fatty acid metabolism, is affected by the deficiency of MetAP. Our study has provided evidence that MetAP may be linked to the essential methionine recycling and purine biosynthesis pathways, suggesting that it could be a useful target for antibiotic development.

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