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An Oxidant- and Organic Solvent-Resistant Alkaline Metalloprotease from *Streptomyces olivochromogenes*

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Abstract Organic solvent- and detergent-resistant proteases are important from an industrial viewpoint. However, they have been less frequently reported and only few of them are from actinomycetes. A metalloprotease from *Streptomyces olivochromogenes* (SOMP) was purified by ion exchange with Poros HQ and gel filtration with Sepharose CL-6B. Apparent molecular mass of the enzyme was estimated to be 51 kDa by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and gelatin zymography. The activity was optimum at pH 7.5 and 50 °C and stable between pH 7.0 and 10.0. SOMP was stable below 45 °C and Ca^{2+} increased its thermostability. Ca^{2+} enhanced while Co^{2+}, Cu^{2+}, Zn^{2+}, Mn^{2+}, and Fe^{2+} inhibited the activity. Ethylenediaminetetraacetic acid and ethylene glycol-bis (β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid, but not phenylmethylsulfonyl fluoride, aprotinin, and pefabloc SC, significantly suppressed the activity, suggesting that it might be a metalloprotease. Importantly, it is highly resistant against various detergents, organic solvents, and oxidizing agents, and the activity is enhanced by H_2O_2. The enzyme could be a novel protease based on its origin and peculiar biochemical properties. It may be useful in biotechnological applications especially for organic solvent-based enzymatic synthesis.

Keywords Oxidant and solvent resistant · Metalloprotease · *Streptomyces olivochromogenes*

Introduction

Demand for biocatalysts able to cope with industrial process conditions is insatiably increasing, and continuous efforts have been focused for the search of such enzymes.

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Microbial proteases represent approximately 40% of the total worldwide enzyme production, and proteases from bacterial sources are more significant compared to animal, vegetable, or fungal proteases [1, 2]. These enzymes are useful in the physiological and commercial fields such as in food processing, tannery, laundry detergent, feather digestion, and in other chemical or pharmaceutical industries [1–3].

Under normal aqueous conditions, proteases disintegrate proteins or selectively hydrolyze specific peptide bonds, but the direction of the reaction can be reversed in the presence of organic solvents or solvents with low water content. Moreover, in non-aqueous media, enzymes can catalyze reactions which are almost impossible in aqueous media, remain more stable, and exhibit novel property such as molecular memory; consequently, enzyme utility is greatly enhanced in non-aqueous media compared to natural aqueous media [4, 5]. Although enzymes application in organic solvents has scores of advantages, they are generally unstable in such media. Therefore, various physical and chemical methods such as chemical modification, immobilization, protein engineering, medium engineering, etc. have been employed to make enzymes suitable for organic media [6]. Naturally organic solvent-stable enzymes thus will be a promising alternative of these expensive and lengthy modification processes.

Furthermore, proteases which are active and stable in alkaline environment are important for biotechnological application, particularly in detergent industry. The performance of laundry detergent proteases is influenced by various factors such as pH and temperature of the washing solution and ingredients of the detergent. Potential detergent protease must be active and stable under harsh conditions such as high temperature, alkaline pH, and in the presence of surfactants or oxidizing agents [1, 7]. Detergent proteases available today such as Subtilisin Carlsberg (SC), Subtilisin Novo (Bacterial Protease Nagase BPN′), Savinase™, Esperase™, and Alcalase™ are stable at high temperature and pH. But most of them are unstable in the presence of modern bleach-based detergent ingredients such as nonionic surfactants (Tween-80), anionic surfactants (sodium dodecyl sulfate, SDS), and oxidizing agent (H_2O_2) [8, 9].

We have reported a novel phospholipase D, an enzyme of industrial importance, from *Streptomyces olivochromogenes* [10]. The strain was also found to produce a profound proteolytic activity in the culture medium. Thus, we were interested in its potential biocatalytic application. The present study is significant because few organic and detergent-stable enzymes have been reported so far and only few of them are from actinomycetes, a large group of organism capable of producing various useful products including antibiotics. Here, we describe purification and biochemical characteristics of an extracellular protease produced by the strain.

