J. Biochem. 125, 690-695 (1999)

Enzymatical Properties of Psychrophilic Phosphatase I[1]

Hiroki Tsuruta and Yasuo Aizono[2]

Laboratory of Biological Chemistry, Department of Biofunctional Chemistry, Faculty of Agriculture, Kobe University, Nada-ku, Kobe, Hyogo 657-8501

Received October 7, 1998; accepted December 21, 1998

Phosphatase I purified from a psychrophile (*Shewanella* sp.) [Tsuruta *et al.* (1998) *J. Biochem.* 123, 219-225] dephosphorylated *O*-phospho-L-tyrosine and phospho-tyrosyl residues in phosphorylated poly(Glu_4,Tyr_1) random polymer (polyEY) and phosphorylated myelin basic protein (MBP) but not phosphoseryl and/or phosphothreonyl residues in phosphorylated histone H1, casein and phosphorylase *a*, indicating that the enzyme showed protein-tyrosine-phosphatase (PTPase, EC 3.1.3.48)-like activity *in vitro*. The enzyme was remarkably inhibited by diethylpyrocarbonate (DEPC), monoiodoacetic acid (MIAA), and monoiodoacetamide (MIAM). Binding of 1 mol of DEPC to 1 mol of the enzyme caused complete inhibition of the enzyme; and 0.88 mol of 1-carboxymethylated histidine per mole of the enzyme was found when 90% of enzyme activity was lost by modification with ^{14}C-MIAA. These results indicated that this psychrophilic enzyme was a PTPase-like enzyme with histidine as its catalytic residue.

Key words: catalytic residue, cold enzyme, protein-tyrosine-phosphatase-like enzyme.

Recent studies on protein chemistry have focused on the relationship between structure and function and the formation of high-order structure. With regard to the former, much information has been accumulated about proteins functioning at intermediate to high temperature, but little is known about proteins functioning efficiently in the low temperature range of 0 to 10°C because of their thermal lability.

Enzyme proteins which function at low temperature, so-called "cold enzymes," are produced by psychrophilic microorganisms and ectothermic organisms living in low temperature environments. To date, several cold enzymes have been purified and characterized, including cold subtilisin S41 [EC 3.4.21.14] of *Bacillus* TA41, cold triose phosphate isomerase [EC 5.3.1.1] of *Moraxella* TA137, cold lipase [EC 3.1.1.3] of *Psychrobacter immobolis* B10 (*1-3*). On the basis of the information obtained, cold enzymes are defined by three general characteristics: (i) high catalytic activity at low temperature, (ii) lower optimal temperature than those of their counterparts in other organisms, and (iii) inactivation at an intermediate temperature (*4*). It was suggested that cold enzymes have a flexible structure, which allows higher activity with lower activation energy at low temperature and also causes the thermal lability (*4*). However, no general information on the structural properties eliciting the unique enzymatic characteristics like high catalytic activity at low temperature has yet been obtained.

To investigate the relationship between structure and function of cold enzymes, we previously isolated phosphatase I and characterized it as a cold enzyme (*5*). Since maximal activity of phosphatase I was observed at pH 6.0, the enzyme belonged to neither the acid nor the alkaline phosphatases. However, the detailed substrate specificity and the catalytic residue of the enzyme are still obscure. Elucidation of these remaining properties are essential steps for investigation of relationship between structure and function of this cold enzyme, *e.g.* the distinctive mechanisms of the catalytic reaction and of the expression of the substrate specificity with the lower activation energy.

In this paper, we describe the substrate specificity and the catalytic residue of phosphatase I, and discuss the finding that the enzyme possesses a protein-tyrosine-phosphatase activity *in vitro*.

MATERIALS AND METHODS

Materials—*O*-Phospho-L-tyrosine, *O*-phospho-L-serine, *O*-phospho-DL-threonine, and other phosphorylated compounds, PCMB, PCMPS, NEM, DEPC, MIAM, and the catalytic subunit of protein kinase A (166 units/mg) were purchased from Nacalai Tesque. PolyEY with an average molecular mass of 37 kDa, dephosphorylated casein, MBP, phosphorylase *b* and phosphorylase kinase (240 units/mg) were from Sigma Chemical; bovine histone H1 with the molecular mass of 21 kDa was from GIBCO BRL, Life Technologies. [γ-^{32}P]ATP (4,500 Ci/mmol) and [1-^{14}C]-MIAA (10 μCi/mmol) were from ICN Biomedicals and

[1] This work was supported in part by Grant-in-Aid for Exploratory Research (No. 09876027) from the Ministry of Education, Science, Sports and Culture of Japan.

