NMR analysis of Staphylococcus aureus Ribosome Binding Factor A (RbfA)

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Staphylococcus aureus ribosomal binding factor (RbfA) is a cold-shock adaptation protein with molecular weight about 14,9 kDA. The RbfA gene is required for normal cell growth at lower temperatures (10-20°C) and its deletion results in slower bacterial growth. At 37°C, RbfA depleted cells show accumulation of ribosomal subunits and 16S rRNA precursor with a significantly reduced polysome profile in comparison with wild-type cells. RbfA has affinity for free 30S ribosomal subunits (but not for 70S ribosomes or polysomes) and required for its maturation. RbfA is essential for efficient processing of 16S rRNA and is suspected to interact with the 5'-terminal helix (helix I) of 16S rRNA. Staphylococcus aureus is a pathogenic microorganism that causes a variety of nosocomial and common infections. A detailed studies of RbfA structure and its role in small ribosomal subunit maturation may be further used as the target for development of compounds to reduce S.aureus viability. We expressed uniformly labeled ¹⁵N, ¹³C RbfA protein in E.coli and acquired 3D NMR spectra at 35°C on a Bruker Avance 700 NMR spectrometer equipped with a cryoprobe. Structural analysis of the RbfA by NMR spectroscopy showed that its structure in solution contains three α -helices and three β -strands ($\alpha 1$ - $\beta 1$ - $\beta 2$ - $\alpha 3$ - $\alpha 4$ - $\beta 3$ topology),), so that the $\alpha 1$ -helix comprises residues 4–25, the α 2-helix comprises residues 57–69, the α 3-helix comprises residues 71–81 and the β -sheet is composed of β 1 (residues 34–40), β 2 (46–52) and $\beta 3$ (89–92). Obtained information we plan to use for further solution structure determination by NMR and by cryo-EM study of RbfA-30S Staphylococcus aureus ribosome.

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