A novel lipolytic enzyme was isolated from a metagenomic library obtained from tidal flat sediments on the Korean west coast. Its putative functional domain, designated MPlaG, showed the highest similarity to phospholipase A from *Grimontia hollisae* CIP 101886, though it was screened from an emulsified tricaprylin plate. Phylogenetic analysis showed that MPlaG is far from family I.6 lipases, including *Staphylococcus hyicus* lipase, a unique lipase which can hydrolyze phospholipids, and is more evolutionarily related to the bacterial phospholipase A₁ family. The specific activities of MPlaG against olive oil and phosphatidylcholine were determined to be 2,957 ± 144 and 1,735 ± 147 U mg⁻¹, respectively, which means that MPlaG is a lipid-preferred phospholipase. Among different synthetic esters, triglycerides, and phosphatidylcholine, purified MPlaG exhibited the highest activity toward p-nitrophenyl palmitate (C₁₆), tributyrin (C₄), and 1,2-dihexanoyl-phosphatidylcholine (C₈). Finally, MPlaG was identified as a phospholipase A₁ with lipase activity by cleavage of the sn-1 position of OPPC, interfacial activity, and triolein hydrolysis. These findings suggest that MPlaG is the first experimentally characterized phospholipase A₁ with lipase activity obtained from a metagenomic library. Our study provides an opportunity to improve our insight into the evolution of lipases and phospholipases.
phospholipase and lipase activities. The novel phospholipase was expressed as a highly soluble form in *Escherichia coli* and then purified to homogeneity and characterized biochemically.

**MATERIALS AND METHODS**

**Isolation and sequence analysis of lipolytic clone from the metagenomic library.** We extracted metagenomic DNA from the tidal flat sediments of the Saemankum area on the western coast of the Republic of Korea and constructed a library with approximately 386,400 *Escherichia coli* EPI300-T1 clones harboring recombinant pCC1fos as described previously (14). Lipolytic clones were screened on an emulsified tricaprylin plate and were observed by a transparent halo around each colony. The recombinant fosmid showing lipolytic activity was finally isolated from an *E. coli* transformant and used for further analysis of the tidal flat metagenomic enzyme. The complete sequence of the lipolytic fosmid was determined by a shotgun sequencing method and deposited in GenBank. Open reading frames (ORFs) in the sequence were identified by ORF Finder at the National Center for Biotechnology Information (NCBI) website, and the sequence similarities of identified ORFs were analyzed using BLASTX (32). Only hits with E values of <0.01 were considered ORFs. The conserved structural and functional domains in protein sequences were analyzed using the NCBI Conserved Domain Database (CDD) (15). The amino acid sequence of the putative lipolytic enzyme was aligned with those of the homologous proteins by using CLUSTALW and the ESPript method (25). The percent identity was calculated and phylogenetic analysis performed using the MEGALIGN program, implemented in Lasergene software (DNASTar, and TreeView V3.2.

**Expression and purification of recombinant lipolytic enzyme.** A putative catalytic domain encoding phospholipase A, *mplaG*, was subcloned between the NdeI and Xhol sites in pET-22b(+)(Novagen), resulting in the plasmid pET-MPlaG. *E. coli* BL21(DE3) harboring pET-MPlaG was cultured at 18°C for 12 h with 0.5 mM isopropyl-β-D-1-thiogalactoside (IPTG) for induction of overexpression. For purification, Ni-nitrilotriacetic acid (Ni-NTA) resin (Qiagen) bound with the crude enzymes was washed with 50 mM Tris-HCl (pH 8.0) containing 5 mM imidazole, and the bound proteins were eluted with 50 mM Tris-HCl (pH 8.0) containing 250 mM imidazole and dialyzed.

