# Characterization of a glucan phosphorylase from the thermophilic archaeon *Sulfolobus tokodaii* strain 7

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## Abstract

A glucan phosphorylase from the hyperthermoacidophilic crenarchaeaon *Sulfolobus tokodaii* strain 7 (stGP) was characterized. The enzyme displayed maximum activity at 75 °C and pH 6.0 and was highly stable at 95 °C. The enzyme showed distinct substrate specificity, with maltose being the minimum primer for glucan synthesis and maltotriose being the minimum substrate for degradation. Interestingly, the sequences of GPs from Crenarchaeota clustered all together and formed a distinct lineage. Compared with previously characterized GPs, stGP (56 kDa) was much smaller. Structure modeling revealed that stGP was more compact than other GPs and it seemed that stGP was more primitive and adaptable to high temperature. Taken together, stGP and its homologues in Crenarchaeota probably constitute a novel group of GPs.
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## 1. Introduction

Glucan phosphorylases (GP, EC 2.4.1.1) catalyze reversible degradation of α-1, 4-glucan into glucose-1-phosphate (G-1-P), which provides readily useable energy *in vivo* for the cells. Glucan phosphorylases are ubiquitous in animals, plants, and microorganisms. The GPs analyzed so far are similar in size and the amino acid sequences are highly conserved. They share similar catalytic mechanism [1]: all GPs require pyridoxal 5'-phosphate for enzymatic activity. However, the regulation mechanisms and natural substrates of the enzymes are varied [2]. The substrates of GPs include glycogen, starch, and dextrin.

GPs are expected to be useful in a broad range of industrial applications, including the conversion of starch to G-1-P or the synthesis of amylose, maltooligosaccharides, and other engineered glucose polymers [3–6]. In previous reports, GPs of *Escherichia coli* [7,8], yeast [9], potato [10], and rabbit muscle [11,12] have been studied in detail regarding to structural and functional relationship of the enzymes. Biochemical properties of some mesophilic and thermophilic bacterial GPs have been investigated [13–17]. However, in archaea, only maltodextrin phosphorylase (MalP) from the euryarchaeon *Thermococcous litoralis* has been characterized up to now [18], although glycogen has been identified in cells of many thermoacidophilic archaea including *Sulfolobus*, *Termoproteus*, and *Thermococcous* [19] and glycogen phosphorylase activities have been detected in the cell extracts of *Thermoproteus tenax* [20] and *Methanococcus maripaludis* [21], little is known about the enzymatic properties of GPs from thermophilic Crenarchaeota, another major subdomain of Archaea.

*Sulfolobus tokodaii* strain 7 was originally isolated from an acidic hot spring in Beppu, Kyushu, Japan in the early 1980s [22,23]. It is an aerobic thermoacidophile growing optimally at pH 2–3 and 75–80 °C. Herein, we report the cloning and expression of the GP gene from *S. tokodaii* strain 7 and the biochemical characterization of the enzyme. Particularly, the thermostability and substrate specificity were investigated. The results show that GPs from thermophilic Crenarchaeota are potentially useful for the synthesis of various expensive glucose polymers in industry.

## 2. Materials and methods

### 2.1. Strains and chemicals

*S. tokodaii* strain 7 was purchased from Japan Microbes Collections and cultured in Allen's mineral medium modified by Brock et al. [24]. The genomic DNA was extracted based on the method reported [25]. Restriction enzymes, Pyrobest DNA polymerase, and the DNA ligation kit (ver.2.1) were obtained from Takara. Maltose, maltotriose, and maltotetraose (with the purity of 98%, 96%, and 96% respectively) and other reagents were purchased from Sigma. Plasmid pET15b (Novagen) was modified with *Nde*I being changed to *Nco*I in order to facilitate protein purification. *E. coli* strains DH5α and BL21(DE3)-CodonPlus-RIL were used as the cloning and expression hosts.

### 2.2. Cloning of GP gene from S. tokodaii strain 7

Based on nucleotide sequence of the putative GP gene (ST0893), sense primer 5′GCACAGGTTATCT**CCATGG**-GGAAACACTT3′ and antisense primer 5′GTCAGAGCTGTA-**GTCGAC**CTTATAAAGCGTATT 3′ were designed (the *Nco*I and *Sal*I sites were indicated in bold). The amplified fragments were digested with *Nco*I and *Sal*I, and ligated into *Nco*I/*Sal*I-digested pET15b. The resultant recombinant plasmid, designated pET15b/stGP, was sequenced to ensure that no unintended mutation had occurred.

### 2.3. Site-directed mutagenesis

The site-directed mutation was introduced by the PCR-based overlap extension method. The PCR products containing the mutations were digested and ligated into pET15b. After sequence confirmation, the recombinant plasmids were transformed into *E. coli* BL21(DE3)-CodonPlus-RIL cells for expression.

