Journal of Microbiology and Biotechnology
Vol. 6. No. 6, 407-413, 1996

Purification and Biochemical Properties of Extracellular Phospholipase A_1 from *Serratia* sp. MK1

KIM, MYUNG KEE AND JOON SHICK RHEE*

Department of Biological Sciences, Korea Advanced Institute of Science and Technology, 373-1, Kusong-Dong, Yusong-Gu, Taejon 305-701, Korea

A novel type of extracellular phospholipase A_1 was isolated from *Serratia* sp. MK1 and purified to homogeneity by ammonium sulfate precipitation, anion exchange and gel filtration chromatography. The purified enzyme was a monomer with a molecular mass of about 43,000 Da. This enzyme showed the highest lipolytic activity toward phosphatidylserine among the phosphoglycerides tested, and preferentially catalyzed the hydrolysis of the ester bond in phosphatidic acid to lyso-phosphatidic acid. Enzyme activity was completely inhibited by the addition of a chelating agent such as EDTA, and inhibited enzyme activity was fully recovered by the presence of Ca^{2+}. This implies that the enzyme requires Ca^{2+} for activity. The enzyme was stable up to 70°C when incubated for 1 h at pH 8.5, and the optimal pH and temperature were 8.5 and 50°C, respectively.

Phospholipase A_1 [E.C. 3.1.1.32], which catalyzes the hydrolysis of *sn*-1 ester bond in phospholipid to fatty acid and lyso-phospholipid, is present in most mammalian cells as well as in various microorganisms. In the food and pharmaceutical industry, phospholipase A_1 is used for the production of lysophospholipid which is a hydrolysis product of phospholipid. Lysophospholipid has greater emulsification activity than phospholipid. Various intracellular phospholipase A_1 have been partially purified from mammalian cells such as rat liver (4) and brain from human and bovine (19). Microbial phospholipases such as membrane bounded phospholipase A_1 from *E. coli* (22) and spore of *Bacillus megaterium* (20) have also been reported. Even though various phospholipase A_1 sources have been reported, the quantity of phospholipase A_1 was limited because most microbial phospholipase A_1 is membrane bound and the available sources of mammalian phospholipase A_1 such as rat liver, brain and heart are very limited.

Although extracellular phospholipase A_1 from *Serratia liquefaciens* (7) was cloned and expressed in *E. coli*, available information on the extracellular phospholipase A_1 from microorganism was less than that for phospholipase A_2 and membrane bound microbial phospholipase A_1 (21, 22).

In our laboratory, to achieve the large scale production of phospholipase A_1, we isolated one of the phospholipase A_1 producing microorganisms, identified as *Serratia* sp. and named it *Serratia* sp. MK1 (11). A characteristic trait of many strains of *Serratia* is that they produce extracellular hydrolases such as nuclease, lipase, protease and chitinase (1, 2, 15-17). Among these extracellular hydrolases, only the characteristics of metalloprotease from *Serratia marcescens* have been investigated in detail using purified enzyme (1, 2), while those of lipase and phospholipase from *Serratia* sp. have not been extensively studied. The present paper describes the purification and characterization of novel extracellular phospholipase A_1 from *Serratia* sp. MK1.

*Corresponding author
Key words: *Serratia* sp. MK1, extracellular phospholipase A_1, phospholipid hydrolysis

MATERIALS AND METHODS

Chemicals

Soybean lecithin was kindly supplied by Nattermann GmBH (Köln, Germany). 60% egg yolk lecithin was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylglycerol (PG), phosphatidic acid (PA), lyso-phosphatidylcholine (lyso-PC), lyso-phosphatidylethanolamine (lyso-PE), sphingomyelin (SP) and cardiolipin (CA) were purchased from Sigma Chemical Co. QAE-Sepharose for ion exchange and Sephacryl S-200 for gel filtration chromatography were obtained from Pharmacia LKB Biotechnology (Uppsala, Sweden). All other chemicals were of analytical grade or better.

pH Titration Method (24)

Phospholipase activity was measured by titrating free fatty acids released by hydrolysis of phospholipid with 10 mM NaOH using a Fisher titration set (pH meter, titrate and burette/dispenser, Pittsburgh, PA). Phospholipid emulsion was prepared by emulsifying 2.5 g phospholipid in 100 ml water for 5 min at maximum speed in a Waring blender, and then adding 1 ml of 300 mM $CaCl_2$ and 78 mM sodium deoxycholate solution to 28 ml of phospholipid emulsion solution. The reaction was initiated by adding the enzyme solution in a reaction vessel maintained at 50°C. One unit of phospholipase activity was defined as the amount of enzyme which liberates 1 μmol fatty acid equivalent per min under assay condition.