Materials and Methods

Materials

Azocasein, Folin–Ciocalteu's phenol reagent, and tyrosine were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sepharose CL-6B was obtained from Pharmacia (Uppsala, Sweden) and Poros HQ was acquired from Applied Biosystems (Foster City, CA, USA). Protease inhibitors pefabloc SC, aprotinin, and ethylenediaminetetraacetic acid (EDTA) were from Roche Applied Science (Mannheim, Germany). All other chemicals and reagents used were of analytical grade.

Bacterial Strain and Culture Conditions

S. olivochromogenes was cultured in a medium supplemented with (g/L) glucose 15.0, oat meal 15.0, K_2HPO_4 3.0, and NaH_2PO_4 3.0. Cultivation was continued for 48 h in a rotary shaker set at 28 °C and 160 rpm agitation.

Protein Assay

Protein concentrations were determined at 595 nm according to previously described methods [11] using bovine serum albumin as the standard protein.

Proteolytic Activity Assay

Protease activity was determined by previously described methods [12, 13] with modifications [14]. In brief, the reaction mixture (total 0.15 mL) containing 0.05 mL of azocasein solution (1 mg/mL azocasein in 10 mM Tris–HCl pH 7.5) and aliquots of enzyme sample (0.1 mL) were incubated at 50 °C for 20 min. The reaction was terminated by adding 0.06 mL 10% (*w*/*v*) trichloroacetic acid followed by immersion in ice water for 10 min. After centrifugation at 10,000×*g* for 20 min, the supernatant (0.15 mL) was mixed with 0.3 mL Folin–Ciocalteu's phenol reagent (0.33 M) and 0.45 mL Na_2CO_3 (10%, *w*/*v*) solution and then incubated at 30 °C for 30 min. Absorbance of the produced dye was read at 660 nm using a UV-1601 spectrophotometer (Shimadzu Corporation, Japan). To assess the enzyme activity, a tyrosine standard curve was constructed with 1–15 mg/L tyrosine and one unit (U) of the enzyme activity was defined as the enzyme amount producing 1 μg tyrosine per minute from azocasein at the assay condition.

Purification of SOMP

Ion Exchange with Poros HQ

S. olivochromogenes culture supernatant was harvested at 48 h by centrifugation at 10,000×*g* for 1 h. Solid ammonium sulfate was added at various saturations (0–30%, 30–75%, and 75–95%) and the fraction with highest specific activity (30–75%) was dialyzed against 10 mM Tris–HCl buffer (pH 7.5) and concentrated with an ultrafiltration membrane YM30 (Millipore Corp.). The dialysate was loaded onto a Poros HQ anion exchanger (10× 1 cm), pre-equilibrated with 10 mM Tris–HCl (pH 7.5). The matrix bound proteins were eluted with zero to 0.5 M KCl in the same buffer at 30 mL/h (2 mL/fraction).

Gel Filtration with Sepharose CL-6B

The active fractions eluted from the ion exchange chromatography were pooled, concentrated, and further purified with Sepharose CL-6B column (70×1.3 cm), pre-equilibrated with 10 mM Tris–HCl buffer (pH 7.5). Proteins were eluted with the same buffer at 24 mL/h (2 mL/fraction).

Gel Electrophoresis

In order to determine protein purity and molecular mass, sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) was carried out using a 10% polyacrylamide resolving gel

according to Laemmli [15]. A low molecular weight marker (Fermentas) was used as reference proteins and molecular mass was estimated by comparing relative mobility of the marker proteins. The gel was stained with Coomassie Brilliant Blue R-250 and destained with a solution containing methanol/glacial acetic acid/distilled water=1:1:8 (by vol.).

Zymography

Gelatin zymography was carried out according to Heussen and Dowdle [16] with minor modifications. Then 1% gelatin (*w*/*v*) was mixed with 10% polyacrylamide gel. After electrophoresis at 150 V and 40 mA for 45 min, the gel was washed with 2.5% (*v*/*v*) Triton X-100 for 1 h, rinsed with distilled water, and incubated in 10 mM Tris–HCl pH 7.5 at room temperature for 24 h. Staining and destaining were performed as in SDS–PAGE. A clear zone against blue background on the gel revealed the protease activity.

