Vol. 183, No. 24

The PPP-Family Protein Phosphatases PrpA and PrpB of *Salmonella enterica* Serovar Typhimurium Possess Distinct Biochemical Properties

LIANG SHI,* DAVID G. KEHRES, AND MICHAEL E. MAGUIRE

Department of Pharmacology, School of Medicine, Case Western Reserve University, Cleveland, Ohio 44106

Salmonella enterica serovar Typhimurium requires Mn^{2+}, but only a few Mn^{2+}-dependent enzymes have been identified from bacteria. To characterize Mn^{2+}-dependent enzymes from serovar Typhimurium, two putative PPP-family protein phosphatase genes were cloned from serovar Typhimurium and named *prpA* and *prpB*. Their DNA-derived amino acid sequences showed 61% identity to the corresponding *Escherichia coli* proteins and 41% identity to each other. Each phosphatase was expressed in *E. coli* and purified to near electrophoretic homogeneity. Both PrpA and PrpB absolutely required a divalent metal for activity. As with other phosphatases of this class, Mn^{2+} had the highest affinity and stimulated the greatest activity. The apparent K_a of PrpA for Mn^{2+} of 65 µM was comparable to that for other bacterial phosphatases, but PrpB had a much higher affinity for Mn^{2+} (1.3 µM). The pH optima were pH 6.5 for PrpA and pH 8 for PrpB, while the optimal temperatures were 45 to 55°C for PrpA and 30 to 37°C for PrpB. Each phosphatase could hydrolyze phosphorylated serine, threonine, or tyrosine residues, but their relative specific activities varied with the specific substrate tested. These differences suggest that each phosphatase is used by serovar Typhimurium under different growth or environmental conditions such as temperature or acidity.

MntH, the *Salmonella enterica* serovar Typhimurium ortholog of the eukaryotic NRAMP proteins (natural resistance-associated macrophage protein), is a highly selective H^+-stimulated Mn^{2+} transporter (6). Subsequently, the mammalian NRAMP1 protein, expressed in monocytes and macrophages, was shown to mediate marked changes in intracellular Mn^{2+} in macrophages (5). Transcription of *mntH* in serovar Typhimurium is induced by hydrogen peroxide and cation starvation, and mutation of *mntH* makes serovar Typhimurium cells more sensitive to killing by peroxide. These data suggest that Mn^{2+} is required for maintenance of some physiological functions and that Mn^{2+} could play a role in virulence, especially in resistance to reactive oxygen species (6). Any role for Mn^{2+} is presumably mediated by its use as an enzyme cofactor. Surprisingly, very few known enzymes are known to be Mn^{2+} dependent. In an effort to understand the role of Mn^{2+} in cell function and pathogenesis, we have begun to characterize each of the few known Mn^{2+}-cofactored enzymes. One such family is the PPP class of protein phosphatases (2, 8, 17).

PPP phosphatases are metalloenzymes that include the catalytic subunits of mammalian PP1, PP2A, PP2B, and related protein phosphatases. Two divalent metal ions in their active sites catalyze a single-step dephosphorylation reaction. In eukaryotes, PPP phosphatases are involved in regulating multiple cellular functions such as cell division, glycogen metabolism, and muscle contraction. Homologs of PPP phosphatases can be discerned in many bacterial and archaeal genomes (for reviews, see references 8 and 17). All bacterial PPP phosphatases characterized to date require Mn^{2+} for maximal activa-

tion (7, 10, 12–16, 18–20). To investigate the role of Mn^{2+} further, we have cloned, purified, and characterized the two PPP phosphatase homologs evident in the extant genome of serovar Typhimurium. As predicted each is a Mn^{2+}-dependent protein phosphatase; however, the two enzymes have markedly different properties suggestive of distinct physiological roles.

MATERIALS AND METHODS

Standard procedures. Protein concentrations were measured with the bicinchoninic acid protein assay kit from Pierce (Rockford, Ill.). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed as described by Laemmli (9), and the gels were stained as described by Fairbanks et al. (4).

Cloning and sequencing *prpA* and *prpB* from serovar Typhimurium. The *prpA* and *prpB* genes were cloned from MM1255 (wild-type serovar Typhimurium LT2) by PCR. Primers (Genosys, The Woodlands, Tex.) were based on available serovar Typhimurium genomic sequence fragments (http://genome.wustl.edu/bacterial.salmonella/shtml). The upstream and downstream primers for *prpA* were 5′-GCTTACTAAGCAAATGCATCTGCACACGC-3′ and 5′-CCTGCGCAGCAGGATCCCGATTTTTAACCC-3′, respectively. The upstream and downstream primers for *prpB* were 5′-GGCCTACTTTTTATGCATAATACCAGACTC-3′ and 5′-GAAGGCTATATCGGATCCCATAATGCAGGG-3′, respectively. Restriction enzyme digestion of PCR products gave clones for each gene that began with an *Nsi*I site 30 to 40 bp 5′ of the presumed start codon and ended with a *Bam*HI site 30 to 40 bp 3′ of the presumed stop codon. These were ligated into the *Nsi*I and *Bam*HI sites of pDGK101 to give pDGK235 (containing *prpA*) and pDGK236 (containing *prpB*). Each plasmid contained 2,916 bp of pBluescriptII SK(+) from the *Bam*HI site through the *Sal*I site in the polylinker (Stratagene, La Jolla, Calif.) followed by 505 bp of the serovar Typhimurium *corA* promoter on a *Sal*I-*Nsi*I fragment, followed by the *prpA* or *prpB* insert.

