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Characterization of a Bifunctional HPr Kinase/Phosphorylase from *Leuconostoc mesenteroides* SY1

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The *hprK* gene encoding bifunctional HPrK/P (kinase/phosphorylase) was cloned from *L. mesenteroides* SY1, a strain isolated from *kimchi*. *hprK* was transcribed as a monocistronic gene. His-tagged HPrH16A and HPrK/P were produced in *E. coli* BL21(DE3) using pET26b(+) and purified. HPrK/P phosphorylation assay with purified proteins showed that the kinase activity of HPrK/P increased at slightly acidic pHs. Divalent cations such as Mg^{2+} and Mn^{2+} and glycolytic intermediates such as fructose-1, 6-bisphosphate (FBP) and phosphoenolpyruvate (PEP) increased the kinase activity of HPrK/P, but inorganic phosphate strongly inhibited it. Kinetic studies for the kinase activity of HPrK/P showed that the apparent K_m values were 0.18 and 14.57 μM for ATP and HPr, respectively. The K_m value for the phosphorylase activity of HPrK/P was 14.16 μM for P-Ser-HPr (HPr phosphorylated at the serine residue).

Keywords: HPr kinase/phosphorylase, *hprK*, *Leuconostoc mesenteroides*, catabolite repression

Bacteria prefer a substrate that yields a maximum profit for their growth among various carbon sources [2]. Expression of catabolic enzyme genes is elaborately regulated depending on the physiological states of the cell and the availability of carbon sources. The highly elaborate regulatory mechanisms are collectively known as carbon catabolite repression (CCR) [2]. In low-GC Gram-positive bacteria, HPr is the most important regulatory protein in the CCR mechanism [24]. HPr contains two phosphorylation sites; a histidine residue at position 15 and a serine residue at position 46 [4, 21, 24]. Histidine residue at position 15 is phosphorylated (P-His-HPr) by enzyme I at the consumption of phosphoenolpyruvate (PEP), and the phosphate group is sequentially transferred to enzymes IIa and IIb, components of the PEP-dependent phosphotransferase system (PTS), and eventually to sugars entering into cells *via* IIc [2]. The serine residue at position 46 is phosphorylated by HPrK/P, and phosphorylated HPr at serine residue (P-Ser-HPr) forms a complex with CcpA [24]. The CcpA/P-Ser-HPr complex then binds to a so-called *cre* (catabolite responsive element) in the neighborhood of the promoter region of gene(s) under CCR, preventing transcription of the gene(s) [12, 21]. The ratio of P-Ser-HPr and P-His-HPr is important for the regulation of genes involved in sugar utilization. In this respect, HPrK/P is very important since it modulates the level of P-Ser-HPr inside the cell. Therefore, research on factors affecting HPrK/P activity is important to understand CCR mechanisms. *Leuconostoc mesenteroides* is one of the most important organisms during the early and middle stages of *kimchi* fermentation [10] when sugars from cabbage and other ingredients are utilized by LAB (lactic acid bacteria) and converted into acids and alcohols. Understanding CCR and the functions of genes involved in CCR in *Leuconostoc* species might be useful if high-quality *kimchi* is to be produced by precisely controlling sugar utilization patterns of *L. mesenteroides*. Previously, we characterized the *ccpA* gene [19] and *ptsHI* genes [18] from *L. mesenteroides* SY1, an isolate from *kimchi* [14]. In this communication, we characterized the *hprK* gene from the same strain and confirmed its regulatory roles by showing the ability of HPrK/P to phosphorylate HPr and dephosphorylate P-Ser-HPr. As far as we know, this is the first report on the HPrK/P from heterofermentive LAB.

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MATERIALS AND METHODS

Bacterial Strains and Growth Conditions

L. mesenteroides SY1 was grown in MRS broth or on MRS plates (1.5% agar, w/v) at 30°C. *E. coli* strains were grown in Luria-Bertani

(LB) broth at 37°C with vigorous agitation. 5-Bromo-4-chloro-3-indolyl-β-D-galactoside (X-gal) was included at a concentration of 40 μg/ml and IPTG (isopropyl-β-D-thiogalactopyranoside) was added to the final concentration of 0.5 mM. Antibiotics were used at the following concentrations: ampicillin (Ap), 100 μg/ml; kanamycin (Km), 30 μg/ml for *E. coli*.

Cloning and Sequence Analysis

Chromosomal DNA was isolated from *L. mesenteroides* SY1 as described previously [13]. PCR reaction was performed with *L. mesenteroides* SY1 genomic DNA as a template and using the GeneAmp PCR system 2400 (PE Applied Biosystems, Foster City, U.S.A.). A primer pair of [Leu]HPrK-BamH1F (5'-TGCCGGA-TCCTGCTGCTGGAATAATAGCTTTACG-3') and [Leu]HPrK-BamH1R (5'-CAATGGATCCAAATCTGCAAGAGCATAAACAC-AGTTA-3') were designed based on the *hprK* of *L. mesenteroides* ATCC 8293 in the database (GenBank Accession No. NZ_AABH02000061). The amplified fragment was digested with BamHI and ligated with pBlueScriptII KS(+) (Stratagene, CA, U.S.A.). DNA sequences were determined by the dideoxy-chain termination method using an ABI-PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems). The homology of the deduced amino acid sequence was analyzed using the Blast program at NCBI (http://www.ncbi.nlm.nih.gov/blast/). The sequence alignment was performed with the ClustalW program [11] using a PAM250 matrix.

Northern Blot Analysis

For total RNA extraction, cells were collected by centrifugation at room temperature (RT). The cell pellet was resuspended in 1 ml of TRIzol Reagent (Invitrogen, Carlbad, CA, U.S.A.) and transferred into 2-ml screw-cap tubes (BioSpec Products, Bartlesville, OK, U.S.A.) containing 400 mg of 0.1-mm zirconia-silica beads (BioSpec Products). The mixture was homogenized with a Mini-Beadbeater-8 (BioSpec Products) for 1 min, chilled on ice for 1 min, and centrifuged at 4°C for 5 min. Supernatant was transferred into a new tube and incubated for 5 min at RT. Chloroform (0.3 ml) was added, vortexed for 10 s, and then incubated for 5 min at RT. The sample was centrifuged at 12,000 ×*g* for 5 min at 4°C and then the upper phase was transferred into a new tube. Cold ethanol (0.5 ml) was added and stored at −20°C for 1 h and then centrifuged at 12,000 ×*g* for 15 min. The DNA pellet was washed with cold 75% ethanol and then resuspended with DEPC (diethyl pyrocarbonate)-treated H_2O after brief drying.