[2] To whom correspondence should be addressed. Tel/Fax: +81-78-803-1290, E-mail: aizono@kobe-u.ac.jp

Abbreviations: DEPC, diethylpyrocarbonate; MBP, myelin basic protein; MES, 2-(*N*-morpholino)ethanesulfonic acid; MIAA, monoiodoacetic acid; MIAM, monoiodoacetamide; NEM, *N*-ethylmaleimide; PCMB, *p*-chloromercuribenzoate; PCMPS, *p*-chloromercuriphenylsulfate; pNPP, *p*-nitrophenylphosphate; pNP, *p*-nitrophenol; polyEY, poly(Glu_4,Tyr_1) random polymer; PTK, protein-tyrosine-kinase; PTPase, protein-tyrosine-phosphatase.

© 1999 by The Japanese Biochemical Society.

Downloaded from http://jb.oxfordjournals.org/ at Changhua Christian Hospital on October 1, 2012

American Radiolabeled Chemicals, respectively. Silica gel plates (20×20 cm, 250 μm thick) were from Whatman. DEAE-Cellulofine A-500m was from Seikagaku Kogyo. Other chemicals used were of analytical grade. Rats (male Wistar ST, SPF, 7 weeks old) were from Nihon SLC. Carboxymethylated histidine derivatives were prepared by the reaction of histidine with a 5-fold excess of MIAA in 1 ml of 0.1 M ammonium bicarbonate, pH 8.5 for 48 h at 25°C with stirring (*6*).

Purification of Phosphatase I and PTK—Phosphatase I was purified to homogeneity from a psychrophile (*Shewanella* sp.) as described (*5*). The enzyme had a specific activity of 58 units. The molecular weight of the purified phosphatase I was determined to be 38,366 by MALDI-TOF mass spectrometry. The molecular extinction coefficient at 280 nm of this enzyme was 7.75×10^4 $cm^{-1}\cdot mol^{-1}\cdot liter^{-1}$.

PTK was highly purified from the membrane fraction of rat spleen by the reported procedure up to anion exchange chromatography on a DEAE-Cellulofine column (*7*). The purified PTK containing protein-tyrosine-kinase II and III showed a specific activity 849 units per mg protein. PTPase activity was not detected in this fraction.

Determination of Protein—The amount of phosphatase I was determined spectrophotometrically on the basis of the molecular extinction coefficient.

Preparations of Phosphorylated Proteins—Tyr-residues of polyEY and MBP were phosphorylated as follows. After incubation of 0.4 mg of polyEY or MBP with 169 units of the purified PTK in 0.5 ml of 50 mM Tris-HCl, pH 7.8, containing 50 mM $MgCl_2$, 10 μM sodium vanadate, 60 μM ATP, and 0.88 nM [γ-^{32}P]ATP (2.1×10^7 cpm) at 30°C for 2 h, the reaction mixture was dialyzed against 0.1 mM sodium vanadate-50 mM Tris-HCl, pH 7.5, and then against 25 mM MES-NaOH, pH 6.0. Ser- and/or Thr-residues in bovine histone H1 (0.3 mg) or casein (0.5 mg) were phosphorylated with 1.3 units of the catalytic subunit of protein kinase A in 0.5 ml of 50 mM Tris-HCl, pH 7.0, containing 0.1 mM EGTA, 10% (v/v) glycerol, 0.1% (v/v) 2-mercaptoethanol, 10 mM magnesium acetate, 100 μM ATP, and 1.46 nM [γ-^{32}P]ATP (3.5×10^7 cpm) at 30°C for 16 h. Phosphorylase *a* was prepared by incubation of 0.5 mg of phosphorylase *b* with 1.2 units of phosphorylase kinase in 0.5 ml of 66 mM sodium glycerophosphate, pH 8.6, containing 16 mM magnesium acetate, 16 mM calcium chloride, 100 μM ATP, and 1.46 nM [γ-^{32}P]ATP (3.5×10^7 cpm) at 30°C for 10 min. Each reaction mixture was dialyzed by the same method as that used for phosphorylated polyEY. By these methods, 0.44, 0.37, 0.61, and 1.0 mol, and 14.7 nmol of phosphorus (^{32}P) were incorporated with 1 mol of polyEY, MBP, histone H1 and phosphorylase *a*, and 1 mg of casein, respectively.

