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Research paper

Enzymatic and regulatory properties of the trehalose-6-phosphate synthase from the thermoacidophilic archaeon *Thermoplasma acidophilum*

Yanyan Gao [1], Ying Jiang [1], Qiulei Liu, Ruiming Wang, Xinli Liu, Bo Liu*

College of Food and Bioengineering, Qilu University of Technology, Jinan, Shandong 250353, PR China

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ABSTRACT

Trehalose-6-phosphate synthase plays an important role in trehalose metabolism. It catalyzes the transfer of glucose from UDP-glucose (UDPG) to glucose 6-phosphate to produce trehalose-6-phosphate. Herein we describe the characterization of a trehalose-6-phosphate synthase from the thermoacidophilic archaeon *Thermoplasma acidophilum*. The dimeric enzyme could utilize UDPG, ADP-Glucose (ADPG) and GDP-Glucose (GDPG) as glycosyl donors and various phosphorylated monosaccharides as glycosyl acceptors. The optimal temperature and pH were found to be 60 °C and pH 6, and the enzyme exhibited notable pH and thermal stability. The enzymatic activity could be stimulated by divalent metal ions and polyanions heparin and chondroitin sulfate. Moreover, the protein was considerably resistant to additives ethanol, EDTA, urea, DTT, SDS, β-mercaptoethanol, methanol, isopropanol and *n*-butanol. Molecular modeling and mutagenesis analysis revealed that the N-loop region was important for the catalytic efficiency of the enzyme, indicating different roles of N-loop sequences in different trehalose-6-phosphate synthases.

1. Introduction

Trehalose is a non-reducing disaccharide in which the two glycosyl moieties are linked together by an α,α-1,1 bond [1]. It exists in many organisms and can be used as a carbon and energy source in metabolism. Moreover, the sugar has remarkable stress protection properties to serve as a protectant against various stress conditions such as desiccation, temperature, salinity, alkalinity and oxidation [2]. There are at least five pathways for trehalose biosynthesis in Bacteria, whereas only the TPS/TPP pathway is present in Eukaryotes. To date, four pathways (TPS/TPP, TreY–TreZ, TreT and TreS) for trehalose biosynthesis have been found in Archaea. The TPS/TPP pathway, which is distributed in all three domains of life, consists of a two-step catalysis mechanism. Firstly, the glycosyl is transferred from UDPG to glucose 6-phosphate (Glc-6-P) with the production of trehalose 6-phosphate (T6P) by the trehalose-6-phosphate synthase (TPS: EC 2.4.1.15). Subsequently, T6P is dephosphorylated to produce trehalose by the trehalose 6-phosphate phosphatase (TPP: EC 3.1.3.12) [3]. Furthermore, the TPS and TPP genes are fused together to encode a fused trehalose-6-phosphate synthase/phosphatase (TPSP) protein in almost all eukaryotic genomes, whereas the two genes are usually clustered in one operon but encoded separately in bacterial genomes. Interestingly, the only characterized TPS/TPSP gene in archaeal genomes from the hyperthermophilic crenarchaeon *Thermoproteus tenax* shows a unique operon organization with a glycosyltransferase (GT) and a mechanosensitive channel (MSC) gene, and also encodes a TPSP fusion enzyme. The GT is required for the bifunctional activity of the TPSP. The authors also suggested a monophyletic origin of eukaryotic and prokaryotic fused TPSPs during evolution [4]. Although many TPSs/TPSPs have been well characterized from Eukaryotes and Bacteria such as fungi, plants and bacteria [5–7], current knowledge about TPS in Archaea is comparatively lacking.

Rao et al. [8] reported the crystal structure of a TPP from the thermoacidophilic euryarchaeon *Thermoplasma acidophilum*, and its kinetics properties suggested that the enzyme is involved in the TPS/TPP trehalose biosynthesis pathway. In the genome of *T. acidophilum*, an ORF (TA1210) was found to be adjacent to the TPP gene (TA1209), which was assumed to encode the TPS. Our research aims

Abbreviations: (UDPG), UDP-glucose; (Glc-6-P), glucose 6-phosphate; (TPS), trehalose-6-phosphate synthase; (TPP), trehalose-6-phosphate phosphatase; (TPSP), trehalose-6-phosphate synthase/phosphatase; (T6P), trehalose 6-phosphate; (ADPG), ADP-glucose; (GDPG), GDP-glucose; (GT), glycosyltransferase; (MSC), mechanosensitive channel.

* Corresponding author. Tel.: +86 13806402782; fax: +86 531 89631192.
E-mail address: ertrdfgg@aliyun.com (B. Liu).

[1] These authors contribute equally to this paper.

