Structural studies of *Staphylococcus aureus* hibernation promoting factor homolog SaHPF by high-resolution NMR spectroscopy

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is any strain of *Staphylococcus aureus* that has developed resistance to beta-lactam antibiotics. One of the main target of antibiotic in bacteria is the ribosome. Recent development of cryo-EM allow to determine structure of the ribosome and its complexes with protein factors at high resolution and interpret mechanism of interaction and structure rearrangement at molecular level. Bacteria slow down protein synthesis during stress conditions by converting ribosomes into translationally inactive 100S dimers, enter a stationary phase. In this phase, bacterial cells are resistant to external stresses, which allows them to resist antimicrobial agents. Expression of stationary-phase proteins, such as ribosome hibernation promoting factor (SaHPF), results in formation of 100S dimer, which leads to "ribosome hibernation" that aids cell survival. Structure of SaHPF protein with molecular weight 22 000 Da is unknown. Sequence analysis has shown that this protein is combination of two homolog proteins obtained in E.coli - ribosome hibernation promoting factor HPF (11 000 Da) and ribosome modulation factor RMF (6,500 Da). Recently, crystal structures of E. coli 70S-EcHPF complex [1] and Thermus thermophilus 70S-EcHPF or 70S-EcRMF hybrid complexes [2] revealed that the binding of these two proteins to the intersubunit cavity overlaps with the tRNA binding sites or mRNA exit tunnel respectively. Cryo-electron microscopy studies have also demonstrated that neither EcHPF nor EcRMF are involved in the contacts between two ribosomes in the dimer. However, S. aureus specific SaHPF is almost twice bigger than its *E. coli* counterpart. It shares similarity with EcHPF only on its N-terminal region, while C-terminal domain suggested to be homologues to EcRMF protein, although there is no direct evidence for this. We produced a recombinant expression, synthesis and purification protocols of ^{13}C, ^{15}N-labeled SaHPF protein for structural studies by high-resolution NMR spectroscopy and made screening for appropriate buffer conditions for 3D NMR experiments of SaHPF protein. We believe that the structure of SaHPF will provide us new insights about the formation of ribosome dimers in staphylococci, which may bring new horizons in treatment diseases of this severe pathogen.

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