RNA (20 μg) was separated on 1.2% agarose-formaldehyde gel, transferred to a Hybond-XL nylon membrane (Amersham Bioscience, Uppsala, Sweden), and hybridized at 65°C with a ^{32}P-labeled *hprK*-specific probe (483 bp) as described previously [16]. The probe was PCR amplified by using a primer set of hprK483-F (5'-GATATT-TCTCGCCCTGGATT-3') and hprK483-R (5'-CTCCAATCAGCCT-CTCTTCA-3').

Primer Extension

An oligonucleotide primer, *hprk*-extension1 (5'-GGGCGAGAAAT-ATCTGCAGTAGTAATTTCTCGG-3'), was 5' end labeled with [γ-^{32}P]dATP using T4 DNA polynucleotide kinase (Promega, Madison, U.S.A.). Then, oligonucleotide was added to 50 μg of total RNA and the annealed primer was extended with AMV reverse transcriptase (Promega) according to the method provided by the manufacturer. The extended product was analyzed on a sequencing gel (6% acrylamide gel) adjacent to DNA sequencing ladders generated from the extension of the *hprk*-extension1 primer with pHPRK25 as the template.

Overproduction and Purification of HPrK/P and HPrH16A

The *hprK* gene was cloned into pET26b(+) for overexpression in *E. coli*. The *hprK* gene was amplified using a primer pair of *hprK*-expF2[NdeI] (5'-GGAATTCCATATGGCACAAAATTCAGTAACA-GTAAAACAATT-3' and *hprK*-expR[XhoI] (5'-GGGGCTCGAGG-TCATTTTTTTCGTCCCCTGCAT-3'). The amplified fragment was digested with NdeI and XhoI, and ligated with pET26b(+). *E. coli* BL21(DE3) (Novagen, Madison, U.S.A.) cells harboring the recombinant plasmid were grown overnight at 37°C and inoculated into fresh medium (1%, v/v). When the A_{600} of the culture reached 0.8, IPTG was added to the final concentration of 1 mM, and the culture was further incubated for 3 h at 30°C. Cells were recovered by centrifugation at 5,000 ×*g* for 20 min, and washed twice with 20 mM Tris-HCl (pH 7.4) and resuspended in 2 ml of the same buffer. Cells were disrupted by sonication for 1 min followed by cooling on ice for 1 min, and this cycle of sonication-cooling was repeated 4 times. Disrupted cells were centrifuged (12,000 ×*g*, 10 min) and the supernatant was obtained and used as protein extract. His-tagged proteins were purified by affinity column chromatography using a HiTrap Chelating HP column (Amersham Biosciences) according to the protocol provided by the manufacturer. HPrH16A, a mutant HPr where the histidine residue at the 16th amino acid was replaced by alanine, was prepared as described previously [18].

HPr Kinase Assay

HPrH16A was used as a substrate for phosphorylation reaction catalyzed by HPrK/P. By using HPrH16A, PEP-dependent enzyme I-mediated phosphorylation at the histidine residue was prevented [4]. The assay mixture for *in vitro* phosphorylation contained 50 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 10 mM fructose-1,6-bisphosphate (FBP), 10 μCi (3,000 Ci/mmol) [γ-^{32}P]ATP, 20 μM HPrH16A, and 1 μM HPrK/P in a final volume of 10 μl. The reaction mixture was incubated for 5 min at 30°C and the reaction was terminated by the addition of Laemmli loading buffer and heating for 5 min at 80°C. SDS-PAGE was done using an 18% gel. Gel was stained with Coomassie blue and dried on a Model 583 gel dryer (Bio-Rad, Hercules, U.S.A.). The dried gel was overlaid with Hyperfilm MP (Amersham Biosciences) for autoradiography. The amount of [^{32}P] incorporated into HPr was measured by liquid scintillation counting of excised gel pieces (LS3000; Beskman Instruments, Palo Alto, CA, U.S.A.). To examine the effect of pH, phosphorylation experiments were done at different pHs. Sodium citrate buffer (pH 4.5–6.0), MOPS buffer (pH 6.5–7.0), and Tris-HCl buffer (pH 7.5–8.5) were used at 50 mM concentration. To examine the influence of divalent cations, cations were added in the reaction mixture at different concentrations.

Purification of P-Ser-HPrH16A

One hundred μM HPrH16A was incubated with 8 μM HPrK/P in the presence of 50 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 10 mM FBP, and 4 mM ATP in a total 200 ml reaction mixture for 2 h at 30°C. After inactivation of the enzyme (70°C, 3 min), the degree of phosphorylation was examined by nondenaturing PAGE using an

18% acrylamide gel. P-Ser-HPrH16A was purified using a HiTrap Chelating HP column (Amersham Biosciences).

Phosphorylase Activity of HPrK/P

Phosphorylase activity of HPrK/P was measured in a mixture (10 ml) containing 50 mM Tris-HCl (pH 7.0), 5 mM sodium phosphate buffer (pH 7.5), 10 mM $MgCl_2$, 1 mM HPrK/P, and variable amounts of P-Ser-HPrH16A (3–30 mM). After incubation for 1 h at 30°C, samples were kept for 5 min at 70°C to stop the reaction. Dephosphorylation of P-Ser-HPrH16A was detected by nondenaturing PAGE. P-Ser-HPrH16A and HPrH16A bands intensities were quantified using a GS-710 densitometer (Bio-Rad).

Nucleotide Sequence Accession Number

The DNA sequence reported here was deposited in the GenBank database under the accession number AY615197.

