Identification of a [3Fe-4S]\(^{1+}\) cofactor and functional analysis of the iron responsive regulator RirA from Dinoroseobacter shibae

Maren Behringer, Elisabeth Härtig, Dieter Jahn
Technische Universität Braunschweig | Institut für Mikrobiologie,
m.behringer@tu-bs.de | Telefon +49 (0) 531 391-5857

Introduction

The Roseobacter group of marine bacteria represents one of the major lineages in near surface waters in the global oceans. In many bacteria iron dependent regulation is mediated by the general ferric-uptake regulator Fur, which acts as a repressor under high iron concentrations. D. shibae exhibits not only a gene encoding the Fur regulator, but also possesses genes encoding proteins homolog to the iron responsive regulator Irr, the rhizobial iron regulator RirA and the iron sulfur cluster regulator IscR. These regulators differ in their way to sense iron and thereby enable a fine tuned adaptation to iron availability. For RirA binding of a Fe-S cluster was postulated. In the presence of iron RirA is acting as a repressor of genes involved in iron acquisition.

D. shibae RirA coordinates a [3Fe-4S]\(^{1+}\) cluster

EPR spectroscopy measurement of purified RirA protein identified a [3Fe-4S]\(^{1+}\). Mössbauer whole cell analysis of RirA producing E. coli cells at 20 K, supported the results of the EPR spectroscopy.

The [3Fe-4S]\(^{1+}\) cluster of RirA has no redox function

Cyclic Voltammetry measurements of RirA revealed a stable [3Fe-4S]\(^{1+}\) cluster. No redox changes were detected. Thus, the cluster is not involved in electron transport processes.

The role of RirA for adaptation to iron limitation

The growth rates of wildtype D. shibae DFL12(black) and the ΔrirA mutant strain (red) were compared in salt water medium in the presence of 15 µM Fe(II)SO\(_4\) (solid line) or absence of iron (dashed line). The ΔrirA mutant strain (red) showed a reduced growth compared to the wildtype strain (black).

To prove whether this growth delay is dependent on RirA, the ΔrirA mutant was complemented with a plasmid encoded rirA gene under the control of an constitutive promoter and a N-terminal StreptI tag. The complemented strain (yellow lines) showed almost the same growth behaviour compared to the wildtype strain (black lines). Moreover, the fused StreptI-tag seemed without influence for the function of the RirA regulator.

UV/Vis spectra of RirA protein solutions were recorded under anaerobic conditions. An absorption maximum at 420 nm, typical for Fe-S cluster containing proteins, was observed for recombinantly produced D. shibae RirA (solid black line). The Fe-S cluster appeared to be oxygen sensitive, since exposure to air reduced the absorption drastically (red line).

RirA coordinates an oxygen sensitive Fe-S cluster using three conserved cysteine residues as ligands

UV/Vis spectra of RirA protein solutions were recorded under anaerobic conditions. An absorption maximum at 420 nm, typical for Fe-S cluster containing proteins, was observed for recombinantly produced D. shibae RirA (solid black line). The Fe-S cluster appeared to be oxygen sensitive, since exposure to air reduced the absorption drastically (red line).

The growth rates of wildtype D. shibae DFL12(black) and the ΔrirA mutant strain (red) were compared in salt water medium in the presence of 15 µM Fe(II)SO\(_4\) (solid line) or absence of iron (dashed line). The ΔrirA mutant strain (red) showed a reduced growth compared to the wildtype strain (black).

To prove whether this growth delay is dependent on RirA, the ΔrirA mutant was complemented with a plasmid encoded rirA gene under the control of an constitutive promoter and a N-terminal StreptI tag. The complemented strain (yellow lines) showed almost the same growth behaviour compared to the wildtype strain (black lines). Moreover, the fused StreptI-tag seemed without influence for the function of the RirA regulator.

The growth rates of wildtype D. shibae DFL12(black) and the ΔrirA mutant strain (red) were compared in salt water medium in the presence of 15 µM Fe(II)SO\(_4\) (solid line) or absence of iron (dashed line). The ΔrirA mutant strain (red) showed a reduced growth compared to the wildtype strain (black).

To prove whether this growth delay is dependent on RirA, the ΔrirA mutant was complemented with a plasmid encoded rirA gene under the control of an constitutive promoter and a N-terminal StreptI tag. The complemented strain (yellow lines) showed almost the same growth behaviour compared to the wildtype strain (black lines). Moreover, the fused StreptI-tag seemed without influence for the function of the RirA regulator.

The growth rates of wildtype D. shibae DFL12(black) and the ΔrirA mutant strain (red) were compared in salt water medium in the presence of 15 µM Fe(II)SO\(_4\) (solid line) or absence of iron (dashed line). The ΔrirA mutant strain (red) showed a reduced growth compared to the wildtype strain (black).

To prove whether this growth delay is dependent on RirA, the ΔrirA mutant was complemented with a plasmid encoded rirA gene under the control of an constitutive promoter and a N-terminal StreptI tag. The complemented strain (yellow lines) showed almost the same growth behaviour compared to the wildtype strain (black lines). Moreover, the fused StreptI-tag seemed without influence for the function of the RirA regulator.