Expression, purification and characterization of recombinant protein tyrosine phosphatase from *Thermus thermophilus* HB27

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The low-molecular-weight protein tyrosine phosphatases (PTPase) exist ubiquitously in prokaryotes and eukaryotes and play important roles in the regulation of physiological activities. We report here the expression, purification and characterization of an active and soluble PTPase from *Thermus thermophilus* HB27 in *Escherichia coli*. This PTPase has an optimum pH range of 2.8–4.8 when using p-nitrophenyl phosphate as the substrate. The thermal inactivation results indicate a high thermal stability of this enzyme, with the optimum temperature of 75°C for activity. It can be activated by Mn²⁺, Mg²⁺, Ca²⁺, Ba²⁺, and Ni²⁺, but inhibited by Zn²⁺, Cu²⁺, Cl⁻, and SO₄²⁻. These results suggest that this heat-resistant PTPase may play important roles in vivo in the adaptation of the microorganism to extreme temperatures and specific nutritional conditions.

**Keywords** protein tyrosine phosphatase; *Thermus thermophilus* HB27; characterization

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**Introduction**

Protein tyrosine phosphorylation and dephosphorylation regulate an important control mechanism for multiple central physiological processes, such as cellular phenotypic functions, cell growth, differentiation, proliferation, oncogenic transformation, cell apoptosis, general metabolism, cell cycle regulation and cytoskeletal integrity, etc. [1,2]. Particularly, the intracellular level of protein tyrosine phosphorylation is regulated by a dynamic balance of opposing activities of protein tyrosine kinases (PTKase, EC 2.7.10.1) and protein tyrosine phosphatases (PTPase, EC 3.1.3.48) [3,4]. PTKase transfers a phosphate group from ATP to a protein-forming phosphoprotein (phosphorylation), which results in a change in the structure and function of the protein. On the contrary, PTPase restores the phosphoprotein to its original structure and function by removing the phosphate group attached to a tyrosine residue (dephosphorylation) [5].

Since the first purification of PTPase in 1988 [6], a wide variety of PTPases have been discovered from Eukarya, and more recently bacterial homologs have also been identified. Studies of eukaryotic organisms have revealed that protein phosphorylation is a remarkably versatile and sophisticated mechanism for exerting regulatory control [7]. However, our understanding of protein phosphorylation processes in prokaryotic organisms is still lacking. The catalytic center of these enzymes seems to be conserved from bacteria to eukarya and contains the signature motif C(X)₅R(S/T), encompassing the cysteine of catalytic sites [8]. Protein phosphorylation events have been implicated in the regulation of a number of processes in prokaryotic organisms [9–15]. However, only in relatively few instances has the architecture of the protein phosphorylation cascades in prokaryotic organisms been elucidated in full molecular detail.

*Thermus thermophilus* HB27 (*T. thermophilus* HB27) is an extremely thermophilic, Gram-negative, aerobic, rod-shaped bacterium, which is originally isolated from a natural geothermal environment in Japan and can grow at temperatures up to 85°C, with an optimal growth temperature of 70°C [16]. Therefore, this microorganism can be a good source for heat-stable enzymes and proteins, and has a considerable biotechnological potential, whose physical map of the intact chromosome has been resolved and its complete genome has been sequenced [17,18]. However, the biological function of the corresponding enzymes in strain HB27 can be unequivocally
established only after biochemical characterization. Recently, many thermostable proteins isolated from the members of *Thermus* have been characterized, such as DNA photolyase [19], laccase [20] and lipases/esterases [21].

Although the *Thermus* genus is one of the most widely studied of the thermophilic ones, and many enzymes have been determined in the *Thermus*, the regulatory proteins have not yet been studied extensively in extreme thermophiles. Until now, only two DNA-binding type proteins (the NusG protein [22] and the DNA-binding protein II [22]) have been determined in *T. thermophilus* HB8. In spite of the importance of PTPase in the control of cellular signaling pathways in bacteria, the mechanisms for the regulation of intracellular phosphorylation level, osmoregulation, expression of stress response genes and catabolite repression, etc. are not fully elucidated. It was assumed to possess PTPase activity, however, the PTPase from this microorganism have not been extensively studied *in vitro* and its biological function in *T. thermophilus* HB27 is still unknown. In the present study, we carried out the cloning and heterologous expression of a gene (GeneID: 2775219) encoding the PTPase from *Thermus thermophilus* HB27, along with the establishment of the easy purification procedure for this protein. In addition, we described the purification and biochemical characterization of this PTPase in order to gain the basic knowledge about PTPase from thermophilic bacteria and to use it for future studies and applications.