Inserts in pDGK235 and pDGK236 were sequenced using the above oligonucleotides as primers at the Howard Hughes Medical Institute Biopolymer/Keck Foundation Biotechnology Resource Laboratory, Yale University. Both sequences agree with the serovar Typhimurium LT2 sequence available at the above website. *prpA* and *prpB* map to approximately 40.2 and 62.8 min on the serovar Typhimurium chromosome, comparable to *E. coli prpA* and *prpB* at 41 and 61.5 min, respectively (3).

Expression of ST-PrpA and ST-PrpB. The open reading frames for *prpA* and *prpB* were amplified by PCR with *Pfu* DNA polymerase (Stratagene). The for-

* Corresponding author. Mailing address: Department of Pharmacology, School of Medicine, Case Western Reserve University, 10900 Euclid Ave., Cleveland, OH 44106-4965. Phone: (216) 368-6187. Fax: (216) 368-3395. E-mail: lxs76@po.cwru.edu.

ward primers were 5′-TGGGGATCCATGAACGACAGGAAAAACATGAT
G-3′ for *prpA* and 5′-TGGGAATCCATGGAATTAATTCGTTATGCTFAC-3′
for *prpB*, while the reverse primers were 5′-TGGAAGCTTGCTATTGTATCC
GCGCTAACG-3′ for *prpA* and 5′-TGGAAGCTTTTACTTTATTTTAAAAA
AAGACAGATTG-3′ for *prpB*. To facilitate cloning, a *Bam*HI restriction was
introduced at the 5′ end of the forward primers and a *Hin*dIII restriction site was
introduced at the 5′ end of the reverse primers. The resulting PCR products were
ligated into the pRSET-A expression vector (Invitrogen, Carlsbad, Calif.) and
transformed into *E. coli* DH5α cells (Gibco BRL, Rockville, Md.). After plasmid
purification, both strands of the insert were sequenced to verify the fidelity of the
PCR amplification. Competent *E. coli* BL21(DE3) cells (Promega, Madison,
Wis.) were transformed with plasmid, grown to an optical density at 600 nm of
0.6 to 1, induced with isopropyl-β-D-thiogalactopyranoside (IPTG) at a final
concentration of 0.1 mM for 4 h at 37°C, and harvested by centrifugation. The
pellet was resuspended in 40 ml of buffer A (20 mM Tris [pH 7], 500 mM NaCl,
1 mM phenylmethylsulfonyl fluoride [PMSF], 1 mM imidazole-HCl) with 2 mg of
lysozyme per ml, 100 μg of DNase I per ml, and 100 μg of RNase per ml and
incubated on ice for 30 min. The cells were lysed by being passed through a
French press three times at 8,000 lb/in²; lysates were centrifuged at $17,000 \times g$ at
4°C for 30 min. The supernatant was loaded on a 3-by 5-cm column of Ni-
nitrilotriacetic acid agarose (Qiagen, Valencia, Calif.) charged with NiCl$_2$ and
equilibrated with buffer A. The column was washed with 100 ml of buffer A and
then with 100 ml of buffer B (20 mM Tris [pH 7], 500 mM NaCl, 1 mM PMSF,
40 mM imidazole-HCl). The fusion proteins were then eluted from the column
with 150 ml of buffer C (20 mM Tris [pH 7], 500 mM NaCl, 1 mM PMSF, 80 mM
imidazole-HCl), 10-ml fractions were collected, and 10 μl of each fraction re-
moved and assayed for phosphatase activities, and peak fractions were pooled.
Samples containing ST-PrpA were dialyzed against 3 liters of buffer D (10 mM
Tris-HCl [pH 7], 1 mM dithiothreitol, 1 mM PMSF, 1 mM EDTA) plus 10 mM
NaCl overnight, and loaded on a 3- by 5-cm column of Macro-Prep High S
Support (Bio-Rad, Hercules, Calif.) that had been equilibrated with buffer D
plus 10 mM NaCl. The column was washed with 100 ml of buffer D plus 50 mM
NaCl and eluted with a salt gradient of 50 to 400 mM NaCl in buffer D. Fractions
(5 ml) were collected and assayed for phosphatase activity. Active fractions were
pooled, dialyzed with 1 liter of buffer E (50 mM Tris-HCl [pH 7], 1 mM
dithiothreitol, 1 mM PMSF, 1 mM EDTA, 10% [vol/vol] glycerol) overnight, and
stored at −20°C.