### 2.4. Expression and purification of the wild-type and mutant stGPs

About 1% of overnight culture of *E. coli* BL21(DE3)-CodonPlus-RIL harboring the recombinant plasmid was inoculated into Luria Bertani medium supplemented with 100 μg/ml ampicillin and 34 μg/ml chloramphenicol. The cells were cultured at 37 °C with shaking. When the $OD_{600}$ reached 0.6, isopropyl-β-D-thiogalactopyranoside (IPTG) was added to a final concentration of 0.5 mM. After induction for further 4 h, the cells were harvested by centrifugation, suspended in 100 mM Tris–HCl (pH 8.0), and disrupted by sonication. The crude extract was incubated at 75 °C for 30 min to denature *E. coli* proteins and centrifuged ($10,000 \times g$) for 15 min. The supernatant was filtered using a 0.22 μm pore-sized membrane filter and loaded onto a nickel column (Novogen). The column was eluted with 80 mM Tris–HCl (pH 7.9) containing 150 mM imidazole. The purified enzyme was analyzed by SDS-PAGE.

### 2.5. Activity assay of stGP

The enzymatic activity of stGP was determined according to the method as described previously with minor modifications [26]. For the synthesis assay, a reaction mixture (250 μl) containing 50 mM MES-NaOH (pH 6.2), 0.4% soluble starch, 10 mM G-1-P and an appropriate amount of enzyme was incubated at 37 °C for 10 min. The reaction was terminated by adding 1 ml of 250 mM glycine-HCl (pH 3.0) followed by rapid cooling in icy water. Ammonium molybdate (125 μl, 1%) in 25 mM $H_2SO_4$ and 125 μl of ascorbic acid (1%) in 0.05% $KHSO_4$ were then added to the mixture. After incubation at 37 °C for 30 min, the absorbance at wavelength of 700 nm was measured and the enzymatic activity was calculated. One unit of enzymatic activity was defined as the amount of the enzyme that produced 1 μmol of phosphate per minute.

For the phosphorolysis assay, the reaction mixture (1 ml) containing 50 mM MES-NaOH (pH 6.2), 0.5% soluble starch, 1 mM $MgCl_2$, 5 mM $NaH_2PO_4$, 2.5 μM glucose-1,6-diphosphate, 0.015% NADP, 0.5 U of glucose-6-phosphate dehydrogenase, 0.5 U of phosphoglucomutase and enzyme was incubated at 37 °C for 30 min. The enzymatic activity was determined by the production of NADPH in the reaction mixture which was calculated according to the change of absorbance at 340 nm. One unit represents the amount of enzyme, which catalyzes the production of 1 μmol of G-1-P per minute.

### 2.6. Effect of temperature and pH on enzyme activity and stability

The effect of temperatures on the enzymatic activity of stGP in the synthetic direction was measured at pH 6.2 using starch as the substrate. To determine the thermostability of stGP, the enzyme in 50 mM MES-NaOH (pH 6.2) was pre-incubated for different lengths of time at various temperatures ranging from 75 °C to 95 °C. The residual activity was determined as described above.

The optimum pH of stGP was determined at 75 °C using the synthesis assay. The buffers used were as follows: 50 mM acetate sodium (pH 3.5, 4.0, 4.5, 5.0), 50 mM MES-NaOH (pH 5.5, 6.0, 6.5), 50 mM HEPES-NaOH (pH 6.5, 7.0, 7.5), and 50 mM Tris–HCl (pH 7.5, 8.0, 8.8).

### 2.7. Substrate specificity

The kinetic parameters of the synthetic and phosphorolytic activities of stGP towards different substrates were determined by incubating the purified enzyme and five different concentrations of substrates (glucose, maltose, maltotriose, maltotetraose and starch; 0.5 mM to 10 mM) in 50 mM MES-NaOH buffer (pH 6.2) at 37 °C. In the synthetic direction, the final concentration of the enzyme in the reaction mixture is 0.26 μM, and the amount of released phosphate was measured as described above in the synthesis assay. In the phosphorolytic direction, the reaction mixture containing 0.24 μM enzyme and different concentrations of substrates was incubated at 37 °C for 30 min. The level of NADPH produced was monitored at 340 nm as described in the phospho-

rolysis assay. All experiments were performed in triplicates and the kinetic parameters were determined using Lineweaver–Burk plot.

The synthetic products using maltose (G2), maltotriose (G3) and maltotetraose (G4) were visualized by silica gel thin-layer chromatography (TLC). Reaction mixture (250 μl) containing 50 mM MES-NaOH (pH 6.0), 10 mM substrate, 10 mM G-1-P, and 0.3 μM of purified enzyme was incubated at 75 °C for the time as specified. *N*-Butanol–methanol–ammonia–$H_2O$ (5:4:2:1 [v/v/v/v]) was used as the developer.