Bioluminescent Assay of Phospholipase A_1 Activity Using Luminescent Marine Bacterium *Vibrio harvey* M-17

In enzyme purification steps, bioluminescence method described by Cho (5) was used to determine the phospholipase A_1 activity rapidly. According to this method, dimyristoyl phosphatidylcholine (DMPC) is used as a substrate. Light intensity is measured with a Shimadzu Spectrofluorophotometer Model RF-5000 at wavelength from 400 to 600 nm. Usually a light peak was obtained after 1.5 min. The peak intensity is directly related to the concentration of myristic acid liberated from dimyristoyl phosphatidylcholine. The concentration of myristic acid is in proportion to enzyme activity.

Protein Assay

Protein was determined by the dye reagent method (3) supplied by Bio-Rad using bovine serum albumin as a standard.

Gel Electrophoresis

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis of fractions collected during the various steps of purification were performed as follows; the sample was boiled for 5 min in the presence of an equal volume of loading buffer [Tris (0.5 M) 1 ml; glycerol, 1.6 ml; SDS (10% w/v), 3.2 ml; Bromophenol blue (0.05%), 0.2 ml; water, 1.2 ml; mercaptoethanol, 12.5 μl)]. The sample was then loaded onto 0.7-mm-thick slab gels (pH 8.0) which contained 10% polyacrylamide (w/v), and a stacking gel (pH 6.5) of 4% polyacrylamide (w/v). Electrophoresis was run at a constant current of 20 mA. The presence of protein bands was detected by Coomassie blue staining or by silver staining (8). In the case of native-PAGE, SDS was removed from the loading buffer and sample was loaded without boiling. All other conditions were the same as with general SDS-PAGE.

Activity Staining

Activity staining was performed according to the method described by Lechner *et al.* (13) with a slight modification. Phosphatidylcholine was used as a substrate instead of Tween 20. Phospholipase A_1 was still active after separation by native-PAGE. In order to detect phospholipase A_1 activity, polyacrylamide gel was laid on similarly sized 1.2% agarose gel prepared in 50 mM Tris-HCl buffer (pH 8.0) solution containing 10 mM $CaCl_2$ and 0.5% (w/v) phospholipid. The gel was incubated for 12 h at 37°C. Visible deposits in the agarose (due to precipitation of liberated water insoluble fatty acids) indicated phospholipase A_1 activity.

TLC-FID(Thin-Layer Chromatography Flame Ionized Detector) Method

In order to determine substrate specificity, the TLC-FID method was used as an assay method. Emulsion solution consisted of 10 mM $CaCl_2$, 26 mM sodium deoxycholate, 3.4 mM phosphoglycerides in 50 mM Tris-HCl buffer, pH 8.5. The enzyme reaction was started by adding 0.1 ml of enzyme preparation to 0.4 ml of emulsion solution. Incubation was conducted for 10 min at 50°C with shaking. The reaction was then stopped by the addition of chloroform-methanol (2:1 by volume) and total lipids were extracted. An internal standard, Monomyristin, was added to the extraction solvent and 1 μl of the solution was spotted onto a Chromarod SIII (Iatron Co., Japan). The first development was performed with chloroform-methanol-14% ammonia solution (65:25:2, by volume) until the solvent front migrated half the length of the Chromarod, and, after drying, it was developed further with hexane-ether-acetic acid (80:20:1 by volume). After the second development, each separated lipid component was analyzed quantitatively with an Iatroscan TH-10 (scan speed 30 sec/scan). Palmitic acid was used as a standard fatty acid to quantify the liberated fatty acid.