Materials and Methods

Materials

Tris, dithiothreitol (DTT) and kanamycin sulfate were purchased from Sigma (St. Louis, MO, USA). *p*-nitrophenyl phosphate (*p*NPP) was a product of Amresco (Solon, OH, USA). Other reagents were obtained from standard commercial sources and were of analytical grade unless specially mentioned.

Cloning and construction of recombinant plasmids

The genomic DNA of *T. thermophilus* HB27 was kindly provided by Dr. Zhong-Yin Zhang (Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indiana, USA). Based on the DNA sequence of PTPase reported previously (GeneID: 2775219) [18], two primers (forward: 5’-AAGAATTCAATGGACCGGCCAGGCAGCGGT-3’ and reverse: 5’-AACGTCCACTCAAAGCCGCGGCTTC-3’) were synthesized with *Eco*RI (NEB, Ipswich, MA, USA) and *Sal*I (NEB) restriction digestion sites, respectively, as indicated with underline. The primers were used for the amplification of DNA templates of PTPase. The amplification of target gene was performed using 2.5 units of *Pfu* DNA polymerase (Stratagene, La Jolla, CA, USA) in a 50 μl reaction mixture of PCR buffer, 0.5 μM each primer, 0.2 μM each dNTP, and 0.2 μg genomic DNA as a template on a PCR Thermal Cycler Dice Gradient (Takara, Tokyo, Japan). The cycling conditions consisted of an initial denaturation step for 5 min at 94°C, followed by 30 cycles of 30 s at 94°C, 30 s at 60°C, 1 min at 72°C, and an additional 10 min for final elongation at 72°C. The PCR product was analyzed by 1% agarose gel electrophoresis with a DNA Marker DL2000 (Takara) as molecular weight standard and the target band was recovered with a MEGA-spin agarose gel extraction kit (iNtRON, Gyeonggi-do, Korea). Subsequently, the purified PCR product was digested by *Eco*RI and *Sal*I and inserted into pET-28a(+) (Novagen, Madison, WI, USA) overnight at 4°C in the presence of T4-DNA ligase (Takara). Chemically competent *E. coli* DH5α (NEB) was transformed with the recombinant vector by heat shock at 42°C for 45 s. The transformation products were spread onto semi-solid LB agar plates containing kanamycin (30 μg/ml) and the plates were incubated at 37°C for 16 h. Single white colonies were picked up and analyzed by PCR to verify the success of recombination. The cycling conditions for PCR were as described above. PCR products were electrophoresed on a 1% agarose gel. The recombinant plasmid was sequenced to ensure that no mutation had been introduced and the *Eco*RI and *Sal*I restriction sites were correctly inserted.

Expression and purification of recombinant PTPase

The successful recombinant plasmid was transformed into *E. coli* BL21 (DE3) competent cells (Novagen) by heat shock at 42°C for 45 s. One colony was used for inoculation of 20–100 ml LB medium in the presence of 30 μg/ml kanamycin and grown over night at 37°C. The 25–100 ml culture was inoculated into 200–600 ml LB media containing selective antibiotics. Cultures were induced with isopropyl-1-thio-β-D-galactopyranoside (IPTG, 0.25–1 mM final concentration) at 37°C until optical density (OD<sub>600</sub>) reached 0.6–1.0. Cells were harvested by centrifugation at 15,000 g for 20 min at 4°C after 4 h of induction and pellets were frozen at −70°C.