Results and Discussion

Cloning and Sequence Analysis of *hprK*

A 2,550-bp fragment was amplified using the primer set mentioned in the Materials and Methods. The amplified fragment was cloned into pBlueScriptII KS(+), resulting in a recombinant plasmid, pHPRK25, and the sequence was determined. The nucleotide sequence of *hprK* from *L. mesenteroides* SY1 was very similar to that of *hprK* from *L. mesenteroides* ATCC 8293 (99% identity, nucleotide sequence level) and three ORFs were identified (see Fig. 1A). *orf1* was 372 bp in size and capable of encoding a putative membrane protease of 123 amino acids with a calculated molecular mass of 13,558.41 Da. The pI value was calculated to be 6.51. The second ORF encoding NAD(P)H-dependent glycerol-3-phosphate dehydrogenase (GpsA) was 1,044 bp in size and could encode a protein of 347 amino acid with a calculated molecular mass of 37,548.78 Da. The pI value was estimated to be 6.25. The ORF encoding HPrK/P was 969 bp in size and thus could encode a protein of 322 amino acids with a calculated molecular mass of 35,255.93 Da. The pI value was calculated to be 4.66. *hprK* was spaced by only 1 bp with *orf1* and overlapped with *gpsA* by 38 bp.

Homology of the *hprK* gene from *L. mesenteroides* SY1 was observed with other known *hprK* genes at the amino acid sequence level. The identity scores of the amino acid sequence were 60%, 52%, and 51% for HPrK/P from *Oenococcus oeni* (ZP_00318973.1), *Lb. plantarum* [15], and *Lb. brevis* [5], respectively. The amino acid sequence of HPrK/P of *L. mesenteroides* SY1 was aligned with those from *Oenococcus oeni*, *B. subtilis*, *Lb. casei* [25], and *Staphylococcus xylosus* [17] (Fig. 2). The first conserved motif (155-162th amino acid in SY1 enzyme), Walker A motif (GXXGXGKS/T), corresponds to the structural motif called P-loop, a nucleotide binding site present in many ATPase or GTPase activity-exhibiting proteins such as nucleotide binding proteins, ABC transporters, proteases, chaperons, *etc.* [26]. Specifically, the Walker A motif and surrounding sequence of HPrK/P were closely related to phosphoenolpyruvate carboxykinase [8]. The second

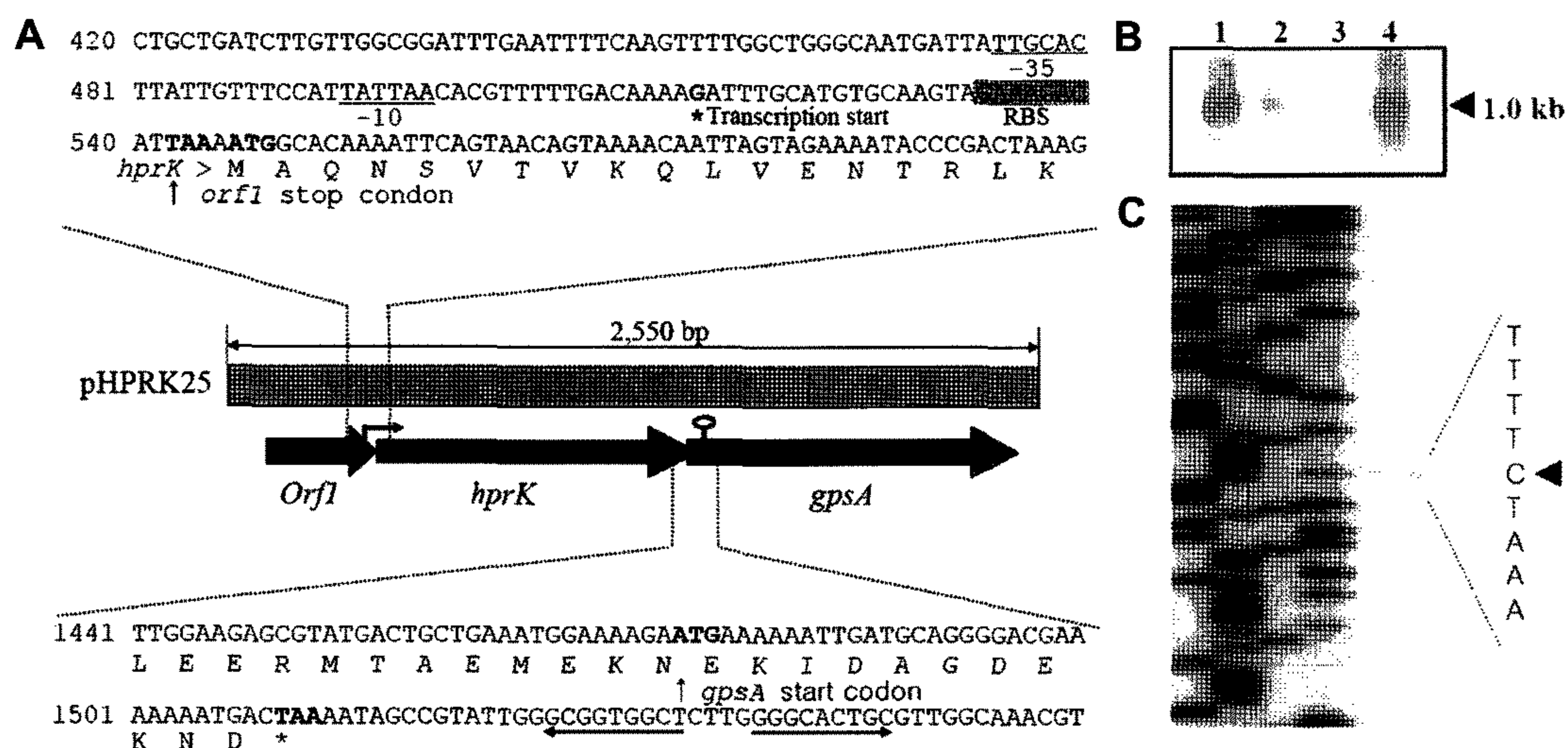

Fig. 1. Genetic organization and transcriptional analysis of the *hprK* gene.