Determination of Phosphatase Activity—Phosphatase activity for phospho-Tyr, phospho-Ser, phospho-Thr, and other phosphorylated compounds was determined in 40 μl of 25 mM MES-NaOH, pH 6.0, containing 2 mM $MgCl_2$, the desired amount of substrate, and 6.0 ng (0.16 pmol) of phosphatase I at 30°C for 5 min. Eight microliters of 40% (w/v) trichloroacetic acid was added to stop the reaction, then the amount of released inorganic phosphorus was determined by the method of Kimelberg and Papahadjopoulos (*8*).

Protein phosphatase activity was assayed by the modified method as described (*9*). The assay was performed at 30°C for different periods of time in 15 μl of 25 mM MES-NaOH, pH 6.0, containing 2 mM $MgCl_2$, 15 μg of bovine serum albumin, 6.0 ng (0.16 pmol) of phosphatase I, and substrate: 2 μg of ^{32}P-polyEY (22.5 pmol phosphorus, 1.6×10^4 cpm), 0.9 μg of ^{32}P-MBP, 2.0 μg of ^{32}P-casein, 1.0 μg of ^{32}P-histone H1, or 2.9 μg of ^{32}P-phosphorylase *a* (each 30 pmol phosphorus, 2.1×10^4 cpm). After addition of 160 μl of 10% (w/v) trichloroacetic acid, the reaction mixture was centrifuged at 15,000×*g* for 2 min. To the supernatant (150 μl), 25 μl of 5% (w/v) ammonium molybdate-4 N H_2SO_4 and 150 μl of isobutylalcohol-benzene (1:1) were added. After centrifugation, the radioactivity in 100 μl of the upper layer was determined with an LSC-5100 liquid-scintillation counter (Aloka).

When the effects of inhibitors on phosphatase I were examined, the remaining activity was determined in 20 μl of 25 mM MES-NaOH, pH 6.0, containing 1 mM pNPP and 2 mM $MgCl_2$ at 30°C for 5 min. After addition of 0.1 N NaOH (0.2 ml), the absorbance at 410 nm was measured. The product of the enzyme reaction was quantitated on a calibration curve obtained with pNP.

Modification of Phosphatase I with DEPC and ^{14}C-MIAA—To estimate the number of amino acids modified with DEPC, phosphatase I (7.7 ng, 0.2 pmol) was incubated with increasing concentrations of DEPC in 10 μl of 25 mM MES-NaOH, pH 6.0, containing 2 mM $MgCl_2$ and 2% (v/v) acetonitrile at 15°C for the indicated periods of time. After addition of 10 μl of 100 mM histidine, pH 7.0, the remaining activity was determined with pNPP as substrate.

Phosphatase I (3 μg, 80 pmol) was incubated with 400 nmol of ^{14}C-MIAA (2.0×10^7 dpm) in 0.5 ml of 25 mM MES-NaOH, pH 6.0, containing 2 mM $MgCl_2$ and 1 M sorbitol at 15°C for 2 h in the dark condition under N_2 gas. The reaction mixture was dialyzed against 5% (v/v) acetic acid to remove excess ^{14}C-MIAA, then lyophilized. The enzyme protein labeled with ^{14}C-MIAA was hydrolyzed in an evacuated and sealed glass tube at 110°C for 24 h with 6 N HCl containing 0.1% (v/v) phenol and 0.25% (v/v) 2-mercaptoethanol.

Thin-Layer Electrophoresis of Amino Acid Derivatives—The hydrolyzate was dried and dissolved in 50 μl of 20 mM HCl, then spotted onto a silica gel plate with or without carboxymethylated amino acids as standards. Electrophoresis was carried out for 2 h at 15°C with a constant current of 6 mA using prydine-acetic acid-water (100v:4v:900v) buffer solution, pH 6.5. After electrophoresis, the plate was applied to a BAS-1000 bio-imaging analyzer (Fuji Photo Film). The standard amino acids were stained with a ninhydrin solution. Carboxymethyl histidine derivatives on the thin-layer plate were identified by their relative mobilities as described (*6*).

RESULTS AND DISCUSSION

Substrate Specificity of Phosphatase I—To examine the substrate specificity of phosphatase I, 40 nmol each of ATP, ADP, AMP, GTP, CTP, UTP, glycerophosphate, phosphatidate, phosphotyrosine, phosphoserine, or phosphothreonine was incubated with the enzyme. The enzyme dephosphorylated phosphotyrosine alone. Accordingly, the kinetic parameters were evaluated by measurement of the activity toward phosphotyrosine in the concentration range

Downloaded from http://jb.oxfordjournals.org/ at Changhua Christian Hospital on October 1, 2012

of 0.2 to 4 mM. The value of K_m was estimated as 1.42 mM from a Lineweaver-Burk reciprocal plot, and the value of k_{cat} was 16.8 s^{-1}. In the previous paper (*5*), phosphatase I dephosphorylated pNPP with the values of $K_m = 0.95$ mM and $k_{cat} = 112\ s^{-1}$.