Frozen pellets were thawed on ice in 30 ml extraction buffer and sonicated three times for 15 s with a pause on ice between each burst. This extraction buffer solution
contained 50 mM Tris–HCl pH 8.0, 50 mM NaCl, 5% glycerol, 5 mM EDTA and 5 mM DTT (freshly added). After sonication on ice, the cell extract was centrifuged at 18,000 g for 20 min at 4°C to remove insoluble materials. Then, the clarified cell lysate was immediately heated at 60°C for 1 h and then centrifuged at 16,000 g at 4°C for 20 min. The supernatant was concentrated to about 5–10 ml with PEG 20,000 in a dialysis tube at 4°C. Then 1 ml sample was loaded onto a Sephadex G-50 column (Pharmacia, Uppsala, Sweden), equilibrated and eluted with 10 mM Tris–HCl (pH 7.5) and 5 mM DTT at a flow rate of 0.4 ml/min. The eluted fractions were analyzed by the absorption at 280 nm, enzyme assay and 15% acrylamide gel SDS-PAGE. All steps during purification were carried out at 4°C unless otherwise indicated.

**SDS-PAGE and protein concentration determination**

Protein purity and size were determined by SDS-PAGE with 15% separating gel and 5% stacking gel according to the method of Laemmli [23]. The purified samples were mixed with 2× SDS loading buffer, heated at 98°C for 5 min, and loaded on a 15% SDS-PAGE. Protein low-molecular-weight (LMW) marker (Amersham, Piscataway, NJ, USA) was used as protein standard. The gel was stained by Coomassie bright blue R-250.

Protein concentration was determined by BCA protein assay kit (Pierce, Rockford, IL, USA), with bovine serum albumin (BSA) as standard. Protein was frozen with 20% glycerol in liquid nitrogen and stored at −80°C. The final concentration of protein was 2.4 μM for most experiments unless specifically mentioned.

**PTPase assay and kinetic parameters calculation**

pNPP is a colorimetric substrate for measuring the activity of protein phosphatases, such as PTPase, serine/threonine phosphatases, Na+/K+ ATPase, and plasma membrane Ca2+-ATPase. Upon dephosphorylation by phosphatases, the reaction yields para-nitrophenol, which becomes an intense yellow soluble product under alkaline conditions and can be conveniently measured at 405 nm on a spectrophotometer.

PTPase assay was carried out in a 200 μl reaction mixture containing 10 mM pNPP as the substrate, 25 mM NaAc-HAc buffer (pH 3.8), 1 mM EDTA and 5 mM DTT. The reaction was initiated by the addition of 4 μl PTPase into 196 μl reaction mixture described above at 30°C and terminated by addition of 1 ml of 1 M NaOH after 10 min incubation. The change in absorption at 405 nm was recorded on a Heλios gamma spectrophotometer (Thermo Scientific Corporation, Waltham, MA, USA) against a blank, prepared in the same way except that water was used instead of the purified enzyme. According to the Lambert–Beer’s law, the PTPase activity can be determined by the following formula: $A = OD_{405} \times V_{\text{total}}/(t \times \varepsilon \times d \times [E])$.

Where, $A$ is the activity of PTPase, $OD_{405}$ is the absorption changes at 405 nm, $t$ is the reaction time in terms of minute, $\varepsilon$ is the molar extinction coefficient of product ($p$-nitrophenol), its value is $1.80 \times 10^{-4}$ M/cm. $d$ is the light path, here it is 1 cm. $V_{\text{total}}$ is the total volume for this assay, here it is 1200 μl. $[E]$ is the amount of PTPase in terms of micromolars of $p$-nitrophenyl phosphate per min at 30°C [24].

To obtain the kinetic parameters, the experimental data were fit to the Michaelis–Menten equation [25]. The best-fit values of the Michaelis constant ($K_m$) and the maximum velocity ($V_{\text{max}}$) in the Michaelis–Menten equation were obtained from a Lineweaver–Burke plot by the program Origin 7.0 (OriginLab Corp., Northampton, MA, USA) using non-linear least-squares regression analysis. All kinetic constants were an average result of at least three measurements.