### 2.8. Homology modeling and structural analysis

Based on the amino acid sequence identity between stGP and *E. coli* MalP (PDB code 1ahp) [27], a three-dimensional homology model of stGP was generated using CPHmodels server 2.0 (http://www.cbs.dtu.dk/services/CPHmodels/). Superimposition of the tertiary structure of the predicted stGP and MalP crystal structure was performed using the Swiss-pdbviewer program [28].

## 3. Results and discussion

### 3.1. Sequence analysis of stGP

From the genomic database of *S. tokodaii*, we found a gene that encodes a putative glucan phosphorylase (ST0893, 480 aa.) with a calculated molecular mass of 56,198 Da. The putative glucan phosphorylase is only about half in size of the GPs characterized so far. Homology search using BLAST program (http://www.ncbi.nlm.nih.gov/BLAST/) revealed that this protein (designated stGP) had high similarity to putative GPs from Crenarchaeota, lower similarity to GPs from Euryarchaeota, and the lowest to those from other organisms (data not shown).

The sequences of GPs from Crenarchaeota display unique features compared with GPs from mammals and bacteria. First, GPs from Crenarchaeota may lack phosphorylation sites and other regulatory elements. Although GPs from Crenarchaeota possess highly conserved cofactor pyridoxal phosphate (PLP) binding and catalytic sites (Fig. 1), they lack the N-terminal regions of mammalian and bacterial GPs. The N-terminal
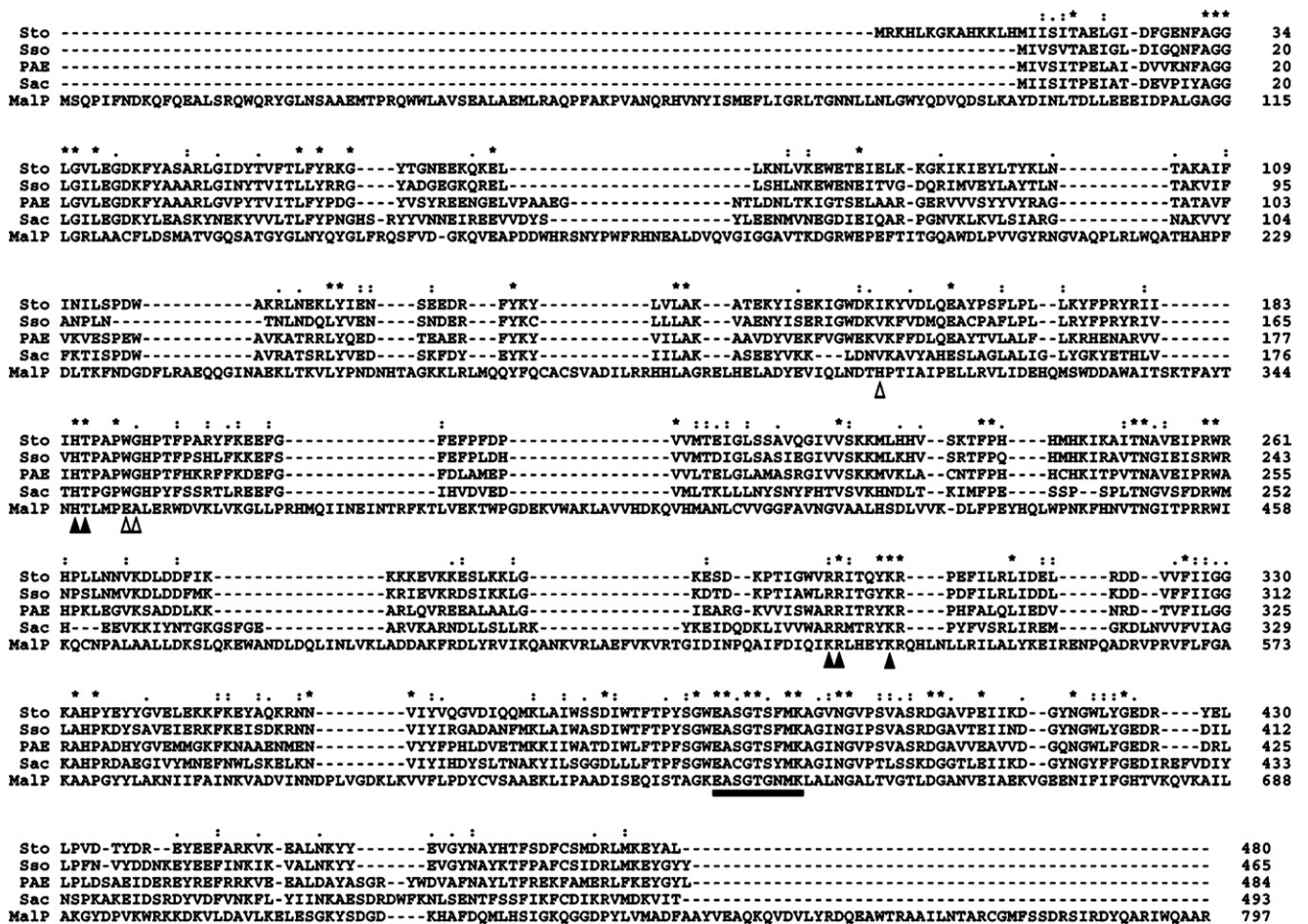