PTPases from human (*10*), bull (*11*), and rat (*12*) have been found to dephosphorylate phosphotyrosine residues in proteins as well as phosphotyrosine. These findings and our experimental result suggested the possibility that phosphatase I could catalyze dephosphorylation of phosphotyrosine residues in proteins.

Dephosphorylation of Phosphorylated Polypeptides by Phosphatase I—We examined the activity of phosphatase I toward ^{32}P-phosphorylated polypeptides. As shown in Fig. 1, 5 pmol and 2 pmol of ^{32}P-phosphorus were released from ^{32}P-tyrosine-polyEY and ^{32}P-MBP, respectively, in 60 min, but not from histone H1, casein, and phosphorylase *a* containing both ^{32}P-serine and/or ^{32}P-threonine residues. Thus, the enzyme evidently dephosphorylated ^{32}P-polyEY and ^{32}P-MBP.

PTPase of rat liver has been purified and characterized with the activity of dephosphorylation of ^{32}P-polyEY as an index (*9*). Our experimental result indicated that psychrophilic phosphatase I had an activity like protein-tyrosine-phosphatase *in vitro*.

Several molecular species of PTPases have been isolated from tissue of mammal, fruit fly, and so on, and it was suggested that almost all PTPases functioned in physiologically important processes like the cell cycle and signal transduction by dephosphorylation of phosphorylated tyrosine residue(s) in proteins (*13*).

In prokaryotes, phosphorylation of histidine and aspartate residues in proteins involved in a "two-component regulatory system" is well known (*14*), but phosphorylation of amino acid residue(s) containing hydroxyl groups, such as serine, threonine, and tyrosine residues, had not been recognized. However, in *Escherichia coli*, it was reported that the heat-shock protein Dna K could autophosphorylate at the position of a threonine residue (*15*). Genes encoding eukaryotic-like protein-Ser/Thr-kinases and protein-Ser/Thr- and -Tyr-phosphatases on a cyanobacterial genome were surveyed (*16*), and the gene (*iph P*) encoding a PTPase was recently isolated from cyanobacterium *Nostoc commune* UTEX 584 and expressed in *E. coli* (*17*). The recombinant protein (Iph P) showed PTPase activity toward phosphorylated tyrosine residues of reduced and carboxyamidomethylated lysozyme (*17*) with optimal activity at pH 5.0. In addition, Iph P dephosphorylated the phosphoseryl groups of phosphorylated casein. It was speculated that the PTPase activity of Iph P might be involved in some aspect of sequestration and transport of iron in *N. commune* UTEX 584 (*17*). Although the function and substrate of phosphatase I *in vivo* are still obscure, this enzyme might play an important role through dephosphorylation of phosphotyrosine residue(s) in intrinsic protein(s) or of phosphotyrosine yielded as a nutrient by degradation of exogenous peptide(s).

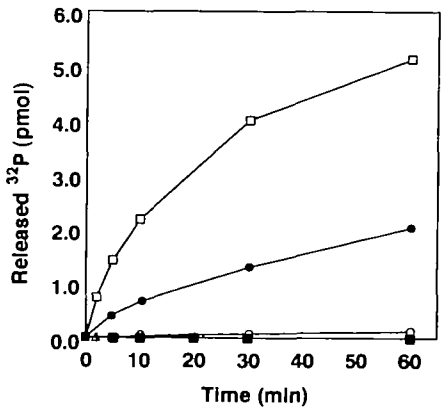

Fig. 1. **Time course of dephosphorylation of phosphorylated polypeptides with phosphatase I.** Activities of phosphatase I (6.0 ng, 0.16 pmol) toward ^{32}P-polyEY (□), ^{32}P-MBP (●), ^{32}P-casein (△), ^{32}P-phosphorylase *a* (■), and ^{32}P-histone H1 (○) were determined for the indicated periods of time as described in "MATERIALS AND METHODS." Activity is represented by the released inorganic phosphorus [^{32}P] as a function of time.

PTPases purified hitherto are activated by reducing reagents like dithiothreitol and 2-mercaptoethanol (*13*), because their catalytic residue is CySH. However, neither of these reducing reagents affected the activity of the present psychrophilic enzyme. Phosphatase I is thus not an SH-enzyme, and its catalytic residue must differ from that of the other known PTPases.

