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Characterization of PNDOR3 homologs as sulfur reductases and identification of genes related to the sulfur-dependent growth in the hyperthermophilic archaeon

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Introduction

Hyperthermophilic archaeon *Thermococcus kodakarensis* and the closely related *Pyrococcus furiosus* prefer amino acids/peptides as energy sources and elemental sulfur (S^0) or polysulfide as a terminal electron acceptor. It has been reported that a homolog of group 3 pyridine nucleotide disulfide oxidoreductase (PNDOR3) in *P. furiosus* (PF1186) had the role in energetic sulfur reduction as NADPH:S^0 oxidoreductase (NSR_{Pf}) with a CoA-dependent manner. Our laboratory had previously characterized four PNDOR3 homologs in *T. kodakarensis* including the counterpart of NSR_{Pf}; TK1299, and detected NSR activity and NAD(P)H oxidase (NOX) activity. However, the specific activity and CoA-dependency of TK1299 were inconsistent with those observed for NSR_{Pf}. While, a counterpart of NSR_{Pf} in *P. horikoshii* (PH0572) was characterized as CoA disulfide reductase participated in maintenance of cellular redox balance. To solve these discrepancies for the PNDOR3 homologs in Thermococcales, this study aimed to re-examine the four PNDOR3 homologs in *T. kodakarensis* along with NSR_{Pf} focusing on NSR and NOX activities in order to get new insights into enzymatic sulfur reduction by PNDOR3 enzymes.

In addition, our laboratory recently established the method for random mutagenesis of *T. kodakarensis.* This technique was applied to screen genes relevant to the sulfur-dependent growth of *T. kodakarensis* by isolation and analyses of mutants showing no or impaired growth on sulfur.

Results and Discussion

i) Enzymatic characterization of PNDOR3 homologs

NSR and NOX activities of four PNDOR3 homologs; TK1299, TK1481, TK0304, and TK0828 in *T. kodakarensis* were re-examined along with PF1186 in *P. furiosus* (NSR_{Pf}). The results are summarized in Table 1. TK1299 as well as PF1186 showed comparable NSR activity with preference to NADPH and strict CoA-dependency. TK1299 was proposed as NADPH: CoA persulfide/disulfide oxidoreductase, and CoA−SH acted as a solubilizer of S^0 forming CoA-persulfide/ disulfide non-enzymatically, as shown in Figure 1. TK1481 exhibited NSR activity with preference to NADPH, but the activity was independent from the addition of CoA, implying that the enzyme directly reacted with S^0. The preference to electron donor was shift to NADH when TK1299 and TK1481 catalyzed oxygen reduction (NOX

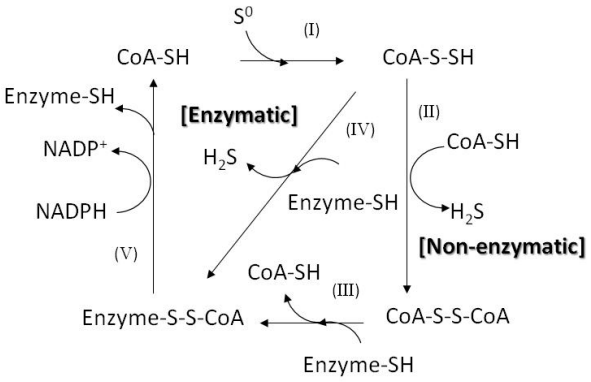

Figure 1. Proposed catalytic cycle of TK1299 relevant to production of H_2S from elemental sulfur

activity), while significant inhibition by CoA-SH was observed for the NADH oxidase activities. TK0304 showed high NOX activity and accepted both NADH and NADPH.

Table 1. NSR and NOX activity determined in NOX homologs

		TK1299		PF1186		TK1481		TK0304		TK0828	
		+CoA	−CoA	+CoA	−CoA	+CoA	−CoA	+CoA	−CoA	+CoA	−CoA
NSR	NADPH	14.2±2.7	NA	4.8±0.9	NA	4.4±0.1	4.2±0.8	0.1±0.05	NA	NA	NA
	NADH	2.5±0.3	NA	0.8±0.2	NA	1.0±0.2	1.4±0.2	NA	NA	NA	NA
NOX	NADPH	0.5±0.05	0.8±0.01	0.6±0.03	0.4±0.04	1.1±0.26	0.96±0.32	3.4±0.2	4.7±0.2	0.6±0.05	0.6±.05
	NADH	0.2±0.03	5.1±0.4	0.3±0.2	6.3±0.4	0.5±0.17	4.18±0.69	1.3±0.1	4.3±0.2	0.3±0.02	0.5±0.05

Enzyme assayed was carried out in 50 mM Tris-HCl pH 8.0 with 10 mM NADPH for NSR or 0.3 mM NADH for NOX, 0.1 mM FAD and 0.2 mM CoA-SH were added when required. NA: no activity.

ii) Screening of mutants showing growth deficiency in sulfur-dependent conditions.

T. kodakarensis was randomly mutagenized by using the transposon-inserted genomic DNA library according to the procedure established previously. At the first trial, opaque plate media containing polysulfide (polyS) were used for the screening. Although many primary candidates forming smaller or no clear zone, attributed to the decrease in consumption of polyS, were obtained from the random mutation library, none of the candidates showed growth impairment in a liquid medium supplemented with polyS. Next, the screening was carried out in a polyS-supplemented liquid medium using a 96-well microtiter plate, and several clones exhibiting growth impairment could be isolated from the random mutagenesis library. The transposon insertion sites in the mutants were mapped on different genes encoding both cytosolic and membrane proteins, and the function of each the identified protein in the observed phenotype was estimated.

In addition to polysulfide, solid S^0 was also examined as a sulfur donor in the cultivation and screening. The author developed a reliable procedure for determining the cellular protein content in the S^0-supplemented medium within a 96-well plate. Several mutants showing actual growth defect on S^0 could be isolated. The transposon-insertion sites were identified, and complementation analysis was performed for a few mutants. The effects of the identified mutation on the S^0-depnedent growth of *T. kodakarensis* were discussed.

Publication

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