**Assay of lipolytic activity.** The activity of MPlaG was determined by pH titration and spectrophotometric methods at room temperature. The hydrolyzing activities of MPlaG toward triacylglycerides, olive oil, and phosphatidylcholine were measured by titrating the free fatty acids by use of a pH titrator (model 842 Titrando; Metrohm). After the pH of the substrate emulsion was adjusted to 8.0 by the addition of 10 mM NaOH solution, an appropriate amount of the enzyme solution was added. The release rate of the fatty acid was measured with a pH titrator for 5 min. One unit of lipase activity was defined as the amount of enzyme liberating 1 μmol of fatty acid per minute (16). MPlaG activity against p-nitrophenyl esters was measured by spectrophotometric detection according to a standard assay method. Unless otherwise indicated, p-nitrophenyl caprate (C<sub>16</sub>), was used as the substrate and 5 mM Ca<sup>2+</sup> was added to the reaction solution. The enzyme activity was determined by continuously monitoring the product, p-nitrophenol, at 405 nm with a DU800 spectrophotometer (Beckman). One unit of activity with p-nitrophenyl esters was defined as the amount of enzyme that released 1 μmol of p-nitrophenol per minute (4). Control reactions were carried out without enzymes for every measurement under different conditions in order to subtract the value for the nonenzymatic hydrolysis of substrates.

**LC-MS analysis of specificity toward phospholipids.** Positional and chain-length specificities of phospholipolytic MPlaG were determined by using 1-stearoyl-2-palmitoyl-phosphatidylcholine and phosphatidylcholine with various carbon chain lengths (C<sub>14</sub>, C<sub>16</sub>, C<sub>18</sub>, and C<sub>18:2</sub>), respectively, as substrates. Purified MPlaG was incubated with 1 mM substrate in 50 mM Tris buffer (pH 8.0) containing 5 mM CaCl<sub>2</sub> and 150 mM NaCl for 12 h at 25°C with shaking. Liquid chromatography-mass spectrometry (LC-MS) was performed for analysis of reaction products, using a Finnigan LCQ Advantage Max ion-trap mass spectrometer (Thermo Fisher Scientific) equipped with an electrospray ionization (ESI) source. High-performance liquid chromatography (HPLC) separation was performed with a Kinetex HILIC column (2.6 μm × 2.1 mm × 100 mm; Phenomenex) and a hydrophilic interaction liquid chromatography (HILIC) guard column (4 × 2.0 mm; Phenomenex). Mobile phase A was 10 mM ammonium formate at pH 3.0, adjusted using formic acid, and mobile phase B was acetonitrile. The gradient elution was performed at a flow rate of 0.2 ml/min, as follows: 0 to 10 min, 10% to 40% mobile phase A (linear gradient); and 10 to 20 min, 70% mobile phase A (isocratic) (35). The column temperature was ambient, and the injection volume was 10 μl. The mass spectra were obtained with m/z values ranging from 100 to 1,200 in negative-ion mode, with 3 microscans and a maximum ion injection time of 200 ms.

**Effects of pH and temperature on the novel lipolytic enzyme.** GTA buffer (100 mM 3,3-dimethylglutaric acid, 100 mM Tris, and 100 mM 2-amino-2-methyl-1,3-propanediol) was used for the enzyme assay. The pH for maximum activity of MPlaG was determined over a pH range of 6.0 to 12.0, using intervals of 1.0 pH unit. To determine pH stability, the enzyme was incubated at various pHs (4.0 to 12.0, at intervals of 1.0 pH unit) for 3 h, and the residual activity was measured. The optimum temperature of MPlaG was measured in pH 8.0 buffer over the range of 5 to 60°C. The melting temperature (*T<sub>m</sub>*<sup>0</sup>) was determined by differential scanning calorimetry (DSC) using VP-DSC (MicroCal). MPlaG was heated from 25 to 80°C in a DSC aluminum pan at a scan rate of 90°C/h. Before measurements were made, the sample was dialyzed against 50 mM Tris buffer (pH 8.0) with or without Ca<sup>2+</sup>. The dialysis buffer was used as a reference solution for DSC. A buffer-reference scan was subtracted from each sample scan prior to concentration normalization. Baselines were created in Origin 7.0 (OriginLab).

**Effects of metal ions, inhibitors, detergents, and organic solvents on the novel lipolytic enzyme.** The enzyme solution was preincubated with various concentrations of metal ions, enzyme inhibitors, detergents, and organic solvents in 50 mM Tris buffer (pH 8.0) for 3 h at 25°C. An adequate amount of the incubation mixture was withdrawn, and the residual enzyme activities were determined as described above.

**Nucleotide sequence accession number.** The complete sequence of the lipolytic fosmid was deposited in the GenBank nucleotide sequence database under accession no. EU285670.