Effects of Various Inhibitors on Phosphatase I—To explore the catalytic residue of phosphatase I in detail, effects of various inhibitors on phosphatase I were examined using pNPP as a substrate. After treatment with the individual inhibitors at pH 6.0 for 1 h, the remaining activity was determined (Table I). The activity was completely inhibited by 50 μM DEPC and strongly inhibited by MIAA and MIAM. More than 83% of phosphatase I activity was lost by 8.0 μM MIAA. Also, under the conditions in Fig. 1, the activity toward ^{32}P-polyEY was strikingly reduced by DEPC and MIAA (data not shown). In contrast, PCMB, PCMPS, and NEM exerted little effect on the activity.

Based on the observation that optimum pH of phosphatase I was 6.0 (*5*), we presumed that the essential residue for the activity was His, judging from the pK of the imidazole group. Concerning the actions of the inhibitors, it has been shown that MIAA and MIAM modify the sulfhydryl group of the CySH residue and the imidazole group of

TABLE I. **Effects of inhibitors on phosphatase I activity.** Phosphatase I (6.0 ng, 0.16 pmol) was incubated with indicated concentrations of inhibitors in 10 μl of 25 mM MES-NaOH, pH 6.0, containing 2 mM $MgCl_2$ at 15°C for 60 min. The remaining activity was determined as described in "MATERIALS AND METHODS." The value of 100% corresponds to 0.45 nmol of pNP per min.

Inhibitors	Concentration	Remaining activity (%)
None		100
PCMB	1.0 mM	82
PCMPS	1.0 mM	96
	4.0 mM	87
NEM	1.0 mM	97
	4.0 mM	83
MIAA	8.0 μM	17
	16 μM	6
	1.0 mM	2
MIAM	1.0 mM	2
DEPC	10 μM	10
	50 μM	0
	1.0 mM	0

Downloaded from http://jb.oxfordjournals.org/ at Changhua Christian Hospital on October 1, 2012

the His residue (*18*), that DEPC reacts specifically with the His residue to yield *N*-carbethoxyhistidine (*19*), and that PCMB, PCMPS, and NEM modify the CySH residue alone in enzyme proteins (*13, 20, 21*).

These findings indicated that the catalytic residue of phosphatase I is a His residue, not the CySH residue of other PTPases (*13*).

The Number of Amino Acid Modified with DEPC—As shown in Table I, the activity of phosphatase I was completely lost by DEPC at concentrations more than 50 μM. However, the possibility that this inactivation was caused by conformational change resulting from modification of amino acid(s) other than the catalytic residue could not be excluded. Therefore, we attempted to evaluate the number of amino acids involved in the inhibition of the enzyme by kinetic analysis. Figure 2A shows the time courses of the inhibition of the enzyme in the presence of several concentrations of DEPC. Inhibition of the enzyme with DEPC followed pseudo-first-order kinetics, with a $t_{1/2}$ (the time required for 50% reduction of phosphatase activity) of 3.3 min in the presence of 2.0 mM DEPC. The finding of pseudo-first-order kinetics suggests that the inhibition involves either single group or, more precisely, equivalent groups. To examine this possibility, the data represented in Fig. 2A were replotted according to Levy *et al.* (*22*). As shown in Fig. 2B, the plots of log $t_{1/2}$ as a function of log [DEPC] yielded a straight line with a slope of the absolute value of 1.007. This value of the slope corresponds to a number equal to the average order of the reaction as a function of the concentration of DEPC (*22*). This result indicated that 1 mol of DEPC on the average bound to 1 mol of the enzyme protein when complete inhibition occurred. As DEPC specifically modifies of His-residues, it is reasonable to conclude that the modification of a single His residue by DEPC resulted in inhibition of this enzyme.

Identification and Quantitation of Modified Amino Acid with ^{14}C-MIAA—The experimental results in Table I and Fig. 2 suggest that the catalytic residue of phosphatase I is a His residue. However, identification of the DEPC-modified histidine *i.e.* carbethoxyhistidine, by analysis for amino acid is difficult because of the instability of this compound. Accordingly, we examined the identification and quantitation of modified His residue after treatment with ^{14}C-MIAA. The dose-dependent curve and the time-course for inhibition of the enzyme by this reagent were evaluated. About 90% of phosphatase I was inhibited by incubation