ST-PrpB samples were concentrated with a Centriprep-10 column and loaded
on a 2- by 65-cm column of Superose 6 (Amersham Pharmacia Biotech, Pisca-
taway, N.J.) equilibrated with buffer F (10 mM ammonium formate [pH 6], 1 mM
PMSF, 1 mM EDTA). The column was eluted with 180 ml of buffer F; 1-ml
fractions were collected and assayed for phosphatase activity. Peak fractions
were pooled, concentrated with a Centriprep-10 column, resuspended with buffer
E, and stored at −20°C. All purification procedures were carried out at 4°C
except gel filtration chromatography, which was performed at room temperature.
Expression and purification of ST-PrpA and ST-PrpB were monitored by West-
ern hybridization with anti-Xpress antibody (Invitrogen, Carlsbad, Calif.).

Preparation of phosphorylated MBP. Serine/threonine- or tyrosine-phospho-
rylated myelin basic protein (MBP) was prepared with [γ-³³P]ATP as specified by
the manufacturer (New England BioLabs, Beverly, Mass.). Peptide substrates
were purchased from Promega.

Phosphatase assay. Purified phosphatase protein (200 ng) was incubated at
37°C in 30 μl of solution containing 50 mM imidazole (pH 6.5 for ST-PrpA and
pH 8 for ST-PrpB), 1 mM MnCl$_2$, 10 μg of bovine serum albumin per ml, BSA,
and 2 μM [³³P]phospho-MyBP. The initial rate of the reaction was linear for at
least 30 min for all substrates. The reaction was terminated by the addition of 100
μl of 20% (wt/vol) trichloroacetic acid, mixed, and centrifuged for 3 min in a
microcentrifuge at maximum speed. ³³P radioactivity was determined using 50 μl
of supernatant liquid. A modified molybdic acid extraction method (7) was used
to verify the reaction product as inorganic phosphate. The peptide substrates
were assayed as specified by the manufacturer (Promega).

pNPP phosphatase activities were assayed at 37°C in 200 μl of solution con-
taining 50 mM imidazole (pH 6.5 for ST-PrpA and pH 8 for ST-PrpB) 1 mM
MnCl$_2$, and 1 mM pNPP. ST-PrpA or ST-PrpB was added to start the reaction.
After 30 min, the reaction was stopped with 800 μl of 0.5 M sodium borate (pH
9). Released *p*-nitrophenol was measured at 410 nm on a Beckman DU-64
spectrophotometer. Most phosphatase assays were performed on proteins car-
rying the His$_6$ tag and linking sequence. However, removal of the fusion domains
of ST-PrpA and ST-PrpB with enterokinase did not change their affinity for
Mn²⁺ or other properties tested. For pH optimum experiments, controls indi-
cated that the composition of the buffer had no significant influence on phos-
phatase activity. Final concentrations of inhibitors used were 10 mM EDTA, 10
mM sodium pyrophosphate, 50 mM NaF, 10 mM sodium potassium tartrate, 1

mM tetramisole, 1 mM sodium orthovanadate, 150 μM trifluoperazine, 10 μM
microcystin-LR, 5 μM okadaic acid, and 1 mM AppppA (diadenosine tetraphos-
phate).

Nucleotide sequence accession numbers. The *prpA* and *prpB* sequences have
been deposited with GenBank under accession no. AY149950 and AY049951,
respectively.

RESULTS

Cloning of *prpA* and *prpB*. Genes for two PPP family protein
phosphatases identified in extant serovar Typhimurium
genomic sequence by a BLAST (1) search were cloned by PCR
amplification from serovar Typhimurium genomic DNA. De-
rived amino acid sequences were each 61% identical to their
respective homologs in *E. coli* (Fig. 1); therefore, the serovar
Typhimurium genes were designated *prpA* and *prpB*. The pre-
dicted protein product of *prpA*, ST-PrpA, consisted of 222
amino acids with a predicted molecular mass of 25,879 Da and
an isoelectric point of 8.8. ST-PrpB was predicted to have 218
amino acids, a molecular mass of 25,024 Da, and a pI of 4.6.
The serovar Typhimurium proteins were each 61% identical to
their respective *E. coli* homologs and 41% identical to each
other (Fig. 1).