Effect of pH and Temperature

Effects of pH and temperature were evaluated with azocasein under standard assay conditions described earlier. pH optimum was determined by carrying out the enzyme assay at 50 °C and various pH values (3.0–12.0) using 100 mM standard buffers: citric acid–sodium phosphate (pH 3.0–7.0), Tris–HCl (pH 7.5–9.0), $NaHCO_3$ (pH 9.6–11.0), and KCl–NaOH (pH 12.0). Temperature optimum of SOMP was assessed by carrying out the assay from 4 to 80 °C at pH 7.5. Similarly, temperature stability of SOMP was examined by incubating the enzyme samples at 45, 50, and 55 °C for 15 to 60 min. pH stability of the enzyme was determined by incubating the enzyme samples with various 100 mM buffers (pH 3.0–12.0) at 4 °C for 8 h. The remaining activities in both cases were measured under standard assay protocol and calculated considering the initial activity (prior to exposing various temperatures or pH) as 100%.

Effect of Protease Inhibitors and Oxidizing and Reducing Agents

The effects of various enzyme inhibitors were examined by incubating the protease sample with EDTA, ethylene glycol-bis (β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), aprotinin, pefabloc SC, or phenylmethylsulfonyl fluoride (PMSF) for 1 h at room temperature before measuring the remaining activity against azocasein. In the same way, effects of oxidizing agents H_2O_2 and sodium perborate, and reducing agent β-mercaptoethanol, were observed.

Effect of Metal Ions, Detergents, and Organic Solvents

The effect of various metal ions such as KCl, $CaCl_2$, $MgCl_2$, $MnSO_4$, $CuCl_2$, $ZnCl_2$ $FeSO_4$, and $CoCl_2$ were investigated by adding them to the reaction mixtures. Suitably diluted enzyme samples were incubated with detergents such as Triton X-100, Tween-20, Tween-80, and SDS at room temperature for 1 h and then the residual activities were measured under the standard assay condition. Organic solvent stability of SOMP was evaluated according to previously described methods [17, 18] with slight changes. Enzyme samples were mixed with organic solvents (25%, *v*/*v*) in Eppendorf tubes, made airtight with parafilm, and incubated in a rotary shaker set at 160 rpm and 28 °C for 72 h and then the residual activities were measured. Methanol, ethanol, butanol, isopropanol, hexane,

dichloromethane, acetonitrile, ethylacetate, acetone, diethylether, xylene, and dimethylsulfoxide (DMSO) were used to determine the enzyme stability.

Determination of Kinetic Parameters

K_m and V_{max} values of the enzyme were determined by measuring the activity with various concentrations of substrate azocasein (0.1–10 mg/mL). Kinetic constants were calculated from the Lineweaver–Burke plot [19].

Results

Enzyme Purification and Molecular Weight

A proteolytic enzyme from *S. olivochromogenes* was purified to electrophoretic homogeneity by a three-step protocol entailing $(NH_4)_2SO_4$ fractionation, anion exchange chromatography (Fig. 1a), and size exclusion chromatography (Fig. 1b). The purification summary of SOMP is summarized in Table 1. The purification protocol resulted in a 12.8-