Determination of Molecular Mass by Mass Spectrometer

Molecular mass of the phospholipase A_1 was determined by matrix assisted laser desorption ionization (MALDI) mass spectra. A Kratos Kompact (Kratos Analytical, Manchester, U.K.) time-of-flight (TOF) mass spectrometer equipped with a nitrogen laser (337 nm, 3ns pulse) was used for laser desorption. The accelerating voltage in the ion source was set to 30 kV. Data were acquired in the linear mode of operation and each spectrum was produced by accumulating data collected from 30 laser shots. Time-to-mass conversion was achieved by internal calibration using standards of cytochrome C and bovine serum albumin obtained from Sigma Chemical Co. All experiments were performed using sinapinic acid (Aldrich Chemical Co., Milwaukee, WI) as a matrix. A mixture of 1% trifluoroacetic acid (TFA) solution containing the internal standards and a protein sample was applied to a stainless-steel probe tip. Matrix-protein solutions were allowed to "air dry" and crystallized on the matrix surface prior to the introduction into the mass spectrometer (25).

Culture and Preparation of Enzyme Extract

A slightly modified M9 minimal salt medium was used to produce phospholipase A_1. The basic components of M9 minimal salt medium were: 15.12 g $NaHPO_4$ $12H_2O$, 3.0 g KH_2PO_4, 0.5 g NaCl, 0.246 g $MgSO_4$ $7H_2O$ and 0.0147 g $CaCl_2$ $2H_2O$ per 1 liter water. Based on the results of our previous works, 5 g/l of xylose and 1 g/l of ammonium sulfate were used as carbon and nitrogen sources, respectively. 1 μM of ferrous sulfate was added to the enzyme production media (11).

Seed cultures were cultivated aerobically for 12 h at 30°C with shaking at 250 rev/min using an orbital shaker (New Brunswick Scientific, Edison, NJ) in nutrient broth (Difco, Detroit, MI). A portion of the preinoculum culture was then transferred to 500 ml of production media as described above in a 3 liter flask. When phospholipase A_1 activity reached a plateau after 14 h of cultivation, cells were removed by centrifugation at 5,600×g for 20 min (Sorvall RC5-C, 4°C). Ammonium sulfate was added to the cell free extract to 30% saturation and the precipitate was discarded. The precipitate was formed by further addition of ammonium sulfate to 75% saturation. Precipitate was collected by centrifugation at 10,000×g at 4°C for 20 min. The precipitate was dissolved in 20 mM ethanolamine buffer (pH 10.0) and dialyzed against the same buffer (2 liters) for 18 h with 3 changes.

Enzyme Purification

The purification method consisted of sequential chromatography on QAE-Sepharose and Sephacryl S-200. The homogeneity of the preparation was detected by SDS-PAGE. All buffers were filtered through a 0.45 μm Millipore filter. All purification steps were performed at 4°C.

Ion Exchange Chromatography

The dialyzed enzyme solution was applied to a QAE-Sepharose column (2.2×15 cm) pre-equilibrated with buffer A (20 mM ethanolamine buffer pH 10.0). After washing with the same buffer, bound phospholipase A_1 was eluted by a linear gradient of 0-100% buffer B (1 M sodium chloride in buffer A). Elution was performed with a flow rate of 50 ml/h.

Table 1. Summary of the procedure for the purification of extracellular phospholipase A_1 from *Serratia* sp. MK1.

Step	Total protein (mg)	Total activity (units)	Specific activity (units/mg)	Recovery (%)	Fold purification
Enzyme extract	324	3480	10.7	100	
75% Ammonium sulfate	178	2040	11.5	58.6	1.1
Ion exchange	113	1480	13.1	42.5	1.2
Gel filtration	34	714	21	20.5	2.0

Gel Filtration Chromatography

The fractions showing a phospholipase A_1 activity after ion exchange chromatography were pooled and concentrated by ultrafiltration and then applied to a Sephacryl S-200 column (2.2×95 cm) equilibrated with buffer C (75 mM $CaCl_2$ and NaCl in buffer A). Elution was performed at a flow rate of 50 ml/h.

