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TOPIC(s) : Enzyme discovery and engineering

Identification and characterization of archaeal and bacterial F420-dependent thioredoxin reductases

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PURPOSE OF THE ABSTRACT
The thioredoxin pathway is an antioxidant system present in most organisms, in which thioredoxin reductase plays a key role. Recently, it was shown that it can also be used in biocatalysis for cofactor regeneration [1]. Most known thioredoxin reductases are NADP(H)-dependent, enabling regeneration of NADPH. Yet, in 2016, a new type of thioredoxin reductase was discovered in archaea which utilizes instead a deazaflavin cofactor, F420. For this reason, the respective enzyme was named deazaflavin-dependent flavin-containing thioredoxin reductase (DFTR). To explore the potential of DFTRs, we set out to express and study microbial DFTRs. First, we identified and characterized two new archaeal representatives. A detailed kinetic study, which included pre-steady state kinetic analyses, revealed these two DFTRs are highly specific for F420H2 while displaying marginal activity with NADPH. A detailed structural analysis led the identification of two key residues that tune cofactor specificity of DFTRs. This allowed us to define a DFTR-specific sequence motif that enabled for the first time the identification and experimental characterization of a bacterial DFTR. All discovered DFTRs are highly thermostable and efficient in utilizing F420 as cofactor. Thus, they provide an additional tool for the regeneration of this deazaflavin cofactor.

FIGURES

FIGURE 1

FIGURE 2

KEYWORDS

Deazaflavin | F420 | Thioredoxin reductase | Cofactor specificity

BIBLIOGRAPHY

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