Fig. 1. Amino acid sequence alignment of stGP homologues and MalP. The alignment was carried out using the Clustal W program with manual adjusting. The abbreviations and accession numbers of these sequences are as follows: Sto, *S. tokodaii* strain 7, NP_376798; Sso, *S. solfataricus* P2, NP_343877; PAE, *Pyrobaculum aerophilum* str. IM2, NP_560721; Sac, *Sulfolobus acidocaldarius* DSM 639, YP_255003; MalP, *E. coli* maltodextrin phosphorylase, YP_026218.1. The pyridoxal phosphate binding site (E-A-S-G-T-G-N-M-K) is underlined, and the conserved residues for catalysis and substrate binding are indicated by filled triangles (H346, K534, R535, K540 in MalP). The amino acids contacting glucose residue three and four of the oligosaccharide in MalP are indicated by open triangles (H310, E351, A352).

regions of other GPs contain phosphorylation sites and other regulatory elements [29], although they exhibit a very low level of identity. It was proposed that the regulatory elements of mammalian glgPs, such as binding sites for allosteric activators and inhibitors were acquired during evolution [30,31]. Secondly, the residues which are generally believed to be involved in substrate recognition and binding in other GPs are not conserved in GPs from Crenarchaeota (Fig. 1). Particularly, the conserved amino acids H310, E351, and A352 in *E. coli* MalP and the corresponding residues H341, E382, and A383 in rabbit muscle phosphorylase b, which contact glucose residues three and four of the substrate directly in both GPs, cannot be identified in GPs from Crenarchaeota. Thus, GPs from Crenarchaeota may have unique enzymatic properties.

## 3.2. Cloning, expression and purification of stGP

To characterize the enzymatic properties of stGP, the gene was amplified and the protein was over-expressed in *E. coli*. The recombinant protein was expressed at high level in soluble form and was purified by heat treatment and metal-chelating affinity chromatography (Ni$^{2+}$-NTA column). SDS-PAGE analysis of the protein is shown in Fig. 2. The size of the protein on the gel was in good agreement with the calculated molecular
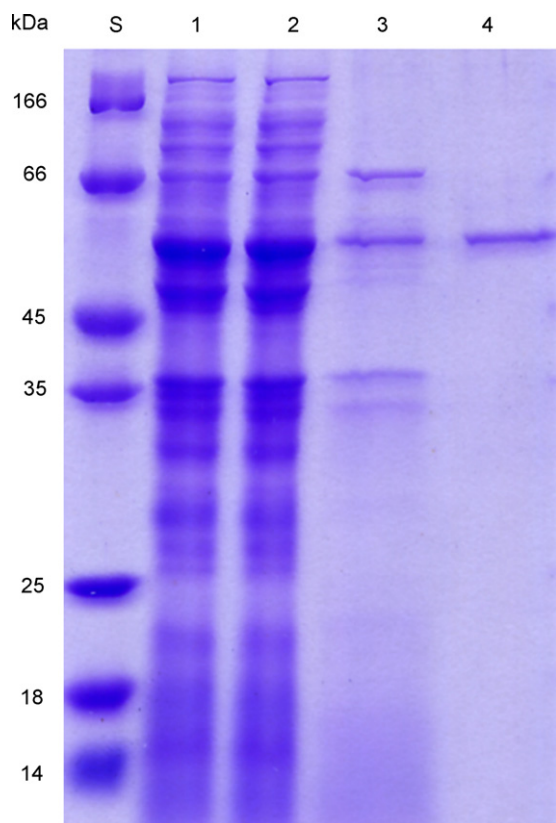