RESULTS AND DISCUSSION

Purification of Phospholipase A_1 from *Serratia* sp. MK1

Table 1 summarizes the purification of *Serratia* sp. MK1 phospholipase A_1 according to the method described in Materials and Methods. Phospholipase A_1 from *Serratia* sp. MK1 was purified approximately 2-fold over the enzyme extract with a 20.5% yield. The protein fractions collected from the QAE-Sepharose column were resolved into two major peaks and the first peak which was eluted at 0.3-0.4 M NaCl gradient (Fig. 1) showed lipolytic activity on phospholipid. In protein purification steps, phospholipase A_1 activity was determined by the bioluminescence method as described in methods using dimyristoyl phosphatidylcholine (DMPC) as a substrate. When the major fractions were further subjected to the Sephacryl S-200 gel filtration column, the proteins were resolved into two major peaks (Fig. 2). The enzyme activity was present in the second major peak. SDS-PAGE was performed to confirm the purity of phospholipase A_1 from *Serratia* sp. MK1 (Fig. 3, lane 4). The active phospholipase A_1 was detected as a single band on activity staining (Fig. 3, lane 5). As shown in Fig. 3, phospholipase A_1 band in the ammonium sulfate precipitation fraction was a major band and purification

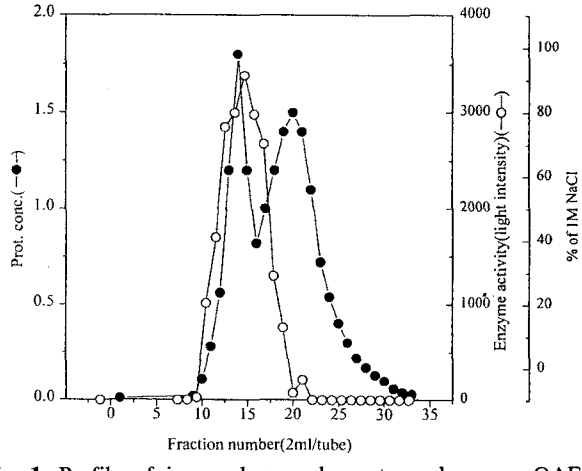

Fig. 1. Profile of ion exchange chromatography on a QAE-Sepharose column (2.2×15 cm) with a gradient of 1 M sodium chloride in 20 mM ethanolamine buffer (pH 10.0).

steps for this enzyme were very simple. But the first enzyme purification step showed a large drop in enzyme activity.

Biochemical Properties of Phospholipase A_1 from *Serratia* sp. MK1

Determination of molecular mass using mass spectrometer. A mass spectrometer is extensively applied for the determination of molecular mass of large, polar and labile compounds such as proteins, nucleic acids and polysaccharides (6, 10). Molecular weight determination by gel filtration chromatography and SDS-PAGE might involve a relatively large calibration error due to the differences in the degree of hydration and the friction ratio of protein. By contrast, the use of a mass spectrometer excludes these drawbacks and determines the accurate molecular mass of the protein because the protein is analyzed according to the absolute mass/charge ratio.

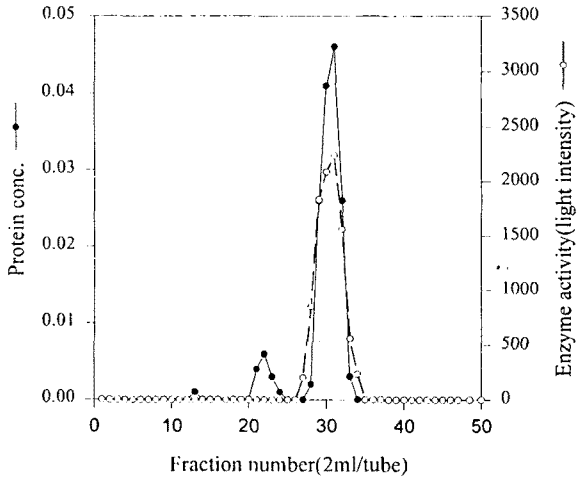

Fig. 2. Profile of gel filtration chromatography on a Sephacryl S-200 column (2.2×95 cm) with 20 mM ethanolamine buffer containing 75 mM of $CaCl_2$ and NaCl, respectively (pH 10.0).