**RESULTS**

**Isolation and annotation of metagenomic fosmid conferring lipolytic activity.** From the metagenomic library of the tidal flat sediments on the Korean west coast, we isolated a fosmid clone from an *E. coli* transformant displaying lipolytic activity on an emulsified tricaprylin plate. The fosmid clone (GenBank accession no. EU285670) had 28,845 bp of metagenomic DNA, contained a total of 15 ORFs with E values of <0.01 (based on ORF Finder at the NCBI website), and showed database hits with broad identity (22% to 88%) (Table 1). Three of the 15 ORFs, namely, ORFs 8, 13, and 15, were found to have a conserved transposase domain (Table 1). A number of proteins (46%) did not significantly match sequences in common protein databases such as Pfam (7) and COG (24), indicating that the parent organism of the 15 ORFs had a genetic makeup that was different from that of the cultured organisms available in the public databases.

**Sequence and phylogenetic analyses of metagenome-derived lipolytic enzyme.** Among all of the ORFs, an ORF encoding a putative phospholipase A of 565 amino acids, with a deduced molecular mass of 61,186 Da, was found. A BLAST search against the sequence databases at GenBank found that only the C-terminal 202 residues showed the highest similarity (31% identity) to phospholipase A (GenBank accession no. ZP_06052044) from Gri-
m. hollisae CIP 101886. Additionally, the N-terminal 26 residues were predicted to be a signal peptide by SignalP 3.0 (2). The amino acid sequence from Ala27 to Gly287 was repeated after amino acids Thr158 to Gly287 as determined by analysis using the TRUST tandem repeat detection tool (23). These results indicate that the ORF should consist of two domains: an unknown functional domain and a catalytic domain, comprising 287 amino acids of the N-terminal region and 278 amino acids of the C-terminal region, respectively. The putative catalytic domain, starting at Leu288, was designated MPlaG. The Ser435-Asp496-His550 catalytic triad, which is typical of the α/β-hydrolase superfamily, was predicted by multiple sequence alignment with similar phospholipases retrieved from the GenBank database (Fig. 1A) (3). As a catalytic nucleophile, the Ser435 residue was part of a G-X-S-X-G motif considered to be one of the identifying features of α/β-hydrolases (28). In addition, residues in the vicinity of this common motif corresponded well to the characteristic amino acid pattern of phospholipase A: [LIV]-[KG]-[LIVFY]-[LIVMST]-G-[HYWV]-S-[YAG]-G-[GSTAC] (where brackets indicate alternative amino acids and braces indicate excluded amino acids) (19). Meanwhile, a phylogenetic tree was established from the amino acid sequences of MPlaG and the homologous proteins (Fig. 1B). MPlaG and its phospholipase homologues were compared with lipase and phospholipase families, and they did not belong to any known lipase family and were more phylogenetically related to the bacterial phospholipase A family.

**Purification and characterisation of metagenome-derived MPlaG.** The heterologically expressed MPlaG enzyme was purified by a single step of Ni-NTA affinity chromatography (see Fig. S1 in the supplemental material). The purified MPlaG enzyme exhibited a specific activity of 169.4 ± 1.8 U mg⁻¹ when p-nitrophenyl palmitate was used as the substrate. Subsequently, purified MPlaG was used for biochemical characterisation.

We determined the effects of pH and temperature on the activity of MPlaG and found that MPlaG displayed characteristics of a cold-adapted alkaline enzyme. When the purified enzyme was assayed for the hydrolysis of p-nitrophenyl caprate, it exhibited maximum activities at pH 8.0 (Fig. 2A) and 25°C (Fig. 2B). The activity of MPlaG retained 39% of the maximum at 5°C and drastically decreased at temperatures above the apparent optimum temperature. The effect of pH on the enzymatic stability of MPlaG was also determined by assaying the remaining activity after pre-incubation of the purified enzyme. MPlaG was remarkably stable (>90% of activity remaining) over a broad pH range (pH 5.0 to 10.0). These results indicate that MPlaG is a cold-adapted alkaline enzyme.