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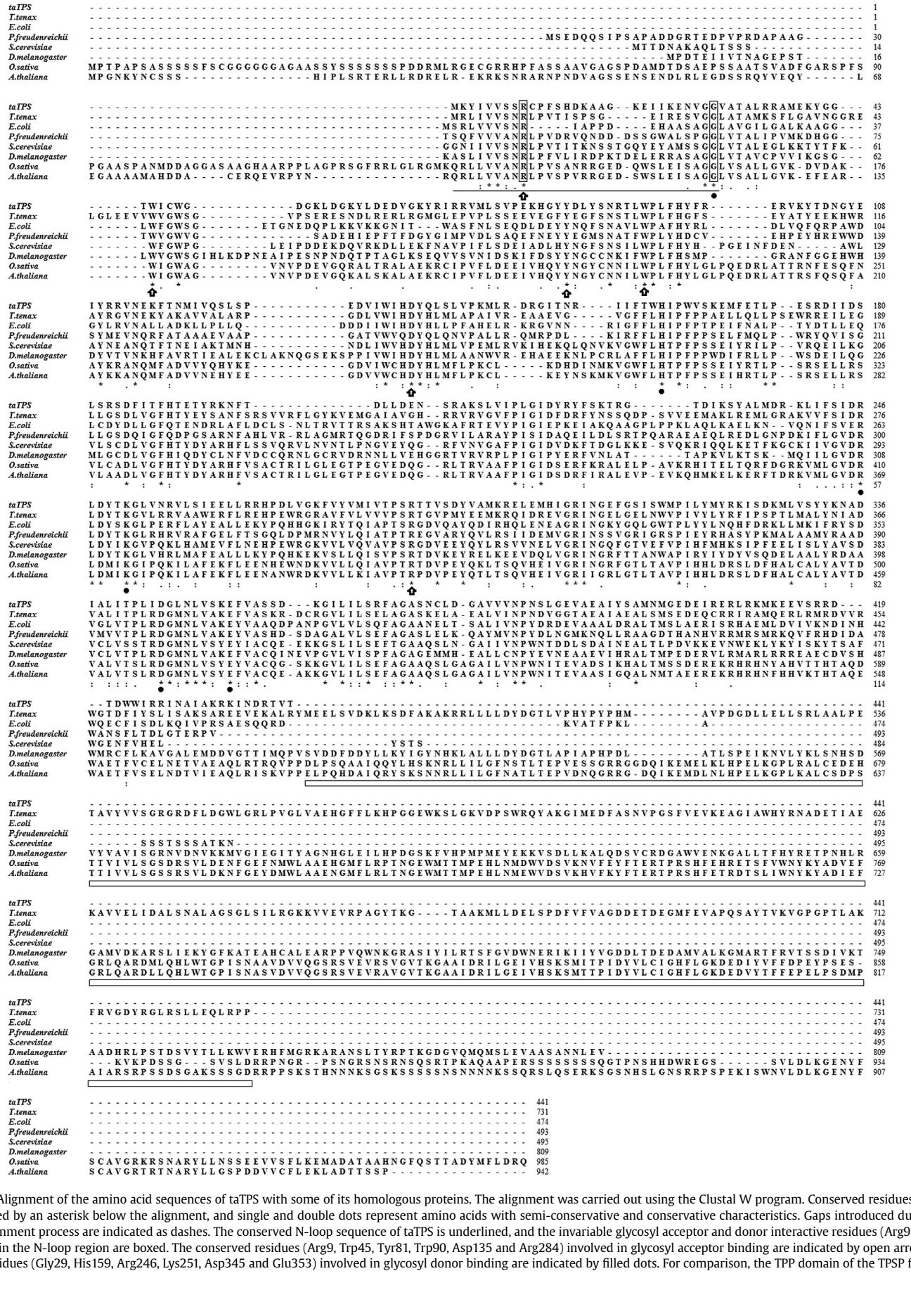

Fig. 1. Alignment of the amino acid sequences of taTPS with some of its homologous proteins. The alignment was carried out using the Clustal W program. Conserved residues are indicated by an asterisk below the alignment, and single and double dots represent amino acids with semi-conservative and conservative characteristics. Gaps introduced during the alignment process are indicated as dashes. The conserved N-loop sequence of taTPS is underlined, and the invariable glycosyl acceptor and donor interactive residues (Arg9 and Gly23) in the N-loop region are boxed. The conserved residues (Arg9, Trp45, Tyr81, Trp90, Asp135 and Arg284) involved in glycosyl acceptor binding are indicated by open arrows, and residues (Gly29, His159, Arg246, Lys251, Asp345 and Glu353) involved in glycosyl donor binding are indicated by filled dots. For comparison, the TPP domain of the TPSP from