A. Genetic map of *hprK* and surrounding genes. The size and orientation of ORFs were deduced from the nucleotide sequence. Sequences corresponding to the upstream regulatory region of *hprK* and the downstream region are shown. Transcriptional start site (TSS) is in bold font and marked by an asterisk. Putative RBS is gray boxed and promoter sequences (-10 and -35 region) are underlined. Start and stop codons are in bold font. Putative transcription terminator is indicated with an arrow. **B.** Northern bolt analysis of *L. mesenteroides* SY1 transcripts. Fifteen microgram of RNA was separated on a 1.2% agarose formaldehyde gel, transferred onto a nylon membrane, and hybridized with a ^{32}P-labeled 483-bp *hprK* probe. Total RNA was prepared from cells grown on MRS medium with 1% glucose (1), fructose (2), sucrose (3), or raffinose (4). **C.** Primer extension result. ^{32}P-labeled oligonucleotide complementary to the *hprK* transcript was extended using avian myeloblastosis virus (AMV) reverse transcriptase. Fifty microgram of total RNA from cells grown on MRS medium with 1% raffinose was used for the hybridization.

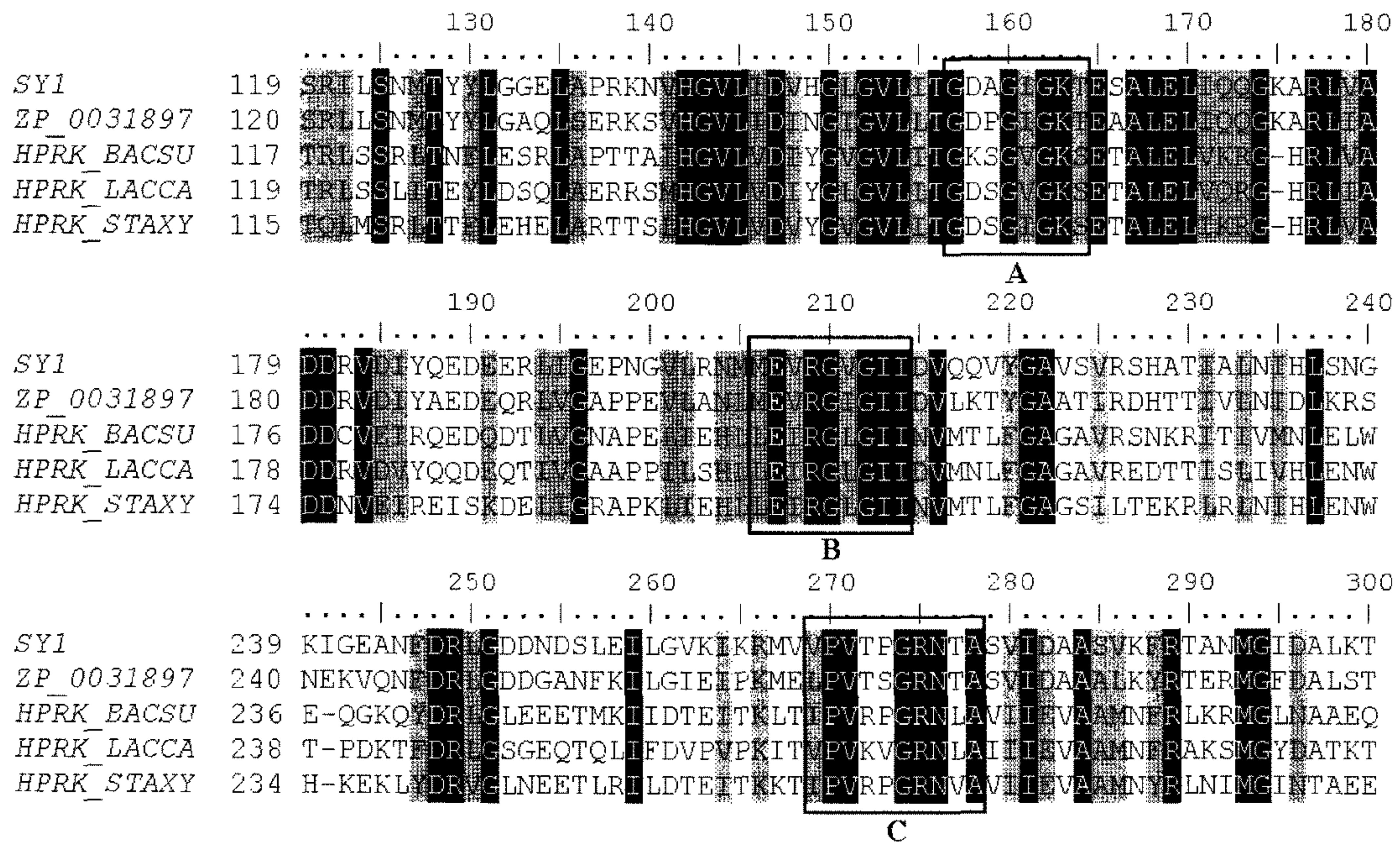

Fig. 2. Multiple sequence alignment of a portion of HPrK/P from various Gram-positive bacteria.
Three conserved motifs are marked by a box. **A.** Walker A motif. **B.** Capping motif. **C.** K3-loop. Amino acid sequence of *L. mesenteroides* SY1 HPrK/P (SY1) was compared with those of *O. oeni* (ZP_0031897), *B. subtilis* (HPRK_BACSU), *Lb. casei* (HPRK_LACCA), and *S. xylosus* (HPRK_STAXY).

conserved motif (204-212th amino acid in SY1 enzyme) is called the HPrK/P signature sequence (I/L/MEI/VRGI/L/M/VGI/VI/L/M). This motif plays an important role in phosphorylase activity, which is also called the capping motif. The third conserved motif (267-276th amino acid in SY1 enzyme) is known as the central K3 loop (V/I/LPVK/R/QXGRNL/M/VA/S), which interferes with the binding of a nucleotide [7]. The K3 loop also makes contacts between the subunits within the HPrK/P trimers. It could be assumed that the HPrK/P of *L. mesenteroides* SY1 is functionally identical to HPrK/Ps of other Gram-positive bacteria, judging from the presence of these three conserved motifs.