The effects of divalent metal ions on the activity of MPlaG were examined at different concentrations of each ion. Over the range of divalent metal ion concentrations tested, Ca²⁺ ions drastically increased the activity of MPlaG (10-fold), whereas Cu²⁺, Zn²⁺, and Ni²⁺ ions inhibited the activity and Co²⁺, Mg²⁺, and Fe²⁺ ions somewhat increased the activity (see Table S1 in the supplemental material). In addition, the enzyme was strongly inhibited by EDTA, a chelator of divalent cations: no activity was detected in the presence of 5 mM EDTA. The thermal unfolding of MPlaG was observed by DSC in the absence and presence of Ca²⁺. The melting temperatures of MPlaG in 2 mM and 5 mM Ca²⁺ were 47.2°C and 49.2°C, respectively. Thus, 5 mM Ca²⁺ caused a remarkable 10.7°C shift in melting temperature compared to the melting temperature of 38.5°C without Ca²⁺ (Fig. 2C). These results indicate that the structural stability of MPlaG is strongly dependent on the presence of Ca²⁺. In fact, the bacterial production and catalytic activity of several lipases are enhanced by Ca²⁺ (5, 17). The increased lipase activity in the presence of Ca²⁺ may be explained mainly by the stabilizing effect of enzyme-bound Ca²⁺ on the transient enzyme-substrate complex (13). Also, for MPlaG, Ca²⁺ should have a structural rather than a catalytic role. In order to investigate the organic solvent tolerance of MPlaG, various organic solvents were added to the enzyme solution, at concentrations of 20, 30, 50, and 60% (vol/vol). Over the range of concentrations tested, MPlaG showed a remarkable stability in the presence of most of the water-miscible organic solvents, with more than 80% of the original activity, but not against water-immiscible organic solvents such as butanol and ethyl acetate (see Table S2 in the supplemental material). Additionally, MPlaG activity was inhibited by concentrations of more than 1.0% (vol/vol) of nonionic and ionic detergents (see Table S3 in the supplemental material).

### Table 1: Annotation of ORFs identified on pFosPlaG

<table>
<thead>
<tr>
<th>ORF</th>
<th>Length (aa)</th>
<th>% G+C</th>
<th>Most homologous protein</th>
<th>Putative source organism</th>
<th>% Identity/% similarity</th>
<th>E value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>291</td>
<td>36.87</td>
<td>RNA polymerase sigma factor</td>
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<td>39/57</td>
<td>2e⁻⁵³</td>
</tr>
<tr>
<td>2</td>
<td>565</td>
<td>44.94</td>
<td>Phospholipase A</td>
<td>Girondia hollisae CIP 101886</td>
<td>31/53</td>
<td>5e⁻⁰⁶</td>
</tr>
<tr>
<td>3</td>
<td>334</td>
<td>47.56</td>
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<td>Moritella sp. PE36</td>
<td>88/96</td>
<td>4e⁻¹⁷</td>
</tr>
<tr>
<td>4</td>
<td>208</td>
<td>44.34</td>
<td>Hypoxanthine phosphoribosyltransferase</td>
<td>Desulfitobacter psychrophila L5v54</td>
<td>71/89</td>
<td>4e⁻⁶⁶</td>
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<tr>
<td>5</td>
<td>90</td>
<td>37.36</td>
<td>Putative regulatory protein</td>
<td>Dictyoglossus thermophilus H-6-12</td>
<td>57/80</td>
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</tr>
<tr>
<td>6</td>
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<td>33.54</td>
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<td>2e⁻²⁶</td>
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<tr>
<td>7</td>
<td>328</td>
<td>36.58</td>
<td>Amino acid ABC transporter periplasmic protein</td>
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<tr>
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<td>Solibacter usitatus Ellin6076</td>
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<tr>
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<td>2e⁻⁵⁷</td>
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<tr>
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<td>38.58</td>
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<td>11</td>
<td>1,095</td>
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<tr>
<td>12</td>
<td>253</td>
<td>39.76</td>
<td>NAD(P)H dehydrogenase</td>
<td>Desulfitobacillus alkenivorans AK-01</td>
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<td>4e⁻⁴³</td>
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<tr>
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<td>435</td>
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<td>Transposase</td>
<td>Marinobacter sp. ELB17</td>
<td>25/47</td>
<td>2e⁻¹¹</td>
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</table>

*ORF* = open reading frame; *G+C* = guanine + cytosine content; *E value* = expectation value.
Because MPlaG showed a relatively high amino acid similarity to phospholipase A in the BLAST search, despite being isolated on an emulsified tricaprylin plate, lipase and phospholipase activities were determined preliminarily by applying the purified MPlaG to preformed holes on emulsified tricaprylin and phosphatidylcholine agar plates, respectively. MPlaG formed transparent clear zones around the holes on both plates, indicating that MPlaG hydrolyzes both lipids and phospholipids.