**Effects of pH and temperature on PTPase activity**

The optimum pH for the activity of PTPase was determined by performing an assay in a series of buffers with an interval of 0.2 pH unit at 30°C for 10 min. All the reaction mixtures were same as previous description except the buffers. Here the buffers for different pH range were 25 mM Gly-HCl (pH 2.2–3.4), 25 mM NaAc-HAc (pH 3.6–5.8), 25 mM MES (pH 6.0–7.0), 25 mM Tris–HCl (pH 7.2–8.4), and 25 mM Gly-NaOH (pH 8.6–10.0), respectively.

The optimum temperature for the activity of PTPase was determined by incubating PTPase with the standard reaction mixture (pH 3.8) at different temperatures (30–90°C) with an interval of 5°C for 10 min. Then the reaction was terminated by addition of 1 ml of 1 M NaOH. The change in absorption at 405 nm was recorded against a blank, prepared in the same way except that water was used instead of PTPase. To eliminate the pH change on activity, the pH value of the buffer was corrected at each given temperature with a HANNA pH 201 Sensitive Glass electrode (HANNA instruments, Woonsocket, RI, USA) before and after each experiment.

The thermal stability experiments of PTPase were performed with an assay after preincubation of PTPase at...
different temperatures (30–90°C) for different time. The relative activities were normalized with the enzymatic activity at 30°C as a reference. Each result was an average of at least three repetitions.

Effects of metal cations and anions on PTPase activity
We know that EDTA (ethylene diamine tetraacetic acid disodium salt-2-hydrate) will interfere with our observations due to its chelation with cations. Thus, before measuring the effects of metal cations on PTPase activity, we studied the effects of EDTA on the activity of PTPase and found there was no difference in the absence and presence of EDTA. Therefore, in this case, we measured the effects of several divalent metal ions (MgCl₂, CaCl₂, BaCl₂, MnCl₂, NiCl₂, CuCl₂, and ZnCl₂) and anions (Cl⁻ and SO₄²⁻) on PTPase activities with the assay in the absence of EDTA at 30°C. The purified PTPase was incubated in different concentrations ions for 120 min except Cu²⁺ and Zn²⁺, for which 30 min incubation with PTPase was applied. A same treatment with water instead of PTPase was used for control. The results were expressed as relative activity with the PTPase activity at 30°C as a reference. Each assay was carried out in triplicate.

Results
Cloning and construction of recombinant PTPase
As described above, the full-length sequence encoding PTPase of *T. thermophilus* HB27 was obtained by PCR. Its full length is 486 bp according to the gene reported. After digestion with EcoRI and SalI and ligation with T4 DNA ligase, the digested PCR product was successfully inserted into pET-28a(+) vector. Thirty-six amino acid residues derived from the vector will be introduced into the PTPase sequence, therefore the molecular weight of recombinant PTPase should be 22.26 kDa according to the program calculated online (http://ca.expasy.org/tools/protparam.html). The result of DNA sequencing showed that it was consistent with the sequence reported (GenBank accession No. AAS81162), which meant we had successfully cloned the PTPase gene from the genomic DNA of *T. thermophilus* HB27.

Expression and purification of recombinant PTPase
In order to improve the expression of soluble recombinant PTPase, we investigated the effects of various parameters such as growth temperatures, induction time, and IPTG concentration. The final parameters were same as those described in Materials and Methods. The purification steps and data were summarized in Table 1. The purified fold was 1.7 and the yield was 92.6% after heat treatment. The final purified fold was 54.3 and the yield was 80.4% after gel filtration. After gel filtration, we found the phosphatase activity emerged with the eluted peak, and the activity profile was almost consistent with the absorption curve at 280 nm (Abs280). As shown in Fig. 1, about 85% purity was achieved after heat treatment (Fig. 1, lane 4), and the final purified enzyme showed more than 95% purity (Fig. 1, lane 5) on 15% SDS-PAGE. The purified PTPase was about 22 kDa, which was in agreement with the calculation of the recombinant PTPase sequence.

