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# 論文 / 著書情報
# Article / Book Information

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| 題目(和文) | |
| Title(English) | 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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| 出典(和文) | 学位:博士（工学）,<br>学位授与機関:東京工業大学,<br>報告番号:甲第9989号,<br>授与年月日:2015年9月25日,<br>学位の種別:課程博士,<br>審査員:福居　俊昭,丹治　保典,和地　正明,松田　知子,平沢　敬 |
| Citation(English) | Degree:Doctor (Engineering),<br>Conferring organization: Tokyo Institute of Technology,<br>Report number:甲第9989号,<br>Conferred date:2015/9/25,<br>Degree Type:Course doctor,<br>Examiner:,,,, |
| 学位種別(和文) | 博士論文 |
| Category(English) | Doctoral Thesis |
| 種別(和文) | 論文要旨 |
| Type(English) | Summary |

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（博士課程）
Doctoral Program

# 論 文 要 旨

THESIS SUMMARY

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| 専攻：<br>Department of | Bioengineering 専攻 | 申請学位（専攻分野）：<br>Academic Degree Requested | 博士<br>Doctor of （ 工学 ） |
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要旨（英文 800 語程度）
Thesis Summary (approx.800 English Words )


Hyperthermophilic archaeon *Thermococcus kodakarensis* prefers proteinaceous substrates as energy sources and elemental sulfur ($S^0$) or polysulfide as a terminal electron acceptor. Despite many previous studies on various aspects, the sulfur reduction system in the archaeal order of Thermococcales including *T. kodakarensis* has been enigma. A previous study proposed that a homolog of group 3 pyridine nucleotide disulfide oxidoreductase (PNDOR3) in a closely related *Pyroccocus furiosus* (PF1186) possibly played a role in energetic sulfur reduction as a CoA-dependent NADPH: $S^0$ oxidoreductase ($NSR_{Pf}$). Our lab also had characterized four PNDOR3 homologs in *T. kodakarensis*, and detected NSR activity as well as NAD(P)H oxidase (NOX) activity. However, some catalytic properties of the PNDOR3 homologs in *T. kodakarensis* were inconsistent with those of the counterparts in *Pyrococcus* spp. 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 insight into enzymatic sulfur reduction by PNDOR3 enzymes. Additionally, the authors tried to isolate and identify genes responsible for the sulfur-dependent growth of *T. kodakarensis* through random mutation technique recently established by our lab.

Enzymatic activities of the four homologs; TK1299, TK1481, TK0304 and TK0828 in *T. kodakarensis* were re-examined together with PF1186 in *P. furiosus* ($NSR_{Pf}$). TK1299 as well as PF1186 showed comparable NSR activity, which was significantly raised when NADH was replaced with NADPH, in a strict CoA-dependent manner. Based on further detailed analyses, TK1299 was proposed as NADPH: CoA persulfide/disulfide oxidoreductase to reduce CoA-persulfide/disulfide that were non-enzymatically formed between CoA−SH and $S^0$. NADPH-dependent NSR activity was also detected for TK1481 but the activity was insignificant toward CoA−SH, indicating the direct interaction between $S^0$ and the enzyme. While, almost no NSR activity was detected for TK0304. Aside from NSR activity, TK1299, TK1481 and TK0304 catalyzed oxygen reduction (NOX activity). The preference of electron donor was shift to NADH for TK1299 and TK1481, whereas TK0304 was likely to accept both NADH and NADPH. Significant inhibition by CoA−SH was observed for the NADH oxidase activities. Neither NSR nor NOX activity was observed for TK0828.

Previously constructed transposon-inserted genomic DNA library was used to randomly mutagenize *T. kodakarensis* with an attempt to isolate and identify mutants having sulfur-dependent growth deficiency. At the first trial of screening, opaque plate media containing polysulfide was applied and many primary candidates forming smaller or

no clear zone, attributed to the decrease in a consumption of polysulfide, were isolated among 288 library mutants. However, none of the candidates showed growth impairment in a liquid medium supplemented with polysulfide, suggesting that size of produced clear zone was not directly relevant to the $S^0$/polysulfide dependent growth. The next screening was carried out in polysulfide-supplemented liquid medium using 96-well microtiter plate, and 8 clones could be isolated out of a total 1,632 mutants. However, further investigation suggested that most of the mutants were sensitive to alkaline condition resulted from the addition of highly alkaline polysulfide solution. Only 1 out of the 8 mutants was the actual sulfur-dependent growth impaired mutant. The mutation site was identified in a gene encoding subunit H of membrane-bound oxidoreductase (MBX), of which result was consistent with the previous studies reporting the importance of MBX under sulfur-dependent growth condition. Another effort to screen the desired phenotypic mutant was done under neutralized pH condition. Out of the total of 864 library clones, 1 mutant having mutation site in *tk2145* encoding a radical SAM protein was isolated, as it showed growth defect in the pH-neutralized medium with polysulfide, while no growth impairment with $S^0$ was observed. The estimated role of the radical SAM protein was probably due to activation or maturation of proteins essential for assimilation of polysulfide.

Beside polysulfide, $S^0$ was used as a terminal electron acceptor in the medium for the screening. The author established a reliable procedure for determining cellular protein content in 96-well plate containing $S^0$-supplemented medium. Finally, 10 clones showing growth defect with $S^0$ could be isolated out of the total of 808 library mutants. The three mutants were clarified to possess identical mutation site in *tk0186* encoding multiple substrate aminotransferase. In the $S^0$-dependent growth condition, TK0186 was considered as one of major aminotransferases required by *T. kodakarensis* for amino acid deamination. The other six mutants shared the transposon insertion within *tk2145*, but the mutation sites were two corresponding to C- and N-terminal regions in TK2145. The growth defect on $S^0$ was actually recovered by gene complementation analysis. The mutation in the last mutant was determined to be within the noncoding regions at the upstream of *tk0810* encoding oligosaccharyl transferase. These results suggested the importance of the hypothetical radical SAM protein and oligosaccharyl transferase in protein modification or maturation of proteins involved in sulfur utilization or the related metabolisms.

備考 ： 論文要旨は、和文 2000 字と英文 300 語を 1 部ずつ提出するか、もしくは英文 800 語を 1 部提出してください。
Note : Thesis Summary should be submitted in either a copy of 2000 Japanese Characters and 300 Words (English) or 1copy of 800 Words (English).

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