Expression and purification ST-PrpA and ST-PrpB. To fa-
cilitate purification by metal-chelate affinity chromatography,
an amino-terminal His$_6$ sequence from the pRSET-A vector
was introduced into the recombinant ST-PrpA and ST-PrpB
carried in pRSRT-A and expressed in *E. coli* after induction
with IPTG. After metal-chelate affinity chromatography, ST-
PrpB was concentrated and further purified by gel filtration
chromatography. ST-PrpA, however, became aggregated when
concentrated. Each was further purified by cation-exchange
chromatography. Both ST-PrpA and ST-PrpB were purified to
near electrophoretic homogeneity (Fig. 2), and their identities
were confirmed by Western blot analysis with anti-Xpress an-
tibody (data not shown). The yield from 1 liter of cells was 1 mg
of ST-PrpA or 3 mg of ST-PrpB.

Properties of ST-PrpA and ST-PrpB. Both ST-PrpA and
ST-PrpB showed divalent metal ion-dependent protein phos-
phatase activity toward [³³P]phosphoseryl MBP (Fig. 3). Al-
though a few other cations supported some phosphatase
activity, Mn²⁺ clearly produced the greatest stimulation.
Surprisingly, ST-PrpA and ST-PrpB displayed different affini-
ties for Mn²⁺ (Fig. 3A). The Mn²⁺ K_a of ST-PrpB was 1.3 μM,
over 50 times lower than the Mn²⁺ K_a of ST-PrpA at 65 μM
(Table 1). Ni²⁺, Co²⁺, and Fe²⁺ could support the phospha-
tase activity of ST-PrpB (Fig. 3C) but with K_a values in the mM
range (Fig. 3A; Table 1). Ni²⁺ was also a weak activator of
PrpA activity. Neither Mg²⁺ nor Ca²⁺ had stimulatory effects
on either phosphatase, while Cd²⁺, Cu²⁺, and Zn²⁺ inhibited
both phosphatases when added concomitantly with Mn²⁺.
Fe²⁺ and Co²⁺ slightly inhibited Mn²⁺-stimulated ST-PrpA
protein phosphatase activity (Fig. 3B).

Both ST-PrpA and ST-PrpB were active toward pNPP over
a pH spectrum from 5 to 10. However, optimal ST-PrpA ac-
tivity occurred at pH 6 to 7, while the optimal pH for ST-PrpB
activity was pH 7.5 to 8.5 (Fig. 4A). Each was active over a
temperature range from 0 to 65°C. Maximal activity was seen
between 45 and 55°C for ST-PrpA but 30 to 37°C for ST-PrpB.
ST-PrpA but not ST-PrpB was heat stable, with PrpA main-
taining substantial activity at 65°C (Fig. 4B). ST-PrpA and

```
ST-PrpA     MNDRKNMMRPEEIYQRIEAKNWRHVWVVGDIHGCFSMLMKRLRECRFDPQQDLLVSVGDL
ST-PrpB     M--------ELIRYADINSDLYRHIWVVGDIHGCYSLLLTRLAQLNFSPDTDLLISTGDN
EC-PrpA     M------KQPAPVYQRIAGHQWRHIWLSGDIHGCLEQLRRKLWHCRFDPWRDLLISVGDV
EC-PrpB     M--------PSTRYQKINTHHYRHIWVVGDIHGEYQLLQSRLHQLSFFPETDLLISVGDN
            *             *   *   .  :**:*: ***** . *   :* .  * *  ***:*.**


ST-PrpA     IDRGPDSLGCLALLRESWMTAVRGNHEQMALDARASSQSTLWLMNGGDWFTRLTAEHAAQ
ST-PrpB     IDRGKENLETLRLLNTPWFISVVGNHEAMALDAFETQDGNFWYVNGGYWYDSVTEKDRQE
EC-PrpA     IDRGPQSLRCLQLLEQHWVCAVRGNHEQMAMDALASQQMSLWLMNGGDWFIALADNQQKQ
EC-PrpB     IDRGPESLNVLRLLNQPWFISVKGNHEAMALDAFATGDGNMWLASGGDWFFDLNDSEQKE
            ****  :.*   *  **.  *.  :* **** **:** :  : .:*  .** *:  :   .. :


ST-PrpA     AEALFILCQRLPWILEVRCRHSTHVIAHADYPASTYQWQKKVDLHQVLWSRERLINK-RG
ST-PrpB     ATELLLTFKQRPHIIEVETSSKKYVIAHADYPDDSYDYGKQVDIDSVLWSRDRLLGSLQG
EC-PrpA     AKTALEKCQHLPFILEVHSRTGKHVIAHADYPDDVYEWQKDVDLHQVLWSRSRLGERQKG
EC-PrpB     ATDLLLKFHHLPHIIEITNDNIKYVIAHADYPGSECLFGKEIAESELLWPVDRVQKSLNG
            *    :    :: *  *:*:           .:******** .  : *..:  .:**. .*:     .*


ST-PrpA     ---GISGRDHFWFGHTPLRRRMDFANVHYIDTGAVFGGQLTLARIQ
ST-PrpB     NIHPIRGADTFIFGHMIVDYTTTFANQIYIDTGSFCSGNLSFFKIK
EC-PrpA     Q--GITGADHFWFGHTPLRHRVDIGNLHYIDTGAVFGGELTLVQLQ
EC-PrpB     ELQQINGADYFIFGHMMFDNIQTFANQIYIDTGSPKSGRLSFYKIK
               * * * ***  .       :.*  *****:  .*.*:: :::
```