Table 1 Summary of the purification of PTPase from *T. thermophilus* HB27

<table>
<thead>
<tr>
<th>Steps</th>
<th>Volume (ml)</th>
<th>Protein (mg/ml)</th>
<th>Total protein (mg)</th>
<th>pNPP phosphatase activity</th>
<th>Purification (fold)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Total (U × 10⁶)</td>
<td>Specific (U × 10³/mg)</td>
<td></td>
</tr>
<tr>
<td>Crude extract</td>
<td>100</td>
<td>13.4</td>
<td>1340</td>
<td>72.8</td>
<td>54.3</td>
<td>1</td>
</tr>
<tr>
<td>Heat treatment</td>
<td>90</td>
<td>8.1</td>
<td>729</td>
<td>67.4</td>
<td>92.5</td>
<td>1.7</td>
</tr>
<tr>
<td>Gel filtration</td>
<td>18</td>
<td>0.6</td>
<td>10.8</td>
<td>54.2</td>
<td>5018.52</td>
<td>54.3</td>
</tr>
</tbody>
</table>

Protein assay and kinetic parameters calculation
According to the description above, a Lineweaver–Burke plot was used to calculate all the kinetic parameters (Fig. 2). The experimental data were fitted with a line as the least squares theory. The inset showed a typical Michaelis–Menten curve when fitted the plot of v vs. [S], which was the basis of a Lineweaver–Burke plot. The calculated \( K_m, V_{max}, \) and \( k_{cat} \) for PTPase were 37.88 ± 0.15 mM, 0.29 ± 0.06 µmol/min and 670.91 ± 3.25 s when using pNPP as the substrate at 30°C, respectively.

This enzyme from *T. thermophilus* HB27 was assumed to have a remarkable thermal stability. So here
we investigated its kinetic properties at 75°C according to previous description. We calculated the $K_m$, $V_{max}$, and $k_{cat}$ value to be 6.01 ± 0.28 mM, 1.42 ± 0.15 µmole/min and 4013.26 ± 5.62 s, respectively. Comparing to these values at 30°C, this result suggests that this enzyme has a higher affinity with pNPP and a higher catalytic efficiency under high temperature. $k_{cat}/K_m$ is often thought of as a measure of enzyme efficiency. Here, the $k_{cat}/K_m$ value is calculated to be about 1.77 x 10^4 M/s at 30°C, as compared with 6.68 x 10^5 M/s at 75°C. With pNPP as the substrate, when compared with other known PTPase (Table 2) [24,26–32], this specific PTPase is more active at 30°C, especially at 75°C.

**Effects of pH on PTPase activity**

As we known, pH plays a very important role on enzymatic activity. Therefore, we investigated the effect of pH on PTPase activity (Fig. 3). The purified enzyme showed a classic bell-shaped activity curve from pH 2.2 to 10.0 and an acidic pH preference when using pNPP as a highly activated substrate. The PTPase activity increased from pH 2.2 to 3.6 and reached the maximum between pH 3.6 and 4.0. A further increase in pH from 4.0 to 10.0 caused a gradual decrease in activity (Fig. 3).

**Effects of temperature on PTPase activity**

To determine the effects of temperature on PTPase from *T. thermophilus* HB27, enzyme activity was measured over a temperature range of 30–90°C. The activity slowly increased from 30 to 75°C (Fig. 4). The optimum temperature was observed to be 75°C. The activity at 30°C was 11% of that at 75°C. After reaching the maximum activity at 75°C, further increase in temperature resulted in a significant decrease in activity. It was remarkable to note that the enzyme exhibited about 63% activity even at 80°C, but there was less than 10% activity at 90°C of that at 75°C (Fig. 4). The enzyme showed high activity (above 50% of maximum activity) in the temperature range of 50–80°C.

Compared with other enzymes researched in our laboratory [33], this PTPase exhibited higher thermal stability. There was no significant decrease in activity after incubation for 4 h at 50 and 60°C. It just lost 34% activity under the same treatment at 70°C. Even after incubation for 1 h at 80°C, this enzyme still retained 30% activity compared with that of native enzyme (Fig. 5).

