




Backbone and side chain NMR assignments for the ribosome binding factor A (RbfA) from *Staphylococcus aureus*

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Abstract

Ribosome binding factor A (RbfA) is a 14.9 kDa adaptive protein of cold shock, which is important for bacterial growth at low temperatures. RbfA can bind to the free 30S ribosomal subunit and interacts with the 5'-terminal helix (helix I) of 16S rRNA. RbfA is important for the efficient processing of 16S rRNA and for the maturation (assembly) of 30S ribosomal subunits. Here we report backbone and side chains ¹H, ¹³C and ¹⁵N chemical shift assignments of RbfA from *Staphylococcus aureus*. Analysis of the backbone chemical shifts by TALOS+ suggests that RbfA contains four α -helices and three β -strands with $\alpha 1$ - $\beta 1$ - $\beta 2$ - $\alpha 2$ - $\alpha 3$ - $\beta 3$ - $\alpha 4$ topology. Secondary structure of RbfA have KH-domain fold topology with $\beta\alpha\alpha\beta$ subunit which is characterized by a helix-kink-helix motif in which the GxxG sequence is replaced by a conserved AxG sequence, where an Ala residue at position 70 forming an interhelical kink. The solution of the structure of this protein factor and its complex with the ribosome by NMR spectroscopy, X-ray diffraction analysis and cryo-electron microscopy will allow further development of highly selective substances for slowing or completely stopping the translation of the pathogenic bacterium *S. aureus*, which will interfere with the synthesis and isolation of its pathogenicity factors.

Keywords RbfA · Cold shock · *Staphylococcus aureus* · Ribosome · Protein NMR · Resonance assignment

Abbreviations

IPTG	Isopropyl-thio- β -D-galactoside
<i>S. aureus</i>	<i>Staphylococcus aureus</i>
RbfA	Ribosome binding factor A
Cryo-EM	Cryo-electron microscopy
PIC	Protease inhibitor cocktail
PMSF	Phenylmethane sulfonyl fluoride
SEC	Size-exclusion chromatography
IMAC	Immobilized metal affinity chromatography

Biological context

Staphylococcus aureus (*S. aureus*) ribosomal binding factor (RbfA) is a cold-shock adaptation protein. It is a member of a large protein family, designated the RBFA family, that includes proteins with single-polypeptide-chain molecular masses ranging from 13 to 15 kDa. Molecular weight of *S. aureus* RbfA is about 14.9 kDa. RBFA family members are found in the proteomes of most archaeobacteria and eubacteria organisms (Huang et al. 2003; Rubin et al. 2003). The *S. aureus* RbfA sequence has 41% sequence identity (71% sequence similarity) with the *H. influenzae* RbfA and 43% (67%) with the *E. coli* RbfA.

The RbfA gene is required for normal cell growth at lower temperatures (10–20 °C) and its deletion results in slower bacterial growth. RbfA is expressed constitutively under normal growth conditions; however, the expression level rapidly increases upon cold shock, due to an up-regulation of the transcription of the *rbfA* mRNA, resulting in a several-fold increase in the amount of 30S-bound RbfA. The elevated levels of RbfA under cold-shock conditions are necessary to overcome the translational block at the

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