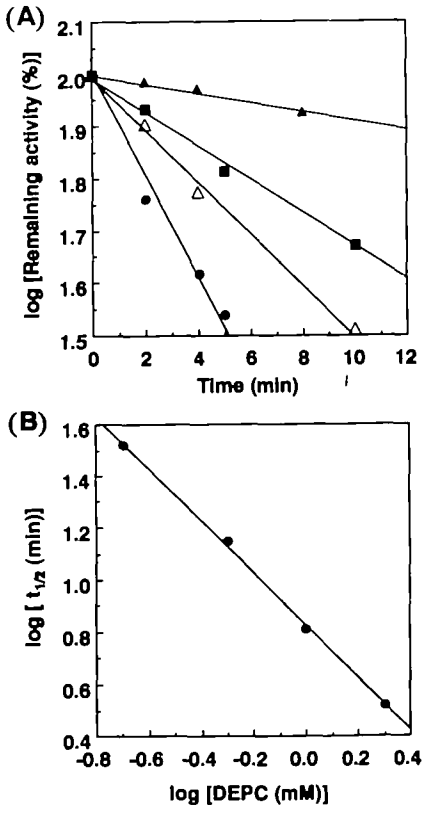

Fig. 2. **Estimation of the number of amino acids modified with DEPC.** (A) Phosphatase I (7.7 ng, 0.2 pmol) was incubated with different concentrations of DEPC: 0.2 (▲), 0.5 (■), 1.0 (△), and 2.0 mM (●). The remaining activity was determined using pNPP as substrate as described in "MATERIALS AND METHODS." The value of 100% corresponds to 0.50 nmol of pNP per min. The time required for 50% reduction of the activity, $t_{1/2}$, was calculated for each DEPC concentration. (B) A double-logarithmic plot of $t_{1/2}$ against DEPC concentration. The slope value is 1.007 with a regression coefficient of 0.999.

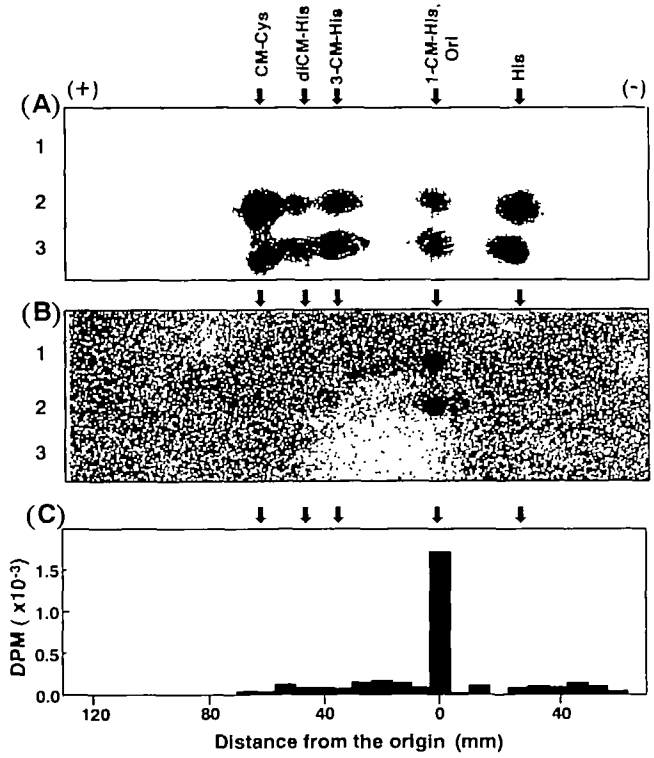

Fig. 3. **Identification and quantitation of the modified amino acid in phosphatase I by ^{14}C-MIAA.** Phosphatase I (3 μg, 80 pmol) was treated with 400 nmol of ^{14}C-MIAA, then hydrolyzed. Half (equivalent to 40 pmol of phosphatase I) of the hydrolyzate alone (lane 1), half with histidine and carboxymethylated amino acids as standard (lane 2), and standard alone (lane 3) were subjected to a thin-layer electrophoresis. (A) Authentic amino acids on a silica gel plate were stained with ninhydrin. (B) Autoradiography. (C) Radioactivity of a segment of the plate of 6.7 mm in width on lane 1 in (B). Arrows indicate the positions of carboxymethylcystein (CM-Cys), 1,3-dicarboxymethylhistidine (diCM-His), 3-carboxymethylhistidine (3-CM-His), 1-carboxymethylhistidine (1-CM-His) on origin (Ori), and histidine (His) on the electrophoresis.