Fig. 2. Purification of recombinant stGP. Samples from all purification steps were analyzed by SDS-PAGE. The gel was stained with Coomassie brilliant blue. Lane 1, crude extract; lane 2, soluble fraction; lane 3, supernatant after heat treatment at 80 °C for 30 min; lane 4, purified enzyme after Ni-NTA affinity chromatography; lane S, the molecular mass standard.
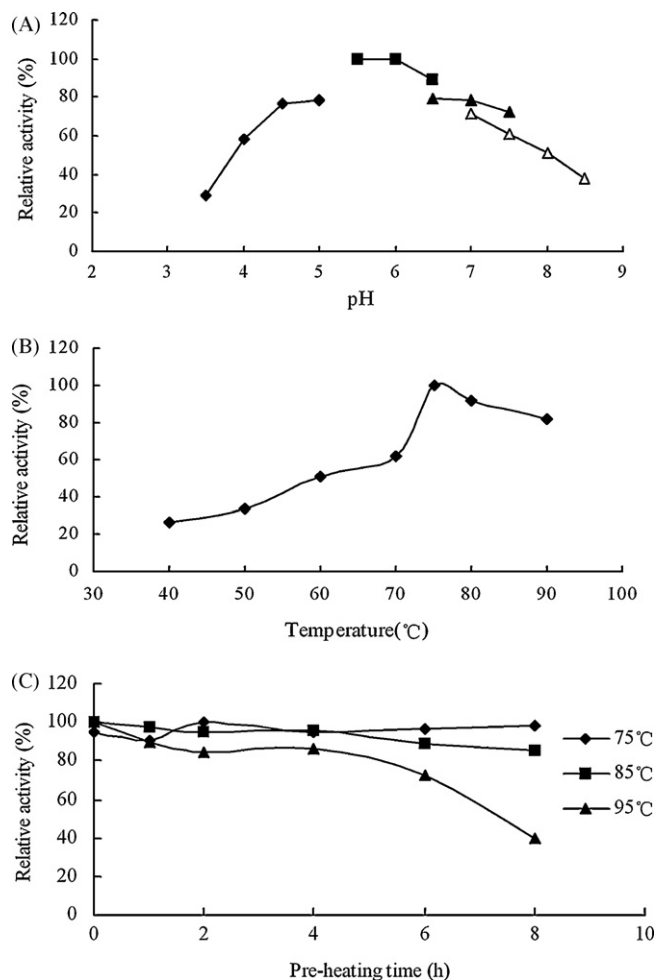


Fig. 3. Effects of pH (a) and temperature (b and c) on stGP synthetic activity. The relative activity was calculated by the percentage of the values against the maximum. (a) The assay was performed using the following buffers (50 mM): acetate sodium (diamonds), MES-NaOH (squares), HEPES-NaOH (filled triangles) and Tris–HCl (open triangles). (b) The activity was determined at temperatures from 40 °C to 90 °C. (c) Thermostability of stGP. The enzyme was pre-heated in 50 mM MES-NaOH (pH 6.2) at 75 °C, 85 °C, and 95 °C respectively for various lengths of time and the activity in synthetic direction was measured as described in the Section 2.

mass. The purified stGP was then used for further enzymatic characterization.

## 3.3. Enzymatic characterization of the recombinant stGP

The pH optimum of stGP was determined in various buffers with pH values ranging from 3.5 to 8.8 (Fig. 3a). The maximum activity was observed at pH 6.0, with more than 50% of the full activity remaining between pH 4 and 7.5. At pH 6.0, the optimum temperature was 75 °C (Fig. 3b). At lower temperatures, the activity dropped quickly. At 40 °C, the activity was only about 26% of the optimal activity.

Thermostability of stGP was studied by measuring the residual activity after incubation at various temperatures at pH 6.2. As shown in Fig. 3c, stGP retained more than 80% of the full activity after incubation at 75 °C and 85 °C for 8 h. Even if the enzyme was incubated at 95 °C for 6 h, more than 70% of the

Table 1
Substrate specificities of the glucan phosphorylase for the synthetic and phosphorolytic reactions[a]

| Substrate | Synthetic direction | | Phosphorolytic direction | |
|---|---|---|---|---|
| | $K_m$ (mM) | $k_{cat}$ (s$^{-1}$) | $K_m$ (mM) | $k_{cat}$ (s$^{-1}$) |
| Glucose | ND[b] | ND | ND | ND |
| Maltose | 1.44 ± 0.31 | 15.2 ± 1.72 | ND | ND |
| Maltotriose | 0.31 ± 0.009 | 24.8 ± 0.68 | 0.79 ± 0.22 | 0.06 ± 0.003 |
| Maltotetraose | 0.478 ± 0.02 | 34.6 ± 0.3 | 0.28 ± 0.06 | 0.16 ± 0.008 |
| Starch | 0.045 ± 0.01% | 19.4 ± 0.77 | 0.013 ± 0.003% | 0.2 ± 0.01 |

All reactions were carried out at 37 °C in 50 mM MES-NaOH buffer (pH 6.2).
[a] Data are means ± S.E.
[b] ND, not detected.

full activity was retained (Fig. 3c). These results demonstrate that stGP is an extremely thermostable enzyme.