FIG. 1. Sequence alignment of serovar Typhimurium and *E. coli* PPP phosphatases. The predicted amino acid sequences of ST-PrpA, ST-PrpB, and the *E. coli* phosphatases were aligned using CLUSTAL W and modified slightly by eye to optimize alignment at the termini. Dashes indicate sequence gaps introduced to optimize the alignment of conserved regions; asterisks indicate identical residues in all four phosphatases; colons indicate either three of four residues identical or all four residues conserved.

ST-PrpB demonstrated a similar specific activity toward [^{33}P]-phosphoseryl MBP, but the activity of ST-PrpA toward [^{33}P]-phosphotyrosyl MBP was less than 10% that of ST-PrpB. In contrast, activity toward synthetic phosphopeptide substrates, whether phosphotyrosyl or phosphothreonyl, was much greater with ST-PrpA than with ST-PrpB (Table 2).

The effect of protein phosphatase and other phosphomonoesterase inhibitors (11, 14, 16) on the activity of ST-PrpA and ST-PrpB was also examined (data not shown). At the concentrations tested, pyrophosphate, the chelating agent EDTA, the nonspecific phosphatase inhibitor NaF, and the tyrosine phosphatase inhibitor vanadate inhibited >90% of activity from either enzyme with [^{33}P]-phosphoseryl MBP as substrate. Diadenosine tetraphosphate (AppppA) inhibited the activity of both enzymes by 20 to 30%, but the acid phosphatase inhibitor tartrate, the alkaline phosphatase inhibitor tetramisole, the protein phosphatase 2B inhibitor trifluoperazine, and the protein phosphatase 1 and 2A inhibitors microcystin-LR and okadaic acid did not inhibit ST-PrpA and ST-PrpB activity.

DISCUSSION

Little is known about the physiological functions of Mn^{2+}. Mn^{2+} is often a functional substitute for Mg^{2+}. Intuitively, one might thus expect to find a fairly large number of Mn^{2+}-dependent enzymes. However, only a handful of Mn^{2+}-dependent metalloenzymes are known. Because of our interest in Mn^{2+} transport and its possible role in pathogenesis (6), we are characterizing several of these Mn^{2+}-dependent enzymes, including the two Mn^{2+}-dependent protein phosphatases of serovar Typhimurium described in this report.

prpA and *prpB* of serovar Typhimurium are clear homologs of the corresponding genes in *E. coli* (13), but their sequences diverge much more than many homologs between such closely related organisms, at only 61% identity between species for both PrpA and PrpB. In contrast, the percent identity between serovar Typhimurium and *E. coli* for other Mn^{2+}-dependent enzymes is 91 to 97% for SodA (Mn^{2+}-superoxide dismutase), GpmM (phosphoglyceromutase), SpoT (AppppA synthase/hy-
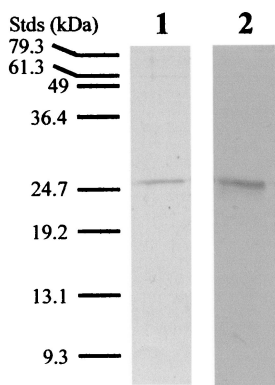
FIG. 2. Analysis of purified ST-PrpA and ST-PrpB by SDS-PAGE. ST-PrpA and ST-PrpB were expressed in *E. coli* and purified by metal-chelate affinity chromatography and ion-exchange or gel filtration chromatography as described in Materials and Methods. Portions of 1 μg of ST-PrpA and 2 μg of ST-PrpB were analyzed by SDS-PAGE on a 15% (wt/vol) acrylamide gel. Lanes: 1, ST-PrpA; 2, ST-PrpB. The positions of protein standard (Stds) are indicated at the left.
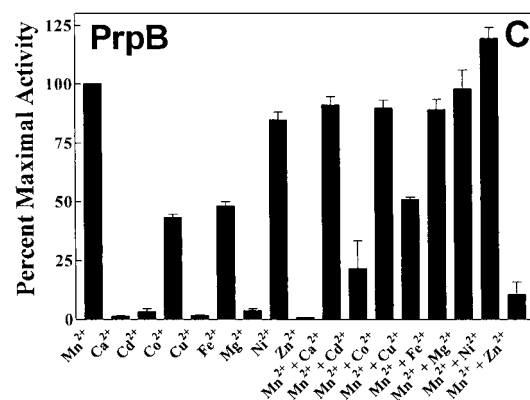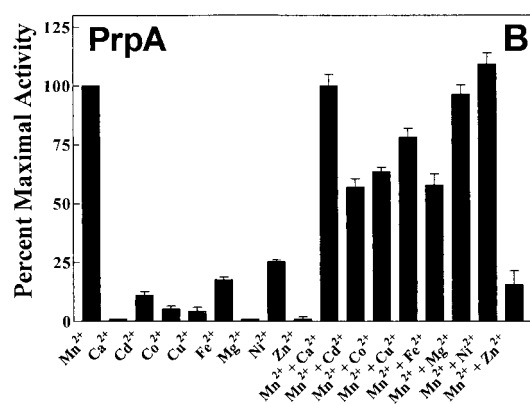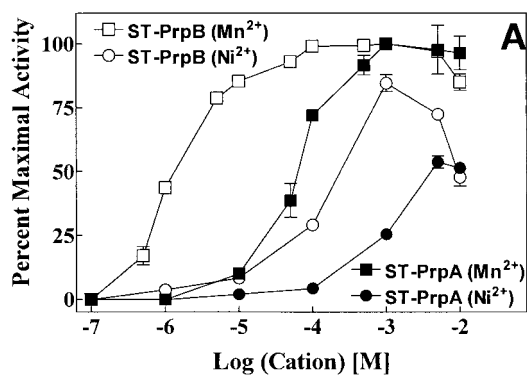
TABLE 1. K_a of ST-PrpA or ST-PrpB for cations

