Biosynthesis of the redox coenzyme F420 in *Thermomicrobia* involves reduction by standalone nitroreductase superfamily enzymes.

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Abstract

Coenzyme F_{420} is a redox cofactor involved in hydride transfer reactions in archaea and bacteria. Since F_{420}-dependent enzymes are attracting increasing interest as tools in biocatalysis, F_{420} biosynthesis is being revisited. While it was commonly accepted for long that the 2-phospho-l-lactate (2-PL) moiety of F_{420} is formed from free 2-PL, it was recently shown that PEP is incorporated in Actinobacteria and that the C-terminal domain of the FbiB protein, a member of the nitroreductase superfamily (NTR), converts dehydro-F_{420} into saturated F_{420}. Outside the Actinobacteria, however, the situation is still unclear because FbiB is missing in these organisms and enzymes of the NTR family are highly diversified. Here, we show by heterologous expression and *in-vitro* assays that standalone NTR enzymes from Thermomicrobia exhibit dehydro-F_{420} reductase activity. Metabolome analysis and proteomics studies confirmed the proposed

biosynthetic pathway in *Thermomicrobium roseum*. These results clarify the biosynthetic route of coenzyme F_{420} in a class of Gram-negative bacteria, redefine functional subgroups of the NTR superfamily, and offer an alternative for large-scale production of F_{420} in *E. coli* in the future.

Beteiligte Forschungseinheiten

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Awards

Braga D and Hasan M contributed equally to this work.

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