Redox Coenzyme F 420 Biosynthesis in Thermomicrobia Involves Reduction by Stand-Alone Nitroreductase Superfamily Enzymes

Braga D, Hasan H, Kröber T, Last D, Lackner G (2020) Redox Coenzyme F 420 Biosynthesis in Thermomicrobia Involves Reduction by Stand-Alone Nitroreductase Superfamily Enzymes *Appl Environ Microbiol* 86(12), e00457-20. PubMed

ILRS Authors

Mahmudul Hasan

Projects

The biosynthesis, biochemistry and physiology of coenzyme 3PG-F420
Details

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 a long time that the 2-phospho-l-lactate (2-PL) moiety of F_{420} is formed from free 2-PL, it was recently shown that phosphoenolpyruvate is incorporated in *Actinobacteria* and that the C-terminal domain of the FbiB protein, a member of the nitroreductase (NTR) superfamily, 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 stand-alone 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 *Escherichia coli* in the future.

IMPORTANCE Coenzyme F_{420} is a redox cofactor of *Archaea* and *Actinobacteria*, as well as some Gram-negative bacteria. Its involvement in processes such as the biosynthesis of antibiotics, the degradation of xenobiotics, and asymmetric enzymatic reductions renders F_{420} of great relevance for biotechnology. Recently, a new biosynthetic step during the formation of F_{420} in *Actinobacteria* was discovered, involving an enzyme domain belonging to the versatile nitroreductase (NTR) superfamily, while this process remained blurred in Gram-negative bacteria. Here, we show that a similar biosynthetic route exists in *Thermomicrobia*, although key biosynthetic enzymes show different domain architectures and are only distantly related. Our results shed light on the biosynthesis of F_{420} in Gram-negative bacteria and refine the knowledge about sequence-function relationships within the NTR superfamily of enzymes. Appreciably, these results offer an alternative route to produce F_{420} in Gram-negative model organisms and unveil yet another biochemical facet of this pathway to be explored by synthetic microbiologists.

Identifier

doi: 10.1128/AEM.00457-20 PMID: 32276981