**Effects of divalent metal ions and anions on PTPase activity**

Different divalent metal cations showed two opposite effects, activation or inhibition on PTPase activity when the concentration was less than 20 mM. It could be noticed that Mn$^{2+}$ was the most effective activator on the enzymatic activity, followed by Mg$^{2+}$ and Ca$^{2+}$. Ba$^{2+}$ showed a similar weak activation with Ni$^{2+}$, which had an activation not more than 130% under the indicated concentrations [Fig. 6(A)]. There were some differences between Mg$^{2+}$ and Mn$^{2+}$ on the activation. The activity increased with the concentration of Mg$^{2+}$ increasing and reached the highest at 1 mM. There were

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**Figure 1** SDS-PAGE analysis of the expression and purification of PTPase. Lane 1, wild type without IPTG induction; lane 2, induction with 1 mM IPTG; lane 3, crude extract; lane 4, the supernatant after heat treatment; lane 5, purified protein after gel filtration; lane 6, protein LMW marker.

**Figure 2** The values of $K_m$ and $V_{max}$ were determined with pNPP as a substrate at 30°C and calculated by a Lineweaver–Burke plot. The inset showed it was a typical Michaelis–Menten reaction. The concentrations of pNPP were 2.5, 5, 10, 20, 30, and 40 mM, respectively.
### Table 2 Kinetic constants for PTPases with pNPP as the substrate

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>$k_{\text{cat}}$ (s$^{-1}$)</th>
<th>$K_m$ (mM)</th>
<th>$k_{\text{cat}}/K_m$ (s$^{-1}$,mM$^{-1}$)</th>
<th>Conditions</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>YOP51</td>
<td>1234</td>
<td>2.40</td>
<td>514</td>
<td>pH 5.0, 100 mM acetate, 150 mM NaCl, 30°C</td>
<td>[24]</td>
</tr>
<tr>
<td>PTPL323</td>
<td>48</td>
<td>0.23</td>
<td>209</td>
<td>pH 5.0, 100 mM acetate, 150 mM NaCl, 30°C</td>
<td>[24]</td>
</tr>
<tr>
<td>YPTPI</td>
<td>1.6</td>
<td>1.18</td>
<td>1.36</td>
<td>pH 5.5, 100 mM acetate, 150 mM NaCl, 30°C</td>
<td>[24]</td>
</tr>
<tr>
<td>rIAR</td>
<td>6.1</td>
<td>0.42</td>
<td>14.5</td>
<td>pH 5.0, 40 mM MES, 30°C</td>
<td>[26]</td>
</tr>
<tr>
<td>VHR</td>
<td>5.14</td>
<td>1.59</td>
<td>3.24</td>
<td>pH 6.0, 100 mM acetate, 50 mM Tris, 50 mM Bis-Tris, 30°C</td>
<td>[27]</td>
</tr>
<tr>
<td>VHR</td>
<td>3.65</td>
<td>7.99</td>
<td>0.457</td>
<td>pH 7.0, 100 mM acetate, 50 mM Tris, 50 mM Bis-Tris, 30°C</td>
<td>[27]</td>
</tr>
<tr>
<td>LARD1</td>
<td>4.1</td>
<td>1.73</td>
<td>2.37</td>
<td>pH 6.0, 100 mM MES, 25°C</td>
<td>[28]</td>
</tr>
<tr>
<td>VH1</td>
<td>0.3</td>
<td>7.7</td>
<td>0.039</td>
<td>pH 5.5, 100 mM acetate, 150 mM NaCl, 25°C</td>
<td>[24]</td>
</tr>
<tr>
<td>Cdc25</td>
<td>0.033</td>
<td>50</td>
<td>0.00066</td>
<td>pH 8.2, 100 mM Tris, 250 mM NaCl, 37°C</td>
<td>[29]</td>
</tr>
<tr>
<td>SH-PTP1</td>
<td>155</td>
<td>148</td>
<td>1.0</td>
<td>pH 5.5, 100 mM MES, 150 mM NaCl, 10 mM DTT, 1 mM EDTA, 23°C</td>
<td>[30]</td>
</tr>
<tr>
<td>SH-PTP1 (PTP1C)</td>
<td>37.4</td>
<td>1.5</td>
<td>25</td>
<td>pH 5.0, 25 mM acetate, 20% glycerol, 1 mM DTT, 1 mM EDTA, 22°C</td>
<td>[31]</td>
</tr>
<tr>
<td>SH-PTP2</td>
<td>0.046</td>
<td>3.6</td>
<td>0.013</td>
<td>pH 5.6, 50 mM 3,3-dimethylglutarate, 50 mM NaCl, 10 mM DTT, 2 mM EDTA, 24°C</td>
<td>[32]</td>
</tr>
<tr>
<td>PTPase*</td>
<td>670.91</td>
<td>37.88</td>
<td>17.7</td>
<td>pH 3.8, 25 mM NaAc-HAc buffer, 1 mM EDTA, 5 mM DTT, 30°C</td>
<td>This study</td>
</tr>
<tr>
<td>PTPase*</td>
<td>4013.26</td>
<td>6.01</td>
<td>667.8</td>
<td>pH 3.8, 25 mM NaAc-HAc buffer, 1 mM EDTA, 5 mM DTT, 75°C</td>
<td>This study</td>
</tr>
</tbody>
</table>