**Substrate specificity of MPlaG.** Because MPlaG showed a relatively high amino acid similarity to phospholipase A in the BLAST search, despite being isolated on an emulsified tricaprylin plate, lipase and phospholipase activities were determined preliminarily by applying the purified MPlaG to preformed holes on emulsified tricaprylin and phosphatidylcholine agar plates, respectively. MPlaG formed transparent clear zones around the holes on both plates, indicating that MPlaG hydrolyzes both lipids and phospholipids.
and phospholipids (data not shown). For the quantitative comparison of phospholipase and lipase activities, the specific activities of MPlaG toward olive oil and phosphatidylcholine from egg yolk were determined by the pH-stat method and were found to be 2,957 ± 144 and 1,735 ± 147 U mg⁻¹, respectively. MPlaG is homologous to phospholipases but displays remarkable lipase activity against olive oil.

To investigate the substrate preference further, the purified enzyme activity was assayed for the hydrolysis of p-nitrophenyl esters, triglycerides, and phosphatidylcholine of different chain lengths (Fig. 3). The results of the pH titration assay against triglycerides showed the highest activity toward tributyrin (C₄), and enzymatic activity dramatically decreased with increasing chain length (Fig. 3A). However, in a spectrophotometric assay using p-nitrophenyl esters, MPlaG was most active toward p-nitrophenyl palmitate (C₁₆) (approximately 112-fold), followed by p-nitrophenyl butyrate (C₄) (Fig. 3B). Specificity of MPlaG toward phosphatidylcholine molecules with 6-, 7-, 8-, and 14-carbon chain lengths was analyzed by LC-MS (Fig. 3C). Since the mobile phase for LC-MS analysis contained ammonium formate and formic acid, a characteristic mass spectrum showed a deprotonated molecular ion ([M/H]+) and solvent adduct ions such as [M + H + HCOOH]⁻ in the negative-ion mode. The catalytic activity for hydrolysis of phosphatidylcholine to lysophosphatidylcholine was the highest for 1,2-dioctanoyl-phosphatidylcholine (diC₈PC) (Fig. 3C). Therefore, MPlaG had diverse chain length specificities according to substrate, showing a high preference for p-nitrophenyl esters with long acyl chains, triglycerides with short acyl chains, and phospholipids with medium acyl chains. The purified enzyme displayed interfacial activity when we measured the hydrolytic activity with increasing concentrations of tributyrin and hydrolyzed triolein (C₁₈:1), which cannot be hydrolyzed by esterases, indicating that MPlaG is not an esterase (Fig. 3A; see Fig. S2 in the supplemental material). Additionally, the site of hydrolysis of MPlaG was determined by LC-MS analysis using 1-oleoyl-2-palmitoyl-phosphatidylcholine (OPPC) (Fig. 4). After incubation of MPlaG with OPPC (molecular weight of 759) at 25°C for 12 h, the LC-MS spectrum revealed that MPlaG degraded OPPC ([m/z 804; [M + H + HCOOH]]⁻) to a by-product of [m/z 540]. If MPlaG cleaved a palmitic acid at the sn-2 position of OPPC, the by-product would be observed at [m/z 567 ([M + H + HCOOH-C₁₆:0]]⁻). However, since the MS spectrum detected [m/z 540 ([M + H + HCOOH-C₁₈:1 ]⁻), corresponding to 2-palmitoyl-lysophosphatidylcholine, MPlaG was identified as a phospholipase A₁ that is able to catalyze hydrolysis of the acyl group from the sn-1 position of phospholipids.