Downloaded from http://jb.oxfordjournals.org/ at Changhua Christian Hospital on October 1, 2012

with a 5,000-fold molar excess of ^{14}C-MIAA at 15°C for 2 h (data not shown). Under the same conditions, 80 pmol of the enzyme was incubated with 400 nmol of the reagent. After hydrolysis with 6 N HCl, the modified amino acid was identified by a thin-layer electrophoresis and quantitated by the measurement of its radioactivity (Fig. 3). As shown in Fig. 3, B and C, 70 pmol of 1-carboxymethyl (CM)-histidine was detected. No radioactivity was detected on the plate when ^{14}C-MIAA alone was subjected to the same manipulation as a control test. This result showed that 0.88 mol of 1-CM-histidine per mol of phosphatase I was modified, and modification was correlated with inhibition of the enzyme by the reagent. It is also noteworthy that no radioactivity was detected at the position of CM-cystein. This showed that the enzyme is not an SH-enzyme.

It was thus directly demonstrated that the catalytic residue of psychrophilic phosphatase I is a His residue, not the CySH residue of known PTPases. In particular, the N-1 nitrogen of the imidazole group of histidine residue might be involved in the catalytic reaction of this enzyme.

A search for the N-terminal amino acid sequence of phosphatase I, Asn-Thr-Ala-Thr-Glu-Phe-Asp-Gly-Pro-Tyr-Val-Ile-Thr-Pro-Ile-Ser-Gly-Gln-, using the BLAST search program on the Internet revealed no other phosphatases containing this sequence (data not shown). This fact supports our conclusion that phosphatase I is a novel enzyme that exhibits PTPase-like activity *in vitro*.

Amino acid sequence alignment of all known PTPases suggests that they contain a common evolutionary-conserved segment of approximately 250 amino acids called the PTPase catalytic domain (*13*). Within this PTPase domain, there is a signature motif (His-Cys-Xaa-Ala-Gly-Xaa-Gly-Arg, where Xaa can be any amino acid) that is present in all PTPases. Mutational and chemical modification experiments indicated that the invariant CySH residue in this signature motif was essential for enzyme activity (*13*) and directly involved in the formation of a covalent phospho-enzyme intermediate (*23*). A recent paper showed that the invariant Arg-residue played a critical role in substrate recognition and transition-state stabilization (*24*).

Also, in respect of the mechanism of the catalytic reaction with protein serine/threonine-phosphatases containing bacteriophage λ protein serine/threonine-phosphatase, which, like PTPases, could dephosphorylate the synthetic substrate pNPP, it was reported that the essential residue for the dephosphorylation activity was a His residue, which interacted with a carboxyl group of an Asp residue by hydrogen bonding (*25*).

From these facts, we speculated that the catalytic domain of phosphatase I contains a His residue in place of the invariant CySH residue in the other PTPases, or that it resembles the catalytic domain of serine/threonine-phosphatases, except for the substrate-binding site.

In this paper, we propose that the psychrophilic phosphatase I is a protein-tyrosine-phosphatase-like enzyme with histidine as its catalytic residue.

However, determination of the primary structures neighboring this His residue and containing the substrate-binding site of this psychrophilic PTPase-like enzyme might be a further essential step for investigation of the relationship between structure and function of this cold enzyme.