The substrate specificity was analyzed by using soluble starch and various maltooligosaccharides as the substrates. In the direction of phosphorolysis, the enzyme was able to phosphorolyze G3 and larger oligosaccharides, but was unable to phosphorolyze G2 (Table 1). In the direction of synthesis, stGP could use G2 and larger oligosaccharides as effective acceptors for chain elongation, but could not use glucose for synthesis. The substrate specificity of stGP was different from those of all known GPs which require minimal oligosaccharides G3 for synthesis and G4 for phosphorolysis [1]. The kinetic parameters of stGP towards substrates with various chain lengths are shown in Table 1. The $K_m$ and $k_{cat}$ of the enzyme towards soluble starch were 0.045% and 19.4 s$^{-1}$ in the synthetic reaction, and 0.013%, and 0.2 s$^{-1}$ in the phosphorolytic reaction. For maltooligosaccharides, the $k_{cat}$ values increased when G3 and G4 were used in both directions. In the phosphorolytic direction, the $K_m$ values decreased about threefolds from G3 (0.79) to G4 (0.28). While $K_m$ values decreased nearly fivefolds from G2 (1.44) to G3 (0.31) in the synthetic direction, no significant difference was found between the $K_m$ values of G3 and G4.

To confirm the substrate specificity, the reaction products using G2, G3, and G4 as the synthetic primers were also analyzed by TLC. As shown in Fig. 4a, maltooligosaccharides of various lengths were generated for the three substrates tested. The enzyme could use G4 and G3 more efficiently than G2 (Fig. 4a, lanes 2, 4 and 6). However, the stGP seemed to be able to use G2 for synthesis. The spot of product G3 in lane 6 was apparent, and larger products could also be visualized. The spots of substrates G2 and G-1-P were weaker than those of the control (Fig. 4a, lanes 5 and 6). Furthermore, after 30 min incubation at 75 °C, some G4 was degraded (Fig. 4a, lane 2), while no degradation product could be detected for G3 in this experiment (Fig. 4a, lane 4). However, when the reaction time was prolonged, degraded product (G2) was also visible for G3 (Fig. 4b, lanes 1, 2 and 4). These results show clearly that the enzyme could use G2 for synthesis and G3 for degradation.

In a previous report, GPs were divided into two groups based on their amino acid sequences and substrate specificities [16]. The first group consists of well-characterized GPs from *E. coli*, potato, rabbit muscle and *Bacillus stearothermophilus*. All of them are already known to use maltotetraose (G4) as the minimum primer for synthesis and maltopentaose (G5) as
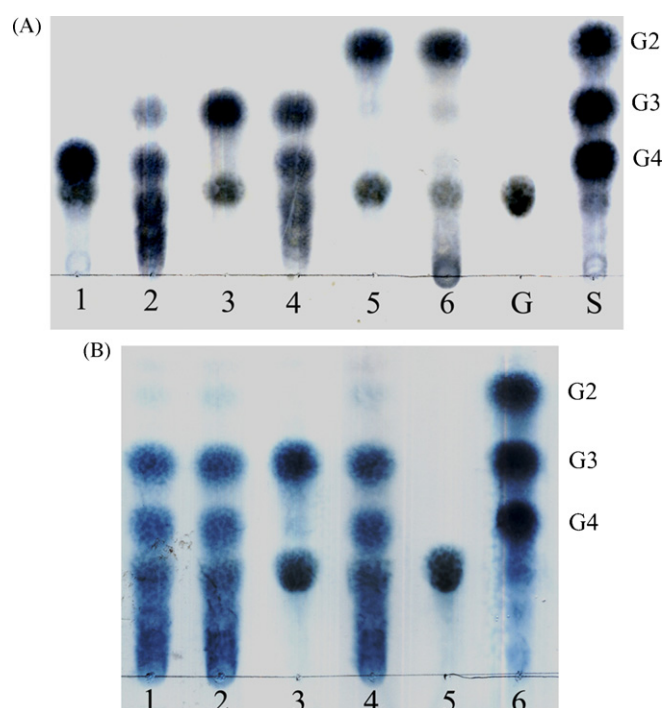
Fig. 4. TLC analysis of the synthetic products using maltose (G2), maltotriose (G3), and maltotetraose (G4) as the substrates. (a) Reaction mixture (250 μl) containing 10 mM substrate (lane 2, G4; lane 4, G3; lane 6, G2), 4.2 μg of purified enzyme and 10 mM glucose-1-phosphate (G-1-P) in 50 mM MES-NaOH buffer (pH 6.0) was incubated at 75 °C for 30 min. Lanes 1, 3 and 5, control without stGP; lane G, G-1-P; lane S, oligosaccharide standards. (b) Reactions with G3 for 45 min (lane 1), 60 min (lane 2) and 75 min (lane 4). Lane 3, control without enzyme; lane 5, G-1-P without substrate; lane 6, oligosaccharide standards.