Phosphatase	K_a (mM) for:			
	Mn^{2+}	Ni^{2+}	Fe^{2+}	Co^{2+}
ST-PrpA	0.065	1	ND^a	ND
ST-PrpB	0.0013	0.14	1.3	5.2

^{a} ND, not determined.

drolase), and PepP (Aminopeptidase P). This could be indicative of a different origin for the phosphatases compared to other Mn^{2+}-dependent enzymes.

The K_a of ST-PrpA for Mn^{2+} (65 μM) is within a factor of 3 of the K_a for Mn^{2+} for the homologous phosphatases PP1-

FIG. 3. Effects of divalent metal ions on the catalytic activity of ST-PrpA and ST-PrpB. (A) The activity of 200 ng each of purified ST-PrpA and ST-PrpB was assayed under standard conditions (see Materials and Methods) using [^{33}P]phosphoseryl MBP as substrate with the indicated concentrations of Mn^{2+} or Ni^{2+}. Results are given as the percent activity observed with 1 mM of Mn^{2+} and the standard error of the mean ($n = 4$). For points with no error bar, the error was smaller than the size of the symbol. For ST-PrpA, 100% activity was equal to 0.0302 pmol of ^{33}P$_i$ released per min, while the corresponding value for ST-PrpB was 0.0304 pmol/min. (B and C) The activity of purified ST-PrpA (B) and ST-PrpB (C) was assayed under the conditions described for panel A, except that, where indicated, the compounds listed were substituted for the activating divalent metal ion, Mn^{2+}. All compounds were present at a final concentration of 1 mM. Iron was maintained in the ferrous state by the addition of ascorbic acid to a final concentration of 1 mM. All results are reported as the percentage of activity relative to that observed with Mn^{2+} and standard error of the mean ($n = 4$).
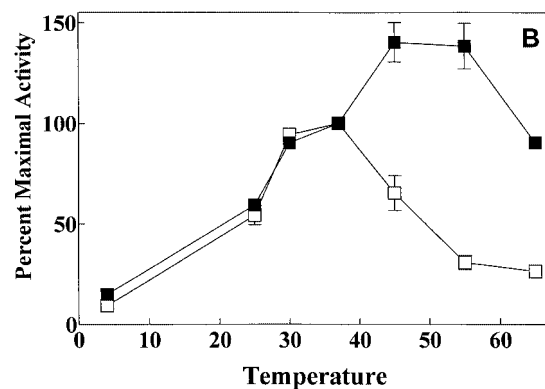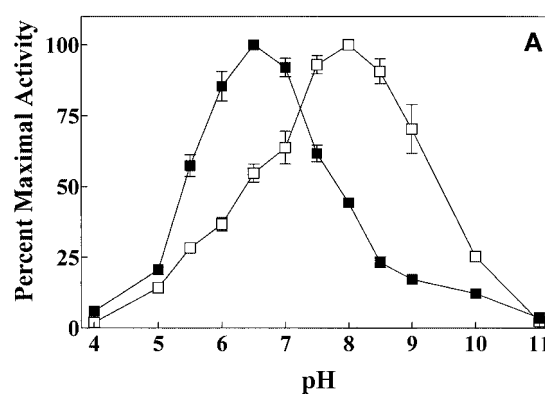