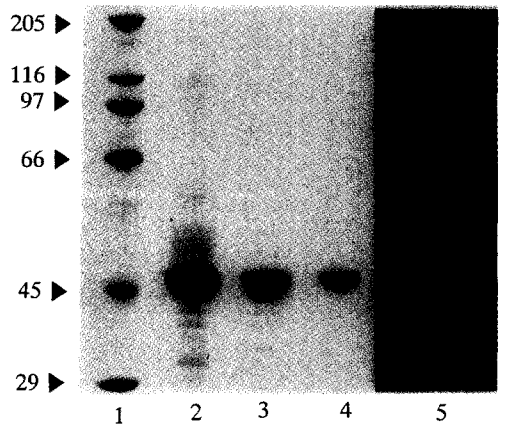

Fig. 3. Polyacrylamide gel electrophoresis and activity staining. Samples during purification was subjected to 10% polyacrylamide gel and stained with Coomassie brilliant blue R. Lane 1, molecular weight standards shown to the left in kDa; lane 2, 70% ammonium sulfate precipitate; lane 3, QAE-Sepharose elute; lane 4, Sephacryl S-200 elute; lane 5, activity staining on phospholipid.

Fig. 4 shows the mass spectrum and the two peaks corresponding 12.3 and 66.5 kDa were standard protein, cytochrome C from bovine heart and bovine serum albumin, respectively. A peak corresponding 43 kDa was phospholipase A_1 from *Serratia* sp. MK1.

Substrate specificity. To survey substrate specificity of the enzyme, each of phosphoglycerides (PC, PE, PS, PG, PI, lyso-PC, lyso-PE, SP, CA) were incubated with the enzyme and the amount of free fatty acids liberated from each of the phosphoglycerides was determined by the TLC-FID method. The free fatty acids produced were quantified using palmitic acid as a standard fatty acid and mono-palmitin as an internal standard. From the results, we can see that phospholipase A_1 from *Serratia* sp. MK1 did not hydrolyze CA and showed the highest lipolytic activity toward PS among the phosphoglycerides tested (Table 2). By contrast, rat liver and brain phospholipase A_1 showed a substrate preference on PE and hepatic lipase showed a substrate preference on PA and PE (4, 12). Particularly, this enzyme showed higher hydrolytic activity toward phosphatidic acid and produced lyso-phosphatidic acid which is used as a potential pharmaceutical reagent (9, 23).

Effects of inhibitors on enzyme activity. Effects of various inhibitors on the phospholipase A_1 activity were examined by assaying the remaining activity after incubating the enzyme at 25°C for 30 min with each inhibitor. As shown in Table 3, phenylmethylsulfonyl fluoride (PMSF) and *p*-chloromercuribenzoate (PCMB)

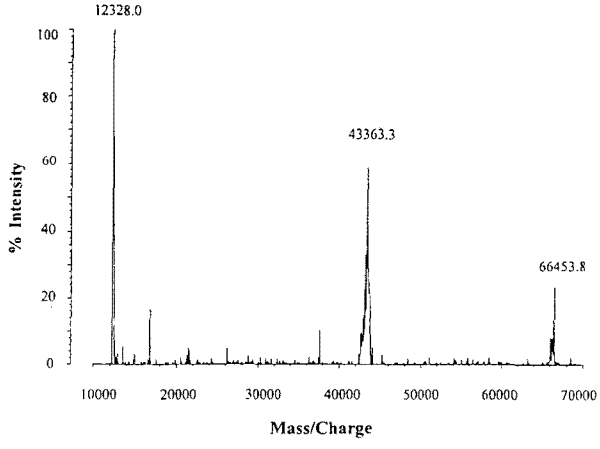

Fig. 4. MALDI-TOF spectrum of phospholipase A_1. Time to mass conversion was achieved by internal calibration using standard proteins such as cytochrome C (12.3 kDa) and bovine serum albumin (66.5 kDa).

Table 2. Hydrolysis rate of various phosphoglycerides by the phospholipase A_1 from *Serratia* sp. MK1.

Substrate	Concentration (mM)	Activity (units/mg protein)
Phosphatidylcholine (PC)	3.4	2.4
Phosphatidylethanolamine (PE)	3.4	0.6
Phosphatidylglycerol (PG)	3.4	3.2
Phsphatidylserine (PS)	3.4	14.5
Phosphatidylinositol (PI)	3.4	8.2
Phosphatidic acid (PA)	3.4	10.2
Lyso-phosphatidylcholine (Lyso-PC)	3.4	3.3
Lyso-phosphatidylethanolamine (Lyso-PE)	3.4	2.8
Sphingomyelin (SP)	3.4	7.2
Cardiolipin (CA)	3.4	0.2

Table 3. Effect of inhibitors on phospholipase A_1 activity.