Transcriptional Analysis

As mentioned above, *orf1*, *hprK*, and *gpsA* were located closely and partially overlapped. The size of the *hprK* transcript was analyzed by Northern blot using a radioactively labeled 483-bp *hprK* internal fragment as a probe. The autoradiogram (Fig. 1B) showed that only a single 1.0 kb transcript hybridized with the probe. The result indicated that *hprK* was not part of an operon but a monocistronic gene. The concentration of *hprK* transcripts was apparently constant in *L. mesenteroides* SY1 cells grown on different carbon sources (1% each of glucose, fructose, and raffinose, w/v) although slightly less in cells grown on sucrose. A primer extension analysis was performed to determine the transcription start site (TSS) of *hprK*. Fig. 1C showed that transcription started at G (nt 516 in Fig. 1) located 31 nt upstream of the start codon. The distance from TSS to the putative rho-independent transcription terminator was 1,010 bp, which was in good agreement with the Northern blot result (Fig. 1B).

Kinase Activity of HPrK/P

Previously, we reported that HPr from *L. mesenteroides* SY1 was phosphorylated at the 47th serine residue by HPrK/P [18]. Using [γ-^{32}P]ATP as the phosphoryl donor, phosphorylation of HPrH16A by HPrK/P was performed under various conditions. To prevent phosphorylation of the histidine residue at the 16th amino acid, HPrH16A was used as the substrate. The degree of phosphorylation at 37°C was higher (1.661±0.047 nM/min P-Ser-HPrH16A) than that at 30°C (0.738±0.041 nM/min P-Ser-HPrH16A) and the kinase activity was higher at slightly acidic pH (Fig. 3B). Different behavior has been reported for Gram-positive bacterial HPrK/Ps. For example, HPrK/P of *Streptococcus mutants* [23] and *S. pyogenes* [22] had optimum activity at pH 7.0. *S. salivarious* HPrK/P showed the maximum activity at pH 7.5 [23]. HPrK/P of *Treponema denticola* had the maximum phosphorylating activity at pH 8.5 [9]. It is suspected that the cytoplasm of *L. mesenteroides* might be in a slightly acidic pH range when grown on easily metabolized carbon sources such as glucose. When these carbon sources are metabolized, the energy level inside the cell increases and it triggers CCR. The kinase activity of HPrK/P is believed to be maximum under the same conditions because P-Ser-HPr is required

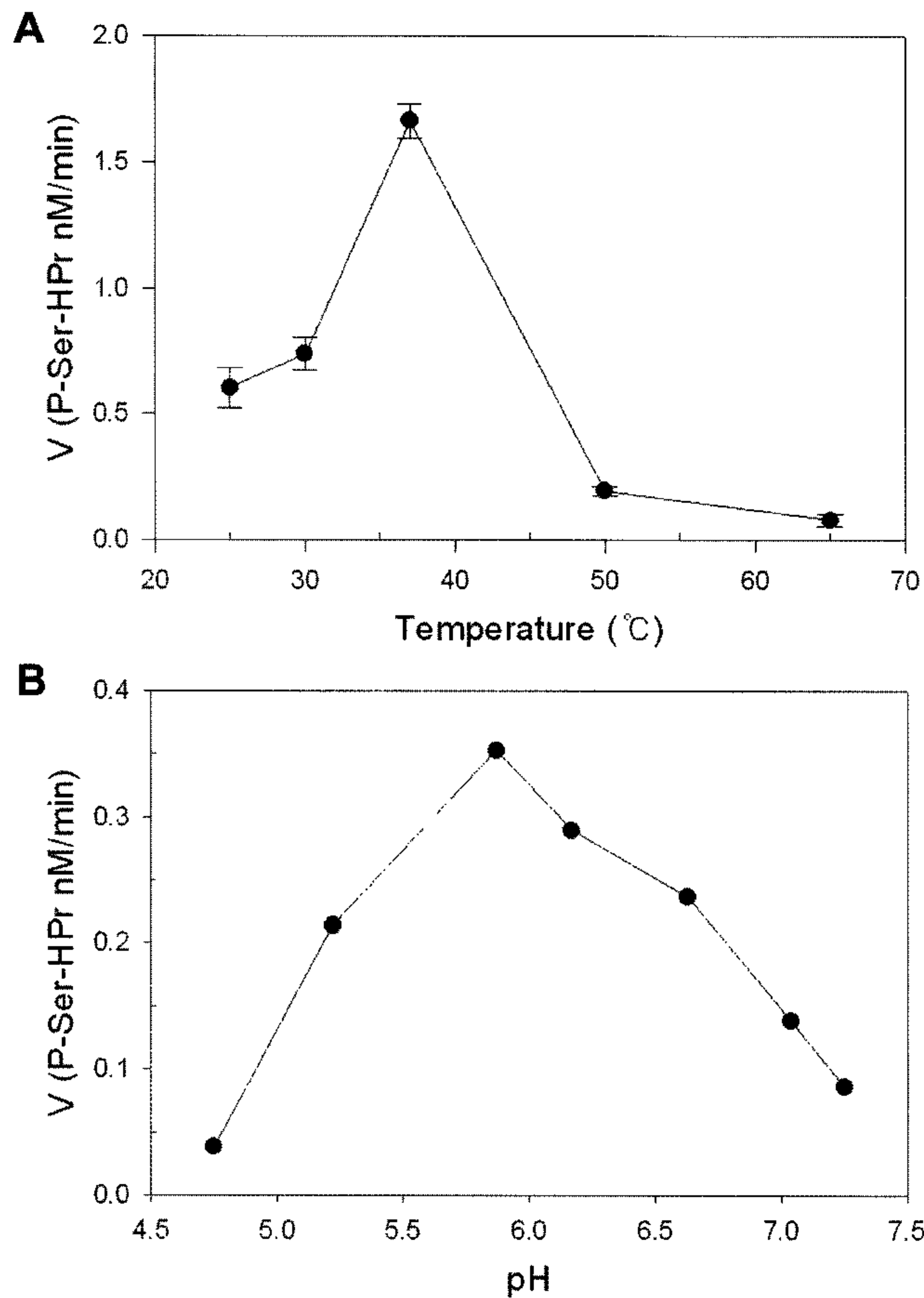

Fig. 3. Properties of purified HPrK/P of *L. mesenteroides* SY1.
A. Effect of temperature on the HPr phosphorylation. **B.** Effect of pH on the HPr phosphorylation. Different pH values were obtained by combining buffers, with a 50 mM final concentration. Sodium citrate buffer (pH 4.5–6.0) and MOPS buffer (pH 6.5–7.0) were used.

to form the CcpA/P-Ser-HPr complex, which in turn binds to *cre* of genes under CCR.