REFERENCES

1. Devail, S., Feller, G., Narinx, E., and Gerday, Ch. (1994) Cold adaptation of proteins. *J. Biol. Chem.* **269**, 17448-17453
2. Rentier-Delrue, F., Mande, S.C., Moyens, S., Terpstra, P., Mainfroid, V., Gorai, K., Lion, M., Hol, W.G.J., and Martial, J.A. (1993) Cloning and overexpression of the triosephosphate isomerase genes from psychrophilic and thermophilic bacteria. *J. Mol. Biol.* **229**, 85-93
3. Aprigny, J.L., Feller, G., and Gerday, Ch. (1993) Cloning, sequence and structural features of a lipase from the Antarctic facultative psychrophile *Psychrobacter immobolis* B10. *Biochim. Biophys. Acta* **1171**, 331-333
4. Feller, G., Narinx, E., Arpigny, J.L., Aittaleb, M., Baise, E., Genicot, S., and Gerday, Ch. (1989) Enzymes from psychrophilic organisms. *FEMS Microbiol. Rev.* **18**, 189-202
5. Tsuruta, H., Tsuneta, S.T., Ishida, Y., Watanabe, K., Uno, T., and Aizono, Y. (1998) Purification and some characteristics of phosphatase of a psychrophile. *J. Biochem.* **123**, 219-225
6. Kleanthous, C., Cullis, P.M., and Shaw, W.V. (1985) 3-(Bromoacetyl) chloramphenicol, an active site directed inhibitor for chloramphenicol acetyltransferase. *Biochemistry* **24**, 5307-5313
7. Brunai, A.M. and Pinna, L.A. (1988) Characterization of four tyrosine protein kinases from the particulate fraction of rat spleen. *Eur. J. Biochem.* **172**, 451-457
8. Kimelberg, H.K. and Papahadjopoulos, D. (1972) Phospholipid requirements for ($Na^+ + K^+$)-ATPase activity: head-group specificity and fatty acid fluidity. *Biochim. Biophys. Acta* **228**, 277-292
9. Hiraga, A., Munakata, H., Hata, K., Suzuki, Y., and Tsuiki, S. (1992) Purification and characterization of rat liver protein-tyrosine phosphatase with sequence similarity to src-homology region 2. *Eur. J. Biochem.* **209**, 195-206
10. Waheed, A., Laidler, P.M., Wo, Y.Y.P., and Van Etten, R.L. (1988) Purification and physicochemical characterization of a human placental acid phosphatase possessing phosphotyrosyl protein phosphatase activity. *Biochemistry* **27**, 4265-4273
11. Lau, K.-H.W., Farley, J.R., and Baylink, D.J. (1985) Phosphotyrosyl-specific phosphatase activity of a bovine skeltal acid phosphatase isoenzyme. *J. Biol. Chem.* **260**, 4653-4660
12. Okada, M., Owada, K., and Nakagawa, H. (1986) [Phosphotyrosine]protein phosphatase in rat brain. *Biochem. J.* **239**, 155-162
13. Walton, K.M. and Dixon, J.E. (1990) Protein tyrosine phosphatases. *Annu. Rev. Biochem.* **62**, 101-120
14. Bourret, R.B., Borkovich, K.A., and Simon, M.I. (1991) Signal transduction pathways involving protein phosphorylation in prokaryotes. *Annu. Rev. Biochem.* **60**, 401-441
15. Zylicz, M., LeBowitz, J.H., MacMcken, R., and Georgopoulos, C. (1983) The dnaK protein of *Escherichia coli* possesses an ATPase and autophosphorylating activity and is essential in an *in vitro* DNA replication system. *Proc. Natl. Acad. Sci. USA* **80**, 6431-6435
16. Zhang, C.-C., Gonzalez, L., and Phalip, V. (1998) Survey, analysis and genetic organization of genes encoding eukaryotic-like signaling proteins on a cyanobacterial genome. *Nucleic Acids Res.* **26**, 3619-3625
17. Potts, M., Sun, H., Mockaitis, K., Kennelly, P.J., Reed, D., and Tonks, N.K. (1993) A protein-tyrosine/serine phosphatase encoded by the genome of the cyanobacterium *Nostoc commune* UTEX584. *J. Biol. Chem.* **268**, 7632-7635
18. Gurd, F.R.N. (1972) Carboxymethylation. *Methods Enzymol.* **25**, 424-438
19. Miles, E.W. (1977) Modification of histidyl residues in proteins by diethylpyrocarbonate. *Methods Enzymol.* **47**, 431-442
20. Robinson, G.W., Bradshaw, R.A., Kanarek, L., and Hill, R.L. (1967) The thiol groups of fumarase. *J. Biol. Chem.* **242**, 2709-2718
21. Nishida, E. and Kobayashi, T. (1977) Relationship between tubulin SH groups and bound guanine nucleotides. *J. Biochem.* **81**, 343-347

Downloaded from http://jb.oxfordjournals.org/ at Changhua Christian Hospital on October 1, 2012

22. Levy, H.M., Leber, P.D., and Ryan, E.M. (1963) Inactivation of myosin by 2,4-dinitrophenol and protection by adenosine triphosphate and other phosphate compounds. *J. Biol. Chem.* **238**, 3654-3659
23. Guan, K. and Dixon, J.E. (1991) Evidence for protein-tyrosine-phosphatase catalysis proceeding via a cystein-phosphate intermediate. *J. Biol. Chem.* **266**, 17026-17030
24. Zhang, Z.-Y., Wang, Y., Wu, L., Fauman, E.B., Stuckey, J.A., Schubert, H.L., Saper, M.A., and Dixon, J.E. (1994) The Cys-$(X)_5$Arg catalytic motif in phosphoester hydrolysis. *Biochemistry* **33**, 15266-15270
25. Zhuo, S., Clemens, J.C., Stone, R.L., and Dixon, J.E. (1994) Mutational analysis of a Ser/Thr phosphatase. *J. Biol. Chem.* **269**, 26234-26238

Downloaded from http://jb.oxfordjournals.org/ at Changhua Christian Hospital on October 1, 2012