Inhibitors	Residual activity	
	Concentration	
	1 mM	10 mM
Control	100	
PCMB	104±2	63±0.4
BPB	98±3	73±4
ME	112±2	95±9
SDS	102±1	126±1
DEP	103±0.4	86±0.6
PMSF	112±1	34±2
IA	101±0.4	95±7
NEM	108±7	78±5

PCMB, *p*-chloromercuribenzoate; BPB, 4-bromophenacyl-bromide; ME, 2-mercaptoethanol; SDS, sodium dodecyl sulfate; DEP, diethyl-pyrocarbonate; PMSF, phenylmethyl-sulphonyl fluoride; IA, iodoacetamide; NEM, N-ethylmaleimide.

inhibited the phospholipase A_1 activity. It was reported that PMSF and PCMB modify His. and Cys. residues in the active site, respectively. But SDS activated the enzyme activity slightly (Table 3).

Effects of metal ions on activity. In order to investigate the effect of metal ions on enzyme activity, various metal ions were added to a metal-free enzyme solution at 10 mM concentration. Metal free enzyme solution was made by chelating the metal ion using 10 mM EDTA. This metal chelated enzyme solution was dialyzed against distilled water for 12 h to remove EDTA. After chelating, enzyme solution showed no enzyme activity, and when 10 mM Ca^{2+} was added to the metal free enzyme solution, 90% of the original activity was recovered (Table 4). Fe^{2+} activated the enzyme slightly, but the other metal ions showed only negligible effects on the enzyme activity. The activation of the phospholipase A_1 was similar to that of the lipase. In the case of the lipase, calcium ion was specific for the

Table 4. Effect of metal ions on phospholipase A_1 activity.

Metal salts (10 mM)	Relative activity (%)
Control	100
EDTA	0
$CaCl_2$	89±5
$FeSO_4$	60±3
$MnCl_2$	16±0.8
$AlCl_3$	18±0.9
$CoCl_2$	5±0.3
$MgCl_2$	3±0.2
$BaCl_2$	4±0.2
$CuCl_2$	1±0.1
NaCl	0
KCl	0
$AgCl_3$	0
$ZnCl_2$	0

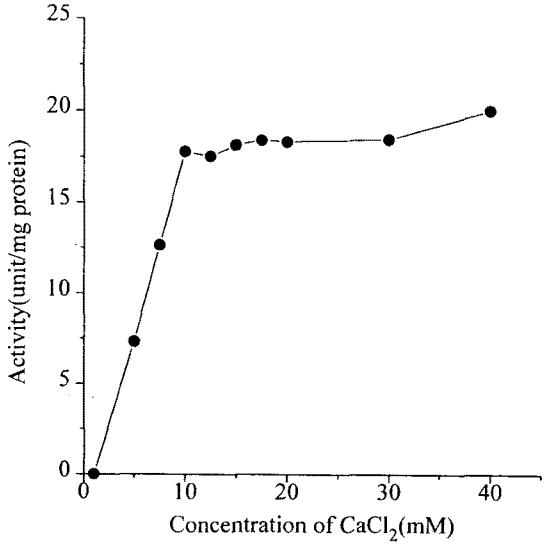

Fig. 5. Phospholipase A_1 activity as function of the cacium concentration.
The metal free enzyme solution was incubated with various concentration of calcium ion for 30 min. The activity was measured at 50°C, pH 8.5 by pH titration method.

activation of the enzyme (14). By contrary, it would appear that the function of the Ca^{2+} is the same as many metallic cations such as Mg^{2+}, Sr^{2+} and Ba^{2+} for many other deacylating phospholipases present in mammalian cells (4, 19). The activation of phospholipase A_1 vs. the increase of calcium ion concentration followed a saturation curve (Fig. 5) while the purified lipase was activated with a sigmoidal pattern (14).