The effect of divalent cations on the kinase activity was investigated (Fig. 4A). Kinase activity was increased by Mg^{2+} as well as Mn^{2+}, and their optimal concentrations were very low (>0.5 mM). Zn^{2+} and Ca^{2+} did not increase kinase activity.

In low G+C Gram-positive bacteria, HPrK/P activity is allosterically activated by FBP [6, 20]. *L. mesenteroides*, a heterofermentative LAB, produces lactate, acetate, ethanol, and CO_2 as major products *via* the pentose phosphoketolase pathway (PKP). Thus, FBP was suspected not to act as an allosteric activator because *L. mesenteroides* SY1 did not produce FBP as a metabolic intermediate. We suspected the existence of another intermediate as an activator. Phosphorylation of HPrH16A by HPrK/P was done at various concentrations of FBP, fructose-6-phosphate (F6P), acetyl phosphate, and PEP (Fig. 4B). Contrary to our expectations, the kinase activity of HPrK/P was activated by FBP *in vitro*. This result supported that the HPrK/P of *L. mesenteroides* SY1 was functionally similar to HPrK/Ps

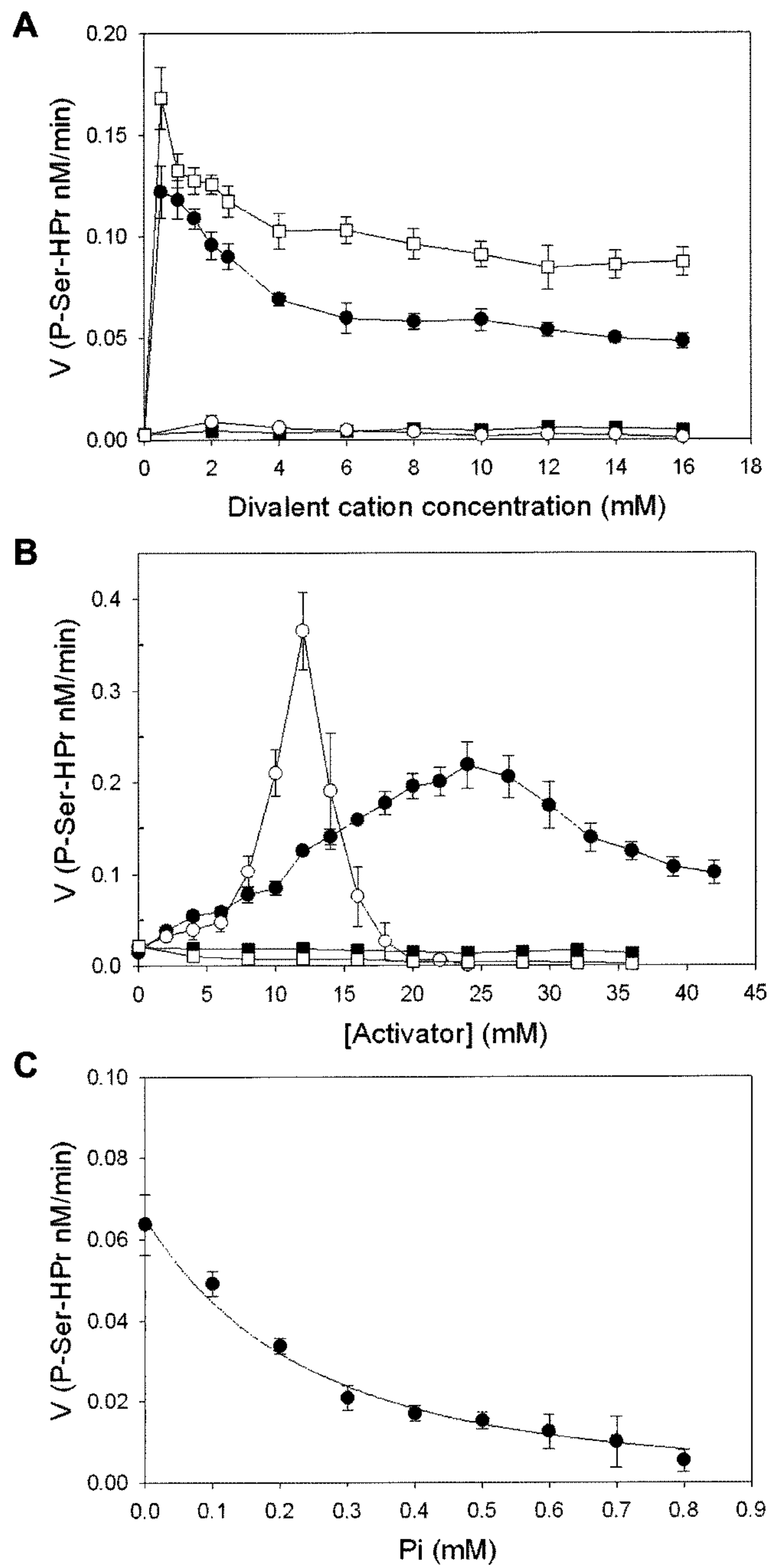

Fig. 4. Effect of different ligands on the kinase activity of HPrK/P.
A. Effect of divalent cations. Cations in the chloride form were added at different concentrations. -●-, Mg^{2+}; -○-, Zn^{2+}; -■-, Ca^{2+}; -□- , Mn^{2+}. **B.** Effect of metabolites. -●-, FBP; -○-, PEP; -■-, fructose-6-phosphate; -□-, acetyl phosphate. **C.** Effect of inorganic phosphate. The reaction mixture contained varying concentrations of potassium phosphate buffer (pH 7.5).