* PTPase from *T. thermophilus* HB27.
no significant changes from 1 to 20 mM. However, the activation of Mn$^{2+}$ was more obvious than that of Mg$^{2+}$. It reached the highest activity at 5 mM, which was higher than that of Mg$^{2+}$. However, the activity was about 3 fold of native enzymatic activity, which was far more than the activation of Mg$^{2+}$. Although the activation started to decrease when the concentration was higher than 5 mM, it still had about 226% activity at 20 mM.

Zn$^{2+}$ and Cu$^{2+}$ showed obvious inhibitions on PTPase activity, which were different from the other cations mentioned above. It was remarkable to notice that Cu$^{2+}$ had a more obvious inhibition than that of Zn$^{2+}$ [Fig. 6(B)]. It resulted in 77.8% activity loss at 1 mM of Cu$^{2+}$, but it only lost 36.7% activity for Zn$^{2+}$ at the same concentration. There was no residual activity at 5 mM of Cu$^{2+}$, but it still had about 24% residual activity at the same concentration of Zn$^{2+}$, and 12% activity left at 10 mM of Zn$^{2+}$.

Cl$^{-}$ and SO$_4^{2-}$ showed some obvious inhibitions on PTPase activity under indicated concentrations. The results were fitted with non-linear functions as the least squares principle. Cl$^{-}$ showed an S-logistic inhibition curve [Fig. 6(C)], which was different from the Hill inhibition curve of SO$_4^{2-}$ [Fig. 6(D)]. It could be found that SO$_4^{2-}$ had a more obvious inhibition than that of Cl$^{-}$. The concentration of 50% activity inhibition (IC$_{50}$) of SO$_4^{2-}$ was less than 40 mM, which was far lower than the value of Cl$^{-}$. The IC$_{50}$ value of Cl$^{-}$ was about 180 mM. There was about 30% residual activity at 50 mM of SO$_4^{2-}$, but it had about 90% activity left at the same concentration of Cl$^{-}$. But when the concentration was more than 300 mM, there were not more than 15% activity left no matter Cl$^{-}$ or SO$_4^{2-}$.

**Discussions**

Many different PTPase have been identified and characterized, such as *Yersinia* PTPase Yop51 [24], human dual-specific PTPase VHR [27], and LMW acid phosphotyrosyl protein phosphatase [34]. As compared with those PTPase, this specific PTPase shows higher specific activity and larger $k_{cat}/K_m$ value with pNPP as the substrate.
A novel protein tryosine phosphatase from *Thermus thermophilus* HB27

A sequence alignment showed that this PTPase was highly homologous to LMW acid phosphatase. Some LMW acid phosphatases have been reported to have PTPase activity [35–37]. They appear to be cytoplasmic in origin, have acidic pH preference, and generally accept pNPP as substrate. This PTPase have more than 60% activity in pH 2.6–6.0 buffers, which may be helpful for the organism to live in an acidic environment.

