

Note

Identification of *sn*-Glycerol-1-phosphate Dehydrogenase Activity from Genomic Information on a Hyperthermophilic Archaeon, *Sulfolobus tokodaii* Strain 7

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***sn*-Glycerol-1-phosphate dehydrogenase is responsible for the formation of *sn*-glycerol-1-phosphate, the backbone of membrane phospholipids of Archaea. This activity had never been detected in cell-free extract of *Sulfolobus* sp. Here we report the detection of this activity on the thermostable ST0344 protein of *Sulfolobus tokodaii* expressed in *Escherichia coli*, which was predicted from genomic information on *S. tokodaii*. This is another line of evidence for the general mechanism of *sn*-glycerol-1-phosphate formation by the enzyme.**

Key words: *sn*-glycerol-1-phosphate dehydrogenase; membrane phospholipid; Archaea; *Sulfolobus tokodaii*

Archaea are distinguished as a group of organisms of a third domain from the domains Bacteria and Eucarya by their small subunit ribosomal RNA sequences¹ as well as various biochemical properties. Polar lipid structures of membranes are one of the most characteristic differences distinguishing Archaea from organisms of the other domains. The glycerophosphate backbones of phospholipids in cells of Archaea and Bacteria are enantiomers, that is, the archaeal backbone is *sn*-glycerol-1-phosphate (G-1-P) and the bacterial one is *sn*-glycerol-3-phosphate (G-3-P).² There has been found no exception as to this difference. This is the most fundamental difference by which Archaea and Bacteria are discriminated. These enantiomers are synthesized from dihydroxyacetonephosphate (DHAP) by G-1-P dehydrogenase in Archaea³ and by G-3-P dehydrogenase in Bacteria.⁴ The gene encoding G-1-P dehydrogenase (*egsA*) has been detected in all the archaeal species of which whole genomes have been sequenced, and has not been found in any bacterial or eucaryal species.⁵

The activity of G-1-P dehydrogenase has been detected in all Archaea so far examined (methanogenic, extremely halophilic, thermoacidophilic, and hyperthermophilic archaea) except for the hyperthermoacidophilic *Sulfolobus* species.⁶ Several attempts to detect the activity in the crude homogenate of *Sulfolobus acidocaldarius* cells or partially purified fractions of it were unsuccessful. The reason is not known, but it is assumed to be a low level of the activity. Also, it can be thought that a different mechanism of G-1-P structure formation might be working in *Sulfolobus* cells. Generalization of the mechanism of G-1-P formation by G-1-P dehydrogenase requires detection of the activity from *Sulfolobus* cells. *Sulfolobus tokodaii* is a member of the *Sulfolobus* genus isolated in hot springs in Beppu, Japan,⁷ which grows optimally at 80 °C and pH 2.5–3.0. The genome of *S. tokodaii* has been sequenced, and an open reading frame (ORF) with similarity to the characterized *egsA* gene has been identified (ST0344).⁸ The primary structures of ST0344 and G-1-P dehydrogenases from *M. thermotrophicus*, *S. acidocaldarius*, and *Aeropyrum pernix* show significant similarities (Fig. 1). This communication reports confirmation of G-1-P dehydrogenase activity on the recombinant ST0344 protein expressed in *Escherichia coli* cells and partial characterization of the biochemical features of this protein.

Construction of expression vector: For polymerase chain reaction (PCR) amplification of ST0344 ORF of *S. tokodaii* strain 7 (JCM10535), primer P1, TTAACATATGGAGCTGAAAGAACATATAAT, and primer P2, TTAACCTCGAGTTAGTCAATAATTCCAGTCT, were designed from the 5' sequence and 3' sequence respectively of ST0344. The PCR products were digested with restriction enzymes *Nde*I and *Xho*I (shown by underlines, New England Biolabs, Beverly, MA) and

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Abbreviations: G-1-P, *sn*-glycerol-1-phosphate; G-3-P, *sn*-glycerol-3-phosphate; DHAP, dihydroxyacetonephosphate; ORF, open reading frame; PCR, polymerase chain reaction; MOPS, 3-(*N*-morpholino)propanesulfonic acid; MES, 2-(*N*-morpholino)ethanesulfonic acid; HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid

treated soluble fraction containing 10 mg of protein. The mixture was incubated for 4 h at 65 °C. After the reaction was completed, the mixture was treated as described by Nishihara *et al.*⁶⁾ to remove protein and