of other Gram-positive bacteria because of the existence of conserved motifs as mentioned above. FBP exhibited the maximal activation at the concentration of 24 mM. At higher concentrations, the activating effect decreased slightly. The optimal concentration (24 mM) is approximately

6-fold higher than that for *B. subtilis* enzyme [20]. Significant activation of HPrH16A phosphorylation was observed when PEP was added in the reaction mixture. PEP exhibited the maximal activation at concentration of 12 mM, but the activating effect rapidly decreased at concentrations higher than 12 mM. F6P and acetyl phosphate did not increase the kinase activity of HPrK/P. One of the candidates of allosteric activator is xylulose-5-phosphate (X5P). Because of the X5P located junction of PKP, it might be an important intermediate modulating the kinase activity of HPrK/P. Unfortunately, we could not test X5P on the kinase activity of HPrK/P because X5P was not commercially available.

Inorganic phosphate (Pi) strongly inhibited the kinase activity of HPrK/P. Pi as low as 2 mM concentration almost totally prevented phosphorylation of HPrH16A by HPrK/P (data not shown). The effect of Pi at 0.1 to 0.8 mM concentrations was investigated (Fig. 4C). The kinase activity decreased by 23% at only 0.1 mM concentration of Pi. When 0.8 mM Pi was added, the enzyme activity decreased by 92%.

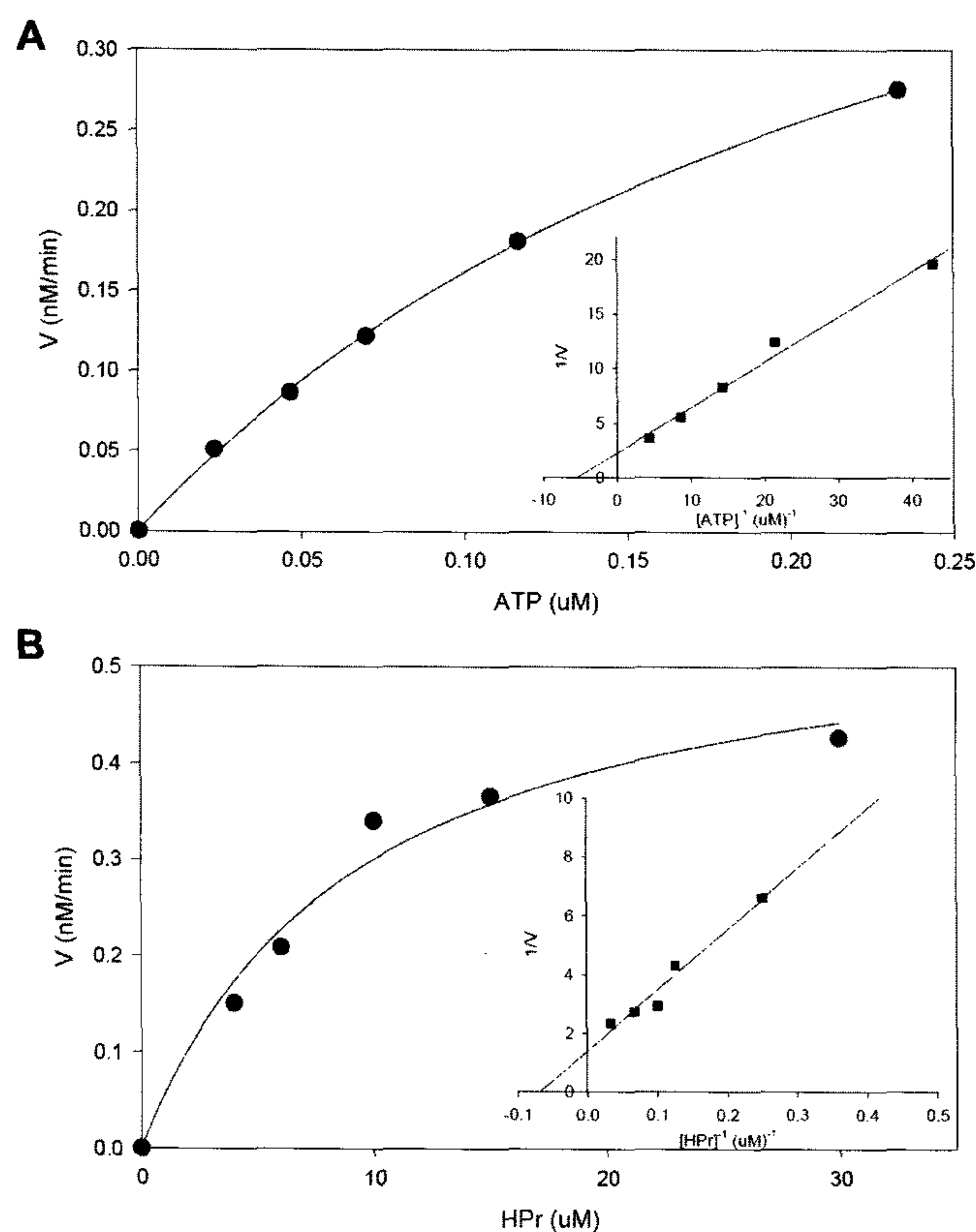

Fig. 5. Kinetic analysis of phosphorylation of HPrH16A by HPrK/P.

A. Effect of ATP concentration on the phosphorylation of HPrH16A with an excess of purified HPrH16A (20 μM). **B**. Effect of HPr concentration on the phosphorylation of HPr with an excess of [γ-^{32}P]ATP (10 μCi [3,000 Ci/mmol]). The apparent K_m for ATP and HPrH16A was determined to be 0.18 and 14.57 μM, respectively. Insets, Lineweaver-Burk regression plots.