Enzyme Characteristics of Purified Phospholipase A_1

Effects of pH on activity and stability. As shown in Fig. 6, phospholipase A_1 showed maximal enzyme activity at pH 8.5. To evaluate the stability of the enzyme for pH, the phospholipase A_1 was dialyzed in distilled

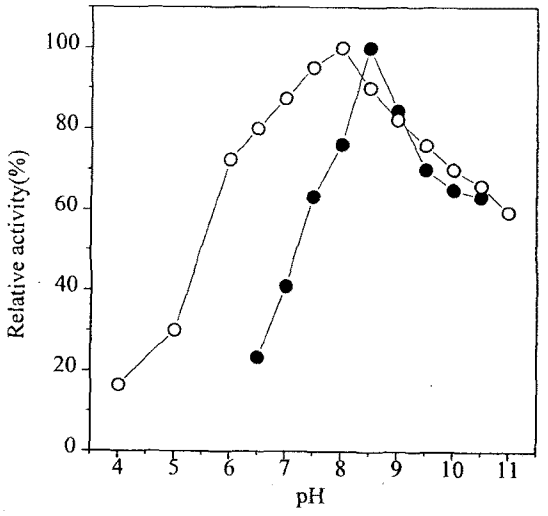

Fig. 6. Effect of pH on the phospholipase A_1 activity and stability.
The enzyme activity was assayed at various pHs (●) and the enzyme stability was measured after incubation of the enzyme at various pHs for 24 h at 25°C (○).

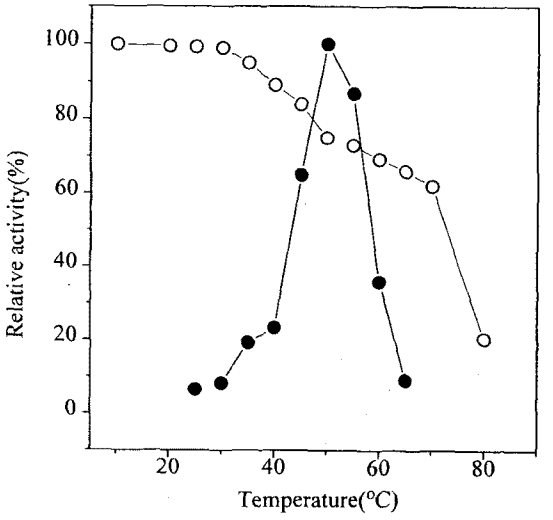

Fig. 7. Effect of temperature on the phospholipase A_1 activity and stability.
The phospholipase A_1 activity was measured at different temperatures (●) and the enzyme stability was measured after 60 min incubation at various temperatures (○).

water and then pre-incubated in Britton and Robinson buffer (18) of different pH's at 25°C for 24 h. The remaining activity was measured at pH 8.5 and 50°C by the pH titration method. The enzyme was quite stable at a pH range between 6-10 (Fig. 6).

Effects of temperature on activity and stability. The effect of temperature on enzyme activity and stability was investigated using the pH titration method. The maximal activity of the enzyme under the conditions employed was observed between 50-55°C when assayed at pH 8.5 (Fig. 7). To investigate thermal stability, the enzyme was incubated at several temperatures for 1 h at pH 8.5 and residual enzyme activity was determined by the pH titration method. The results showed that this enzyme was thermally quite stable up to 70°C, at which 60% of initial enzyme activity was retained after 1 h of incubation (Fig. 7).

