The role of the light-oxygen-voltage (LOV)-histidine kinase Dshi_1135 for regulation of the bacteriochlorophyll a biosynthesis in Dinoroseobacter shibae

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Introduction

The bacterium Dinoroseobacter shibae is a representative of the important marine Roseobacter group, which is one of the major lineages in the global oceans. Besides aerobic and anaerobic respiration, D. shibae is capable to perform aerobic anoxygenic photosynthesis for the generation of organic matter, without producing oxygen and belongs to the aerobic anoxygenic phototrophic bacteria (AAnP). D. shibae possesses a gene cluster encoding the structural components of the photosystem and the light harvesting pigments bacteriochlorophyll a (Bchl a) and spheroidenone. Transcriptome analyses revealed a light-dependent expression of the photosynthetic gene cluster [1]. A D. shibae transposon library was screened with mutants for a defect in Bchl a biosynthesis [2]. Here, the gene locus Dshi_1135, encoding a potential blue-light sensing LOV histidine kinase, was found.

Inactivation of Dshi_1135 results in a complete loss of Bchl a

After identification of Dshi_1135 as potential regulator of Bchl a biosynthesis, initial cultivation experiments and Bchl a extractions followed by UV/Vis measurement were performed.

a) After cultivation of ΔDshi_1135 in liquid culture in the dark, no typical pink pigmentation as in the wild type was observed, indicating missing spheroidenone synthesis.

b) The wild type strain shows typical absorption peaks at 360 nm and 780 nm for Bchl a. Carotenoids are displayed at 580 nm. In ΔDshi_1135 a complete loss of pigments was detected.

Light-dependent expression of bchF-lacZ is regulated by Dshi_1135

bchF encodes an enzyme, which is involved in late Bchl a biosynthesis. To determine the regulatory role of Dshi_1135 and the influence of light on this pathway, a bchF-lacZ system was generated. bchF-lacZ expression in the wild type was only induced in the dark. In contrast, expression was completely abolished in ΔDshi_1135 regardless of the light condition.

Transcriptomic analyses revealed missing gene expression of the photosynthetic gene cluster in ΔDshi_1135

To determine the influence of blue light on gene expression of the photosynthetic gene cluster in the wild type and the ΔDshi_1135 mutant strain, transcriptome analyses were performed. Therefore RNA samples were prepared from the mid log growth phase. In the wild type strain the majority of genes was upregulated, whereas genes were downregulated in the mutant strain. These results are in good agreement with the assumed regulatory role of Dshi_1135 and the influence of blue light during Bchl a biosynthesis.

The potential blue-light sensing LOV histidine kinase
Dshi_1135

Dshi_1135 encodes for a potential blue light sensing LOV (light oxygen or voltage) domain, which is fused N-terminally to a histidine kinase (HK) domain. For signal detection FMN is used as cofactor. When illuminated with blue light, the protein is moved into a signaling state via covalent linkage between the sulfur of a highly conserved cystein residue (red) and the C4 of the isoalloxazine ring of FMN. Upon activation the HK gets autophosphorylated at a highly conserved histidine (yellow) and transfers the phosphate to a so far unknown response regulator. Furthermore, Dshi_1135 shows 42 % identity to the LOV-HK EL346 from Ernythrobacter litoralis [3] and with available structural data we were able to model a protein structure.

Heterologous production of Dshi_1135

Dshi_1135 was produced as a Strepti-tag fusion protein with a relative molecular mass of 52 kDa and purified to apparent homogeneity. All steps were performed under red dim light.

UV/Vis spectra of the protein were recorded. Typical absorption maxima for FMN at 380 nm and 450 nm were observed.

Outlook

- Autophosphorylation assays with [γ-32P] ATP to proof kinase activity of Dshi_1135
- Identification of the cognate RR via phosphotransfer experiments and genome screening of standalone RR
- DNA-binding studies with potential target promoters
- Generation of additional promoter-lacZ systems

References