The kinetics of HPrH16A phosphorylation as a function of both [γ-^{32}P]ATP and HPrH16A concentrations were studied and the results are shown in Fig. 5. In these two experiments, the concentrations of HPrH16A and [γ-^{32}P]ATP were maintained constant at 20 μM and 10 μCi (3,000 Ci/mmol), respectively. Other parameters were those for the ideal conditions for kinase activity of HPrK/P; 50 mM sodium acetate buffer at pH 5.9, 0.5 mM $MgCl_2$, and 30 mM FBP. The reaction followed Michaelis-Menten (hyperbolic) kinetics for both substrates. The apparent K_m values were 0.18 and 14.57 μM for ATP and for HPrH16A, respectively. The K_m values of *Streptococcus salivarious* were 1 and 31 μM for ATP and for HPr, respectively [1]. Thus, the *L. mesenteroides* SY1 enzyme showed 5.5- and 2.1-fold higher affinity for ATP and for HPr, respectively, than *Streptococcus salivarious* enzyme.

HPrK/P Dephosphorylates P-Ser-HPrH16A

HPrK/P dephosphorylates P-Ser-HPr in the presence of inorganic phosphate [3]. Prior to kinetic study on dephosphorylation by HPrK/P, P-Ser-HPrH16A was purified from HPrK/P after phosphorylation of HPrH16A, as mentioned in the Materials and Methods section. P-Ser-HPrH16A was easily purified from HPrK/P by HiTrap

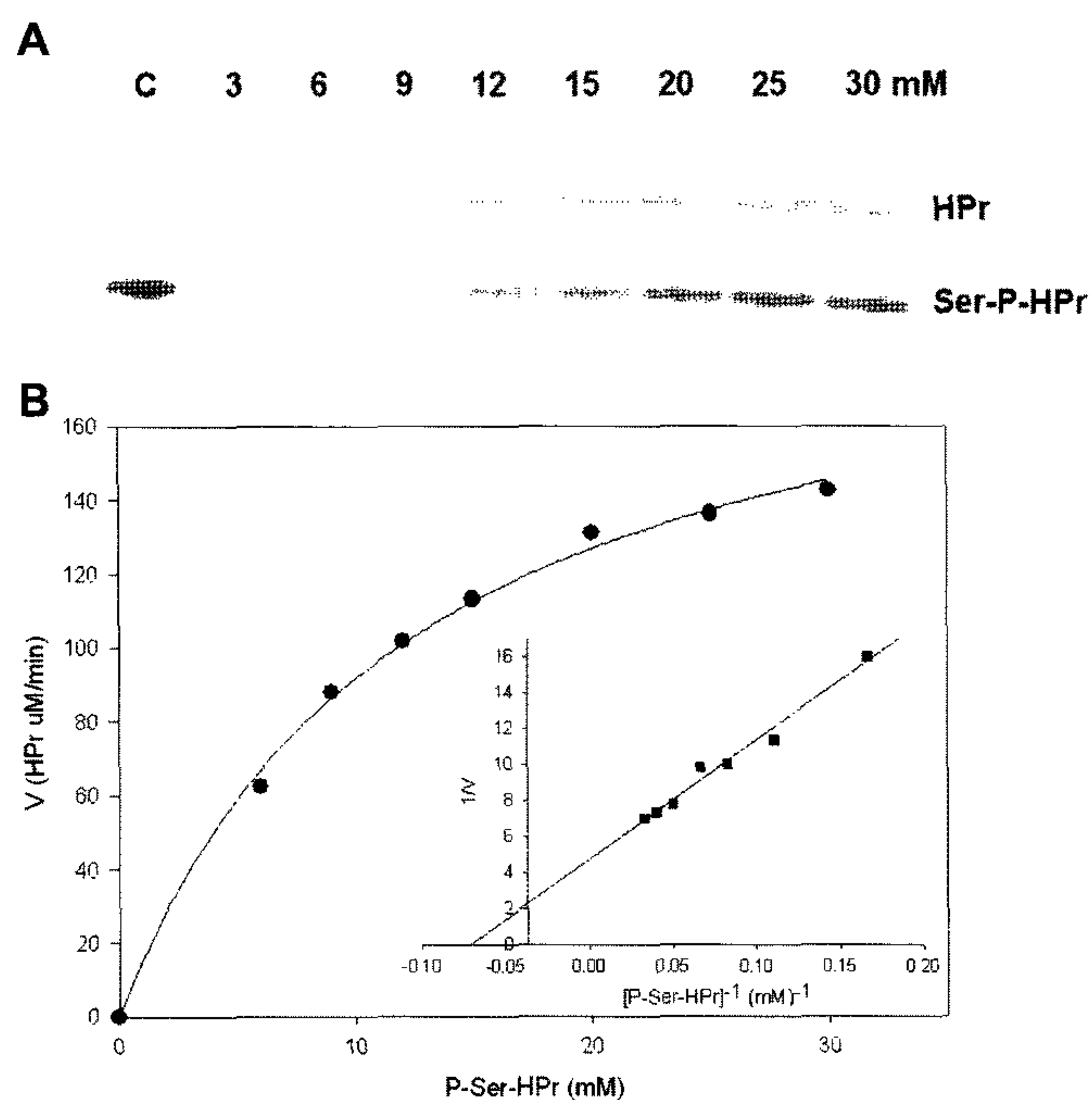

Fig. 6. Kinetic analysis of dephosphorylation of P-Ser-HPrH16A by HPrK/P.

A. PAGE using a nondenaturing 18% gel was employed to check the degree of dephosphorylation of P-Ser-HPrH16A by HPrK/P. Lane C was a control where 30 mM P-Ser-HPrH16A was applied. **B**. Effect of P-Ser-HPrH16A concentration on the dephosphorylation of P-Ser-HPrH16A by HPrK/P with an excess of phosphate (5 mM). The apparent K_m was determined to be 1.46 μM. Inset, Lineweaver-Burk regression plot.

Chelating HP column because P-Ser-HPr16A and HPrK/P were eluted at different imidazol concentrations (HPrK/P: 300 mM; HPrH16A: 100 mM). The kinetics of P-Ser-HPrH16A dephosphorylation as a function of P-Ser-HPrH16A concentration were examined. As shown in Fig. 6, the reaction followed Michaelis-Menten (hyperbolic) kinetics and the apparent K_m and V_{max} values were 1.46 μM and 211.56 μM/min for P-Ser-HPr16A, respectively.

Acknowledgments

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