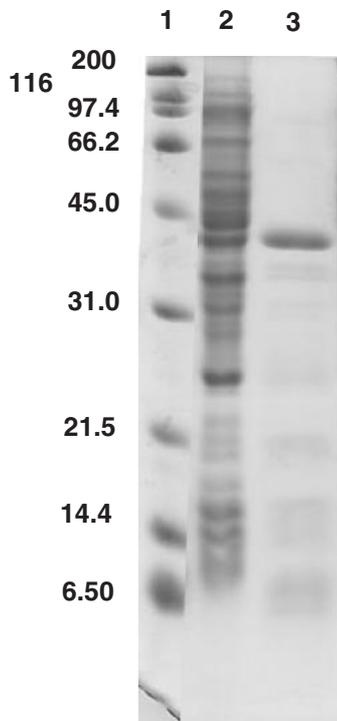


Fig. 2. SDS-PAGE of the Heat-Treated Cell Homogenate of *E. coli* in Which *S. tokodaii* ST0344 Gene Was Expressed.

Protein bands were detected with Coomassie Brilliant Blue R-250. Lane 1, molecular weight markers (SDS-PAGE Molecular Weight Standards, Broad Range, Bio-Rad, Hercules, CA); lane 2, the *E. coli* cell homogenate (5 μ l) in which the ST0344 gene was expressed; lane 3, the supernatant fraction of the heat-treated *E. coli* cell homogenates filtrated with a membrane filter (0.22 μ m pore size). The molecular weight markers were: 6.5 kDa, aprotinin; 14.4 kDa, lysozyme; 21.5 kDa, trypsin inhibitor; 31 kDa, carbonic anhydrase; 45 kDa, ovalbumin; 66.2 kDa, serum albumin; 97.0 kDa, phosphorylase; 116 kDa, β -galactosidase; 200 kDa, myosin.

nicotinamide nucleotides. The coenzyme-free solution was lyophilized and dissolved in 2.2 ml of water. G-1-P and G-3-P in the product were measured by enzymatic methods using purified *M. thermautotrophicus* G-1-P dehydrogenase heterologously expressed in *E. coli* cells, as described previously,¹²⁾ and using rabbit muscle G-3-P dehydrogenase (Boehringer Mannheim, Mannheim, Germany),¹³⁾ respectively. The amounts of G-1-P and G-3-P measured 2.77 μ mol and 0.071 μ mol respectively, revealing that almost all the formed glycerophosphate was G-1-P. These results indicate that the product of the ST0344 gene from *S. tokodaii* was really G-1-P dehydrogenase.

Properties of *S. tokodaii* G-1-P dehydrogenase: The ST0344 protein showed the highest activity at pH 6.2 in MES buffer or MOPS buffer. At pH 6.8, 86–91% of the highest activity was detected. About one third of the highest activity was observed at pH 5.6 (MES) buffer and at pH 7.7 (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) buffer) (Fig. 3A). Precipitate was formed at pH below 5.6 (acetate buffer). The optimum pH of this enzyme was slightly lower than that of the neutrophilic archaeon *M. thermautotrophicus*,¹⁰⁾ or *A. pernix*.¹¹⁾ Since it is assumed that the intracellular pH is 5 to 6, it appears that this pH profile of this enzyme reflects the intracellular pH.

The recombinant ST0344 protein from *S. tokodaii* exhibited thermophilic and thermostable G-1-P dehydrogenase activity. The activity increased as the reaction temperature rose from 37 °C to 85 °C (Fig. 3B). At temperatures higher than 85 °C, activity could not be determined due to precipitation of the enzyme. The activity at 85 °C was 74 times higher than that at 37 °C. The activity of the homogenate remained after heating for 30 min at various temperatures was measured. A slight increase in activity was observed with preincubation at 50 °C to 80 °C (Fig. 3C). The heated protein at 80 °C showed 28% higher activity than the unheated soluble fraction. Heating at 100 °C for 30 min com-