FIG. 4. pH and temperature dependence of ST-PrpA and ST-PrpB. (A) The activity of 2 μg of purified ST-PrpA and ST-PrpB was assayed for phosphatase activity against pNPP under standard conditions (see Materials and Methods), except that the pH was varied as indicated using different buffers. Shown is the relative protein phosphatase activity detected as a function of pH and the standard error of the mean ($n = 3$). 100% activity was defined as 0.84 nmol of p-nitrophenol released per min for ST-PrpA at pH 6.5 and 0.92 nmol/min for ST-PrpB at pH 8. Buffer salts (50 mM) were sodium acetate (pH 4 to 5), imidazole (pH 5 to 8), Tris-HCl (pH 8 to 9), and glycine (pH 9 to 11). (B) The activity of purified ST-PrpA and ST-PrpB was assayed for pNPP phosphatase activity under standard conditions (see Materials and Methods) while varying the temperature. All results are reported as the percent activity relative to that observed at 37°C and the standard error of the mean ($n = 3$). For points with no error bar, the error was smaller than the size of the symbol.

TABLE 2. Protein phosphatase activity of ST-PrpA and ST-PrpB

Substrate	Enzyme activity (pmol min^{-1}·mg^{-1} [$n = 3$])	
	ST-PrpA	ST-PrpB
Phosphoseryl/threonyl MBP	151 ± 6	152 ± 6
Phosphotyrosyl MBP	60 ± 4	733 ± 37
RRA(pT)VA	$18,200 \pm 870$	700 ± 170
END(pY)INADSL	$39,800 \pm 8,000$	$2,240 \pm 120$
DADE(pY)LIPQQG	$8,660 \pm 880$	$1,960 \pm 140$

arch1 from the archaeon *Sulfolobus solfataricus* and PP1-cyano1 and PP1-cyano2 from *Microcystis* sp. (7, 12, 16). In contrast, the Mn^{2+} K_a of 1.3 μM for ST-PrpB is much lower than that for other prokaryotic PPP phosphatases previously characterized. Although Ni^{2+}, Co^{2+}, and Fe^{2+} can support some phosphatase activity, their relative K_a values compared to that of Mn^{2+} indicate that Mn^{2+} is the physiologically relevant cation.

PrpA and PrpB of *E. coli* have also been purified and partially characterized (13). Unfortunately, divalent cation selectivity cannot be compared since this was not determined for the *E. coli* enzymes. The effects of several phosphatase inhibitors on ST-PrpA and ST-PrpB were generally similar not only to each other but also to the effects of the same inhibitors on the homologous phosphatases PP1-cyano1 from *Microcystis aeruginosa* PCC 7820 and PP1-cyano2 from *M. aeruginosa* UTEX 2063 (16). In contrast, although fluoride ion could completely block phosphatase activity in the serovar Typhimurium and *M. aeruginosa* enzymes, it had little effect on the two *E. coli* phosphatases. Vanadate inhibited both phosphatases from serovar Typhimurium but only PrpA from *E. coli* (13). Finally, substrate specificity may differ between serovar Typhimurium and *E. coli*. In both species, PrpA and PrpB have similar activity toward phosphoseryl MBP, but the activities of the *E. coli* phosphatases toward phosphotyrosyl MBP were equivalent whereas ST-PrpB had 10-fold greater activity than ST-PrpA toward phosphotyrosyl MBP. In contrast, ST-PrpA had high activity against phosphotyrosyl peptide substrates. Therefore, until physiological substrates are determined, substrate specificity cannot be determined. Our preliminary data (L. Shi and M. E. Maguire, unpublished results) indicate that mutation of *prpA* or *prpB* markedly alters the peroxide and temperature sensitivity. Proteins whose expression is altered with a change in temperature or exposure to reactive oxygen species might therefore be candidates for substrates for these phosphatases.

Not all bacterial genomes have PPP-class phosphatases. For those that have PPP phosphatases, almost all have only a single gene (17). Serovar Typhimurium and *E. coli* are the only bacteria so far studied to have two PPP phosphatase genes. The *E. coli* phosphatases are also the only bacterial PPP phosphatases for which a function can be assigned. They regulate the transcription of *htrA* via the CpxR-CpxA two-component signal transduction pathway that is responsive to protein misfolding in the periplasm (13). *htrA* encodes a protease that degrades

misfolded proteins, thus avoiding potential cell toxicity. The similarities between the PrpA phosphatase in *E. coli* and serovar Typhimurium suggest that they probably have a similar function in serovar Typhimurium. In contrast, the rather different properties of the PrpB enzymes from the two species suggest the possibility of overlapping but not identical physiological roles.