The temperature effects on enzyme activity cannot only accelerate the reactions catalyzed by enzyme, but also result in protein inactivation or denaturation. When the temperature was lower than 75°C, it showed an increase tendency in that the inactivation effect was not as obvious as the activity increase. But when the temperature was higher than 75°C, thermal-induced inactivation became dominant and the activity decreased quickly with further increase in temperature. The results reported here, showing that this PTPase had an optimal temperature of 75°C that corresponded to this organism’s optimal growth temperature of 70°C. This specific PTPase displayed higher thermostability and activity comparable with that of the enzyme from other species, which may ensure that the microorganism grows at extremely elevated temperatures. Here we may conclude that it may affect the intracellular metabolism as a whole or induce the expression of stress response genes by regulating the phosphorylation level in vivo to meet the requirements of the survival of *T. thermophilus* HB27 under thermal pressure. PTPase was highly stable at high temperatures.

temperature, which enabled us to easily purify the wild-type PTPase because most of the heat-labile proteins present in E. coli cells could be easily removed by heat treatment. Until now, despite the biological importance in regulating signaling transduction pathway, no tertiary structure of PTPase was determined. The easy purification process and high stability of PTPase may become some advantages to further researches of its structure and function. The presence of EDTA did not affect the PTPase activity, suggesting that it does not require metals for activity. Furthermore, the enzyme showed no loss of activity after exhaustive dialysis against buffer containing 5 mM EDTA. The EDTA-dialyzed PTPase turned out to be quite stable at 70°C for 3 h, both in the absence and presence of EDTA. These results indicate that PTPase does not require metals for activity or structural stabilization, although this is usual for the typically non-metal short-chain dehydrogenase/reductase, the classical RADH_{Lsb}-type ADHs that show strong Mg^{2+} dependence [38]. The Mg^{2+}- or Mn^{2+}-dependent protein phosphatase family in eukaryotes are protein-serine/threonine phosphatases that require the presence of an exogenous divalent metal ion, usually Mg^{2+}, for activity [39,40]. While eukaryotic protein phosphatase P family (PPPs) are also metalloenzymes [41], they bind metal ions with sufficient tenacity to render such supplementation unnecessary. ORF221, the first prokaryote-associated member of the PPPs family, requires the addition of exogenous metal ions for activity, especially Mn^{2+} [42].

There are two cysteines located at the active sites (C{\text{Cl}}N{\text{GCR}}s known as signature motif), which are very important to the PTPase activity. Zn^{2+} and Cu^{2+} may bind to these two cysteines, and then result in PTPase inactivation. Likely, Cu^{2+} may induce the oxidation of cysteine, which will result in the same result. Similar results have been reported that Cu^{2+} is a highly potent and reversible oxidizer on the activity of vaccinia H1-related (VHR) PTPase [43].

Both Cl\(^{-}\) and SO\(_4\)\(^{2-}\) showed inhibitions, though different, on PTPase activity, which were also different from that of Zn\(^{2+}\) and Cu\(^{2+}\). They were not as potent as Zn\(^{2+}\) and Cu\(^{2+}\) on the inhibitions of PTPase activity. The inhibition of Cl\(^{-}\) and SO\(_4\)\(^{2-}\) under high concentrations on the activity of PTPase may derive from the ionic strength effects. Our results suggested SO\(_4\)\(^{2-}\) was more effective than Cl\(^{-}\) on the inhibition of PTPase activity at the same concentration, which reflected the difference in the chemical identity of the ions. We have known some metal cations and anions play important physiological roles \textit{in vivo}. Such activation and inhibition of metal ions and anions on PTPase activity may affect the PTPase activities and levels \textit{in vivo}, which further affect signal pathways and metabolic activities.

In conclusion, the gene encoding PTPase of \textit{T. thermophilus} HB27 was successfully cloned and expressed in \textit{E. coli}. The purified enzyme showed remarkable thermophilicity and acidic-pH preference. Therefore, PTPase has many advantages with regard to its preparative application, including ease of purification and long-term stability. While the mechanisms involved in the regulation of intracellular signaling pathways in \textit{T. thermophilus} strains remain elusive, our data suggest that this PTPase has contributed significantly to thermoadaptation of \textit{T. thermophilus} HB27 thriving in extreme environments.

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