the minimum effective substrate for phosphorolysis. The second group includes GPs from *Thermus aquaticus*, *Pyrococcus horikoshii*, *Thermotoga maritima*, *T. litoralis*, *Thermus thermophilus*, *Denococcus radiodurans*, and *Aquifex aeolicus*. In the second group, four enzymes from *T. aquaticus* [12], *T. thermophilus* [10], *T. litoralis* [18], and *A. aeolicus* [22] were found to be able to use G3 for synthesis and G4 for phosphorolysis. In this study, we found that stGP was able to use G2 as the minimum primer for elongation and G3 as the minimum substrate for phosphorolysis. The minimum substrates in both directions were at least one unit shorter than those of any other characterized GPs. In addition, the sequences of GPs from Crenarchaeota clustered all together and formed a distinct lineage (Fig. 5). Thus,
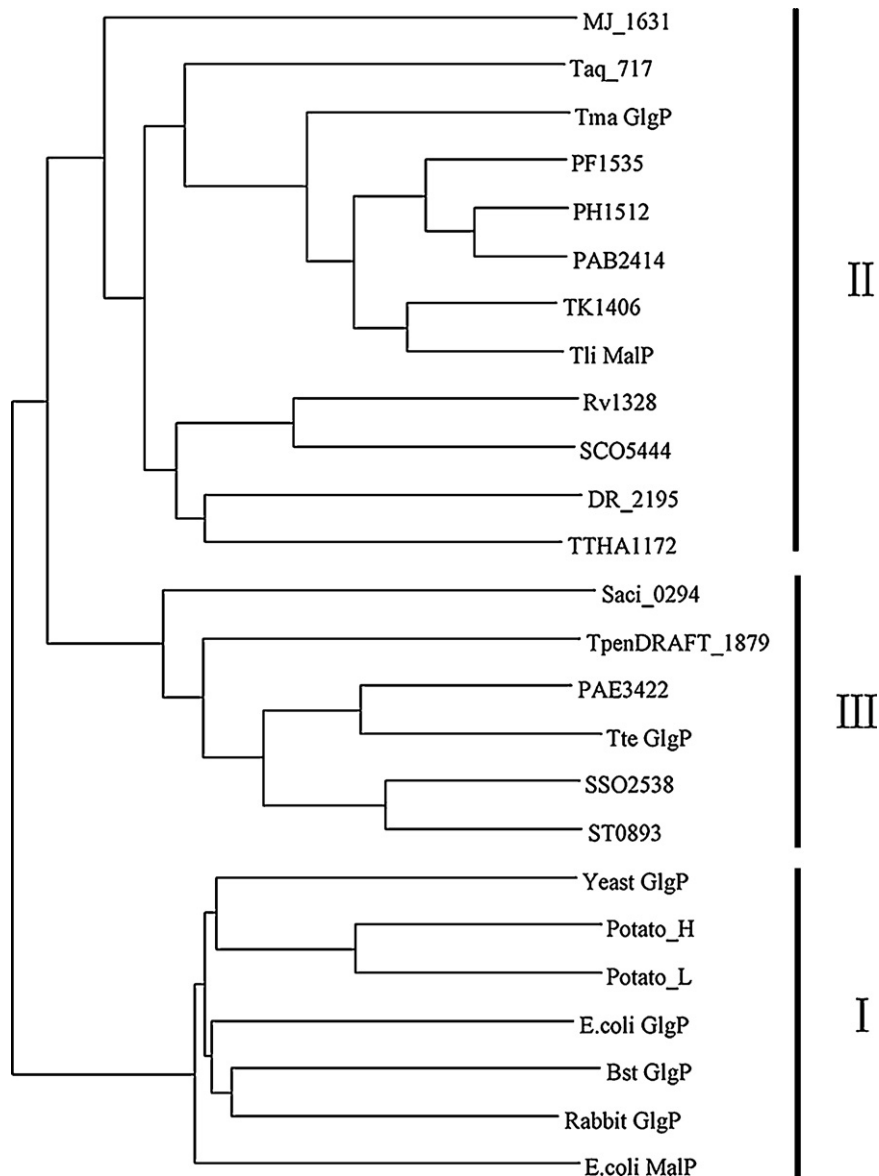
Fig. 5. Phylogenetic analysis of GPs. MJ_1631, glycogen phosphorylase from *Methanocaldococcus jannaschii* DSM 2661; Taq_717, *T. aquaticus*; Tma GlgP, *T. maritima*; PF1535, *Pyrococcus furiosus*; PH1512, *P. horikoshii* OT3; PAB2414, *P. abyssi* GE5; TK1406, *Thermococcus kodakarensis* KOD1; Tli MalP, *T. litoralis*; Rv1328, *Mycobacterium tuberculosis* H37Rv; SCO5444, *Streptomyces coelicolor*; DR_2195, *D. radiodurans*; TTHA1172, *T. thermophilus* HB8; Saci_0249, *S. acidocaldarius* DSM 639; TpenDRAFT_1879, *Thermofilum pendens* Hrk 5; PAE3422, *P. aerophilum* str. IM2; Tte GlgP, *T. tenax*; SSO2538, *S. solfataricus* P2; ST0893, *S. tokodaii* strain 7; Yeast GlgP, *Saccharomyces cerevisiae*; Potato_L and H types; Bst GlgP, *B. stearothermophilus*; Rabbit GlgP, rabbit muscle; *E. coli* GlgP and MalP, *E. coli* K12. The phylogenetic tree was based on the neighbor-joining algorithm and drawn using the Clustal W program. Classification of these GPs (labeled I–III) was based on their amino acid sequences and substrate specificity.

stGP and its homologues in Crenarchaeota may constitute a new group of GPs.