**DISCUSSION**

Phospholipase A is of particular interest for industrial applications because it yields lysophospholipids, which are excellent emulsifying agents for food, cosmetics, and pharmaceuticals (22). However, relatively few phospholipase A enzymes have been characterized, despite their apparent ubiquity and industrial demands. In this study, MPlaG, a novel lipolytic enzyme with unique proper-

FIG 2 Effects of pH, temperature, and Ca²⁺ ions on MPlaG activity. Lipase activity was measured by a spectrophotometric method using p-nitrophenyl caprate (C₁₀) and GTA buffer. (A) To determine pH stability, MPlaG was incubated at various pHs (4.0 to 12.0) for 3 h, and the residual activities were measured. Optimum pH and pH stability are indicated by circles and squares, respectively. (B) The optimum temperature of MPlaG within the range of 5 to 60°C was determined in pH 8.0 buffer. All measurements were repeated three times to confirm reproducibility. The values are shown as relative activities, with a maximum value of 100%. (C) The effect of Ca²⁺ on the temperature of MPlaG thermal unfolding (Tₘ) was determined by DSC. The Tₘ without CaCl₂ is indicated by a solid line, that with 2 mM CaCl₂ is indicated by open circles, and that with 5 mM CaCl₂ is indicated by solid circles.
ties, was isolated from a metagenomic library obtained from tidal flat sediments. Although the specific activity of MPlaG revealed that lipids rather than phospholipids were preferable substrates for the enzyme, the amino acid similarity to phospholipase A from Grimontia hollisae CIP 101886 and the finding of phospholipase activity of MPlaG indicated that it was a phospholipase rather than a lipase. A phylogenetic tree was established from the amino acid sequences of MPlaG and homologous proteins for comparison with other lipase and phospholipase families (Fig. 1B). Judging from eight lipase families containing staphylococcal lipase family I.6, MPlaG and its phospholipase homologues formed a phylogenetically distinct group that does not belong to the known lipase families and is distant from the staphylococcal lipase family, including SHL (Fig. 1B). As a result, MPlaG is evolutionarily closer to the bacterial phospholipase A family, in agreement with the experimental results identifying the phospholipase A activity of MPlaG by LC-MS analysis using OPPC as the substrate.

Previously, staphylococcal lipases from Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Staphylococcus xylosus, Staphylococcus hyicus, and Staphylococcus warneri were reported to form a unique lipase family. Among the staphylococcal lipases characterized so far, S. hyicus lipase (SHL) has considerably higher phospholipase than lipase activity, which distinguishes the enzyme not only from other staphylococcal lipases but also from all bacterial lipases. Moreover, SHL shows low chain length specificity and readily hydrolyzes short-, medium-, and long-chain triacylglycerols as well as \( p \)-nitrophenyl esters, irrespective of their chain length (20). In addition, SHL is dependent on \( \text{Ca}^{2+} \), which is considered to have a function in stabilizing the tertiary structure rather than in the catalytic reaction (26). Although MPlaG and SHL share similar biochemical characteristics, the most important difference is in their primary structures: MPlaG was shown to have relatively low identity (8.2%) with the mature domain of SHL, and even catalytic active site residues were not aligned with each other (data not shown). Thus, based on amino acid sequence similarity, SHL is a lipase and MPlaG is a phospholipase. Furthermore, with respect to substrate specificity, SHL is a phospholipid-preferred lipase, whereas MPlaG is a lipid-preferred phospholipase.

Recently, structure-function relationships of SHL and GPLRP2 with regard to phospholipase activity were elucidated (26, 33). The activity of SHL toward phospholipids comes from a
polar acyl binding pocket, whereas the phospholipase activity of GPLRP2 is based on the short lid domain. MPLaG should have a lid domain, considering its interfacial activation. Therefore, MPLaG may possess a substrate binding pocket similar to that of SHL; at least one of three hydrophobic pockets for acyl chains in the active site of MPLaG may be shallower and/or more polar than the others, and another pocket may be more extended and/or flexible, permitting the binding of diverse chains.

In conclusion, the tidal flat metagenome-derived MPLaG enzyme is the first experimentally confirmed phospholipase A1 with lipase activity. The novel MPLaG was identified as having dependence on Ca^{2+}, cold-adapted alkaline properties, and remarkable stability against organic solvents. The sequence of MPLaG has higher homology with phospholipases A than with other lipases and is phylogenetically close to the phospholipase A1 family. However, the experimental results revealed that MPLaG is a phospholipase A1 that has considerably higher lipase than phospholipase activity. When phospholipases evolve from lipases and vice versa, MPLaG may be an intermediate that is closer to phospholipases in the evolutionary relationships between lipases and phospholipases. Finally, further investigations on the substrate specificities of and relationships between lipases and phospholipases through X-ray crystallography and mutagenesis studies are believed to be necessary and will give us more detailed information on this promising enzyme.

ACKNOWLEDGMENTS

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