REFERENCES

1. Aiyappa, P. S. and J. O. Harris. 1976. The extracellular metalloprotease of *Serratia marcescens*. I. Purification and characterization. *Mol. Cell. Biochem.* **13**: 95-100.
2. Baumann, U. 1994. Crystal structure of the 50 kDa metallo protease from *Serratia marcescens*. *J. Mol. Biol.* **242**: 244-251.
3. Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**: 248-254.
4. Dawson, R. M. C., R. F. Irvine, N. L. Hemington, and K. Hirasawa. 1983. The alkaline phospholipase A_1 of rat liver cytosol. *Biochem. J.* **209**: 865-872.
5. Cho, K. W. 1994. Bioluminescent assay of phospholipase A_2 using luminescent marine bacterium *Vibrio harvey* mutant M-17. *Kor. Biochem. J.* **27**: 522-525.
6. Egge, H., J. Peter-Katalinic, M. Karas, and B. Stahl. 1991. The use of fast atom bombardment and laser desorption mass spectrometry in the analysis of complex carbohydrates. *Pure Appl. Chem.* **63**: 491-498.
7. Givskov, M., L. Olsen, and S. Molin. 1988. Cloning and expression in *Escherichia coli* of the gene for extracellular phospholipase A_1 from *Serratia liquefaciens*. *J. Bacteriol.* **170**: 5855-5862.
8. Hames, B. D. and D. Rickwood. 1981. *Gel electrophoresis of proteins, a practical approach*, p. 1-92. IRL press, Washingto D.C.
9. Howe, L. R. and C. J. Marshall. 1993. Lysophosphatidic acid stimulates mitogen-activated protein kinase activation via a G-protein-coupled pathway requiring $p21^{ras}$ and $p74^{raf-1}$. *J. Biol. Chem.* **268**: 20717-20720.
10. Karas, M. and F. Hillenkamp. 1988. Laser desorption ionization of proteins with molecular masses exceeding 10,000 daltons. *Anal. Chem.* **60**: 2299-2301.
11. Kim, M. K., J. K. Kim, and J. S. Rhee. 1996. Isolation of phospholipase A_1 producing microorganisms. *J. Industrial Microbiol.* **16**: 171-175.
12. Kucera, G. L., P. J. Sisson, M. J. Thomas, and M. Waite. 1988. On the substrate specificity of rat liver phospholipase A_1. *J. Biol. Chem.* **263**: 1920-1928.
13. Lechner, M., H. Märkl, and F. Götz. 1988. Lipase production of Staphylococcus carnosus in a dialysis fermentor. *Appl. Microbiol. Biotechnol.* **28**: 345-349.
14. Lee, Y. P., G. H. Chung, and J. S. Rhee. 1993. Purification and characterization of *Pseudomonas fluorescens* SIK W1

lipase expressed in *Escherichia coli*. *Biochim. Biophy. Acta*. **1169**: 156-164.
15. Matsumae, H. and T. Shibatani. 1994. Purification and characterization of the lipase from *Serratia marcescens* Sr41 8000 responsible for asymmetric hydrolysis of 3-phenylglycidic acid esters. *J. Ferm. Bioeng*. **77**: 152-158.
16. Monreal, J. and E. T. Reese. 1969. The chitinase of *Serratia marcescens*. *Can. J. Microbiol*. **15**: 689-696.
17. Nyeste, L., B. Sevella, J. Holló, and F. Békés. 1976. Kinetic study of extracellular nuclease of *Serratia marcescens*. *Eur. J. Appl. Microbiol*. **2**: 161-168.
18. Perin, D. D. and B. Dempsey. 1974. *Buffers for pH and metal ion control*, p. 155. Chapman and Hall. London.
19. Pete, M. J., A. H. Ross, and J. H. Exton. 1994. Purification and properties of phospholipase A_1 from bovine brain. *J. Biol. Chem*. **269**: 19494-19500.
20. Raybin, D. M., L. L. Bertsch, and A. Kornberg. 1972. A phospholipase in *Bacillus megaterium* unique to spore and sporangia. *Biochem*. **11**: 1754-1760.
21. Renetseder, R., S. Brunie, B. W. Dijkstra, J. Drenth, and P. B. Sigler. 1985. A comparison of the crystal structures of phospholipase A_2 from bovine pancreas and *Crotalus atrox venom*. *J. Biol. Chem*. **260**: 11627-11634.
22. Scandella, C. J. and A. Kornberg. 1971. A membrane-bound phospholipase A_1 purified from *Escherichia coli*. *Biochem*. **10**: 4447-4456.
23. Tigyi, G., D. L. Dyer, and R. Miledi. 1994. Lysophosphatidic acid possesses dual action in cell proliferation. *Proc. Natl. Acad. Sci. USA*. **91**: 1908-1912.
24. Wells, M. A. 1972. A kinetic study of the phospholipase A_2 (*Crotalus adamanteus*) catalyzed hydrolysis of 1,2-dibutyryl-*sn*-glycero-3-phosphocholine. *Biochem*. **11**: 1030-1041.
25. Zoluzec, E. J., D. A. Gage, and J. T. Watson. 1994. Quantitative assessment of cysteine and cystin in peptides and proteins following organomercurial derivatization and analysis by matrix-assisted laser desorption ionization mass spectrometry. *J. Am. Soc. Mass Spectrom*. **5**: 359-366.

(Received July 25, 1996)