### 3.4. Site-directed mutagenesis of the catalytic sites

In order to understand the catalytic mechanism of stGP, residues were selected for site-directed mutagenesis according to the sequence alignment of stGP with other known GPs. As shown in Fig. 1, most residues that play key roles in *E. coli* MalP are identical in stGP and its crenarchaeal homologues. In *E. coli* MalP, H346 (corresponding to H185 in stGP) was considered to be involved in carbohydrate binding, R535 (R303) and K540 (K308) are crucial for catalysis, and K646 (K394) is essential for cofactor binding. However, K534 which was thought to stabilize the 5′-phosphate group of the PLP cofactor in MalP has been substituted by R302 in stGP. To investigate whether these residues are also involved in substrate binding and catalysis in stGP, we tested the activities of the wild-type enzyme and mutants H185A, R303A, and K308A. The mutant stGPs were expressed and purified in the same way as the wild-type (data not shown). The kinetic parameters of the wild-type and mutant enzymes in synthetic direction using G3 as the substrate were summarized in Table 2. Compared with the wild-type, mutation of R303 or K308 to alanine led to significant changes in

Table 2
Kinetic parameters of the wild-type and mutants of *S. tokodaii* strain 7 glucan phosphorylase[a]

| Mutant enzyme | Synthetic direction (G3) | |
| --- | --- | --- |
| | $K_m$ (mM) | $k_{cat}$ (s$^{-1}$) |
| WT | 0.31 ± 0.009 | 24.8 ± 0.68 |
| H185A | 0.69 ± 0.04 | 0.73 ± 0.02 |
| R303A | 0.35 ± 0.02 | 0.16 ± 0.015 |
| K308A | 0.3 ± 0.013 | 0.11 ± 0.007 |

The synthetic activities of the wild-type and mutants were performed at 37 °C in 50 mM MES-NaOH (pH 6.2) using the maltotriose as the substrate.

[a] Data are means ± S.E.

$k_{cat}$. The values dropped about 155 and 225-fold for R303A and K308A respectively, but no change in the $K_m$ values. For H185A mutant, the $k_{cat}$ value decreased about 34-fold and the $K_m$ value also decreased about twofold. These results confirm that residue H185 of stGP is involved in carbohydrate binding, while R303 and K308 are involved in catalysis.

### 3.5. Homology modeling of stGP

The crystal structure of *E. coli* MalP has been determined previously [32]. The enzyme is composed of two domains, the N-terminal domain (residues 19–482) and the C-terminal domain (residues 483–829), which are separated by a catalytic cleft. Based on sequence identity, partial structure of stGP (residues 222–411, including the C-terminal domain and the catalytic cleft), was generated using the structure of *E. coli* MalP as the template. The resulting model was then compared with the structure of MalP. As shown in Supplementary Fig. 1, the overall hypothetical structure and topology of stGP resemble MalP strikingly. Furthermore, the catalytic sites of the two enzymes, which are buried in the center of the enzymes, superimpose precisely (Supplementary Fig. 2). However, many deletions locating at the solvent surface were found in stGP. Lacking these amino acid sequences, stGP therefore has a more compact structure, which could contribute to stGP thermostability [33].

### 4. Conclusion

Amylose is a functional biomaterial in various industries. Approximately 20% amylose is normally contained in starch granules. Since, purification of natural amylose from starch is difficult, other methods such as processing using enzymes are expected to be promising in amylase production. GPs are therefore of commercial interest in the aspect [3]. The application of GPs in the production of G-1-P [4,5], amylase, and other glucose polymers has attracted great attentions. In fact, GP has recently been used for enzymatic synthesis of expensive linear amylose and other glucose polymers with various structures [6]. To obtain GPs suitable for industrial applications, much effort has been made to enhance the thermostability including the use of random and site-directed mutagenesis [34], or directly acquire enzymes from thermophilic organisms [14,15]. So stGP and other crenarchaeal GPs may be good candidates for industrial purpose, not only for its high thermostability, but

also for its ability to use inexpensive maltose as the substrate for synthesis.

### Acknowledgements

### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.molcatb.2007.11.003.

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