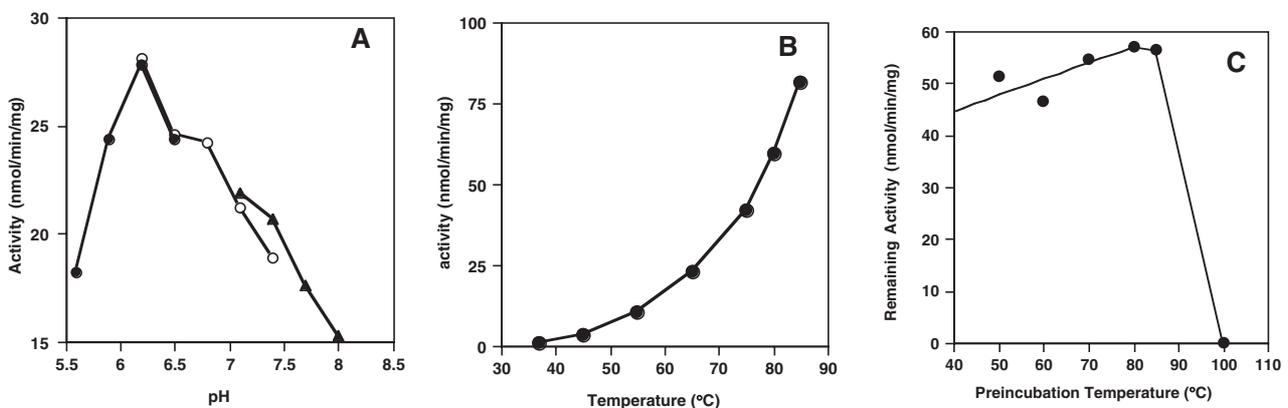


Fig. 3. Properties of G-1-P Dehydrogenase from *S. tokodaii*.

A, pH dependence of G-1-P dehydrogenase activity. Closed circle, MES buffer (pH 5.6–6.5); open circle, MOPS buffer (pH 6.2–7.4); closed triangle, HEPES buffer (pH 7.0–8.0). B, Effect of reaction temperature on G-1-P dehydrogenase activity. C, Effect of preincubation temperature on G-1-P dehydrogenase activity. The enzyme solution was preincubated at the temperatures shown for 30 min before activity was measured at 65 °C.

pletely inactivated the enzyme. Because the recombinant ST0344 protein was prepared by heating of the soluble fraction of *E. coli* cell-free extracts at 80 °C for 20 min, heat treatment might have a more effect on this activity.

The Michaelis constants (K_m) of the enzyme for DHAP and NADPH were determined to be 0.47 mM and 0.067 mM respectively. The V_{max} was 32.8 nmol/min/mg under the assay conditions described above. The K_m values were comparable to those of G-1-P dehydrogenase from *M. thermoautotrophicus*,¹⁰ while the V_{max} value was remarkably low.

Previous attempts to detect G-1-P dehydrogenase activity in crude or partially fractionated homogenates of *Sulfolobus* sp. have been unsuccessful. The reason is not obvious. It is possible that the mechanism of formation of the G-1-P backbone of phospholipids in organisms of the genus *Sulfolobus* are different from that of other Archaea. For example, G-1-P might be synthesized from glycerol by phosphorylation by a new glycerol kinase (G-1-P forming). But the glycerol kinase found in *S. acidocaldarius* catalyzed formation of G-3-P from glycerol and ATP the same as in other Archaea.¹⁴ In this study we detected G-1-P dehydrogenase activity for the first time on the recombinant ST0344 protein of *S. tokodaii*. The ST0344 protein partially purified by heat treatment showed extremely low specific activity. This might be one of the reasons detection of the activity in *Sulfolobus* cell homogenate was unsuccessful. The G-1-P stereo structure of polar lipid backbone of *S. tokodaii* was found to be synthesized by G-1-P dehydrogenase, which extends the generalization of the mechanism of G-1-P formation by this activity. The properties of the enzyme showed a slightly acidophilic and highly thermophilic nature. This is acclimatized to the intracellular environment of the organism, and this suggests, in turn, that the enzyme is in fact working in the cells.

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