REFERENCES

1. **Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman.** 1990. Basic local alignment search tool. J. Mol. Biol. **215:**403–410.
2. **Barford, D.** 1996. Molecular mechanisms of the protein serine/threonine phosphatases. Trends Biochem. Sci. **21:**407–412.
3. **Blattner, F. R., G. Plunkett, C. A. Bloch, N. T. Perna, V. Burland, M. Riley, J. Collado-Vides, J. D. Glasner, C. K. Rode, G. F. Mayhew, J. Gregor, N. W. Davis, H. A. Kirkpatrick, M. A. Goeden, D. J. Rose, B. Mau, and Y. Shao.** 1997. The complete genome sequence of *Escherichia coli* K-12. Science **277:**1453–1474.
4. **Fairbanks, G., T. L. Steck, and D. F. Wallach.** 1971. Electrophoretic analysis of the major polypeptides of the human erythrocyte membrane. Biochemistry **10:**2606–2617.
5. **Jabado, N., A. Jankowski, S. Dougaparsad, V. Picard, S. Grinstein, and P. Gros.** 2000. Natural resistance to intracellular infections. Natural resistance-associated macrophage protein 1 (NRAMP1) functions as a pH-dependent manganese transporter at the phagosomal membrane. J. Exp. Med. **192:**1237–1248.
6. **Kehres, D. G., M. L. Zaharik, B. B. Finlay, and M. E. Maguire.** 2000. The NRAMP proteins of *Salmonella typhimurium* and *Escherichia coli* are selective manganese transporters involved in the response to reactive oxygen. Mol. Microbiol. **36:**1085–1100.
7. **Kennelly, P. J., K. A. Oxenrider, J. Leng, J. S. Cantwell, and N. Zhao.** 1993. Identification of a serine/threonine-specific protein phosphatase from the archaebacterium *Sulfolobus solfataricus*. J. Biol. Chem. **268:**6505–6510.
8. **Kennelly, P. J., and M. Potts.** 1999. Life among the primitives: protein O-phosphatases in prokaryotes. Front Biosci. **4:**D372–D385.
9. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227:**680–685.
10. **Leng, J., A. J. Cameron, S. Buckel, and P. J. Kennelly.** 1995. Isolation and cloning of a protein-serine/threonine phosphatase from an archaeon. J. Bacteriol. **177:**6510–6517.
11. **MacKintosh, C., and R. W. MacKintosh.** 1994. Inhibitors of protein kinases and phosphatases. Trends Biochem. Sci. **19:**444–448.
12. **Mai, B., G. Frey, R. V. Swanson, E. J. Mathur, and K. O. Stetter.** 1998. Molecular cloning and functional expression of a protein- serine/threonine phosphatase from the hyperthermophilic archaeon *Pyrodictium abyssi* TAG11. J. Bacteriol. **180:**4030–4035.
13. **Missiakas, D., and S. Raina.** 1997. Signal transduction pathways in response to protein misfolding in the extracytoplasmic compartments of *E. coli*: role of two new phosphoprotein phosphatases PrpA and PrpB. EMBO J. **16:**1670–1685.
14. **Oxenrider, K. A., M. E. Rasche, M. V. Thorsteinsson, and P. J. Kennelly.** 1993. Inhibition of an archaeal protein phosphatase activity by okadaic acid, microcystin-LR, or calyculin A. FEBS Lett. **331:**291–295.
15. **Rusnak, F., L. Yu, S. Todorovic, and P. Mertz.** 1999. Interaction of bacteriophage lambda protein phosphatase with Mn(II): evidence for the formation of a [Mn(II)]2 cluster. Biochemistry **38:**6943–6952.
16. **Shi, L., W. W. Carmichael, and P. J. Kennelly.** 1999. Cyanobacterial PPP family protein phosphatases possess multifunctional capabilities and are resistant to microcystin-LR. J. Biol. Chem. **274:**10039–10046.
17. **Shi, L., M. Potts, and P. J. Kennelly.** 1998. The serine, threonine, and/or tyrosine-specific protein kinases and protein phosphatases of prokaryotic organisms: a family portrait. FEMS Microbiol. Rev. **22:**229–253.
18. **Solow, B., J. C. Young, and P. J. Kennelly.** 1997. Gene cloning and expression and characterization of a toxin-sensitive protein phosphatase from the methanogenic archaeon *Methanosarcina thermophila* TM-1. J. Bacteriol. **179:**5072–5075.
19. **Voegtli, W. C., D. J. White, N. J. Reiter, F. Rusnak, and A. C. Rosenzweig.** 2000. Structure of the bacteriophage lambda Ser/Thr protein phosphatase with sulfate ion bound in two coordination modes. Biochemistry **39:**15365–15374.
20. **Zhuo, S., J. C. Clemens, D. J. Hakes, D. Barford, and J. E. Dixon.** 1993. Expression, purification, crystallization, and biochemical characterization of a recombinant protein phosphatase. J. Biol. Chem. **268:**17754–17761.