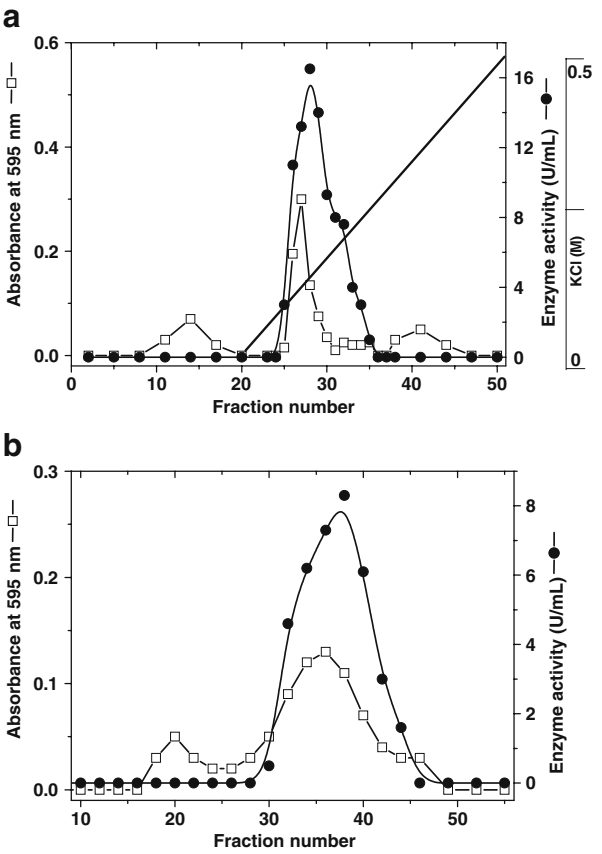

Fig. 1 Elution profile of SOMP from **a** ion exchange with Poros HQ and **b** gel filtration with Sepharose CL-6B column. Protein concentration (*open squares*) and the protease activity (*filled circles*) of each fraction were measured at 595 and 660 nm, respectively. The enzyme activity was expressed in terms of units per milliliter

Table 1 Summary of SOMP purification.

Purification step	Total protein (mg)	Total activity (U)	Specific activity (U/mg)	Yield (%)	Purification (fold)
Crude supernatant	83.9	1,352	16.1	100	1
$(NH_4)_2SO_4$	48.6	1,194.1	24.6	88.3	1.5
Poros HQ	1.1	188	170.9	13.9	10.6
Sepharose CL-6B	0.8	165	206.3	12.2	12.8

fold increase in specific activity with 12.2% activity recovery (yield). Purity and molecular mass of the enzyme were estimated by SDS–PAGE, in which the enzyme migrated as a single protein band (Fig. 2), corresponding to an apparent molecular mass of 51 kDa. In the activity staining step (gelatin zymography), the enzyme appeared as a single band corresponding to the same protein band that appeared in SDS–PAGE (Fig. 2).

Effect of Temperature and pH

Effects of pH and temperature on SOMP activity and stability are shown in Figs. 3 and 4 and compared with other detergent and/or organic solvent-stable proteases (Table 2). SOMP remained active from pH 7.0 to 10.0 with the optimum at pH 7.5. The relative activity at pH 10.0 was about 66%. The enzyme was highly stable at a pH range of 7.0–10.0, retaining more than 80% of its initial activity when measured after 8 h (Fig. 3). Moreover, it showed highest catalytic activity at 50 °C and the activity remained virtually unaltered at 45 °C when measured after 1 h (Fig. 4).

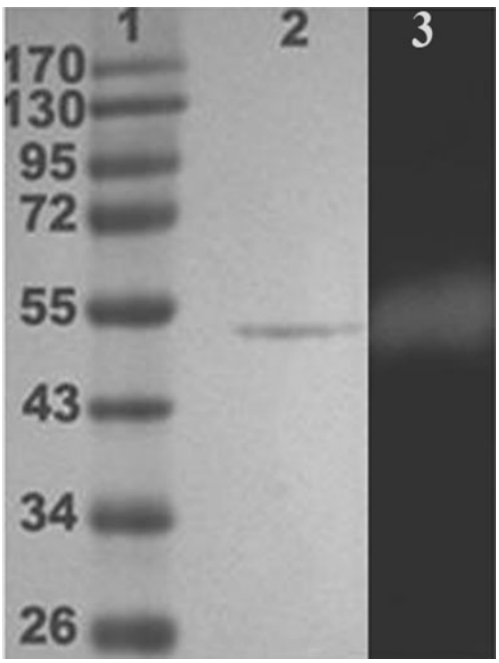

Fig. 2 Electrophoretic analysis of SOMP was carried out in 10% (*w*/*v*) polyacrylamide gel and stained with Coomassie Brilliant Blue R-250. *Lane 1* protein size marker (sizes in kilodaltons are shown at the *left* of each band). *Lane 2* purified SOMP (1.5 μg). *Lane 3* gelatin zymography

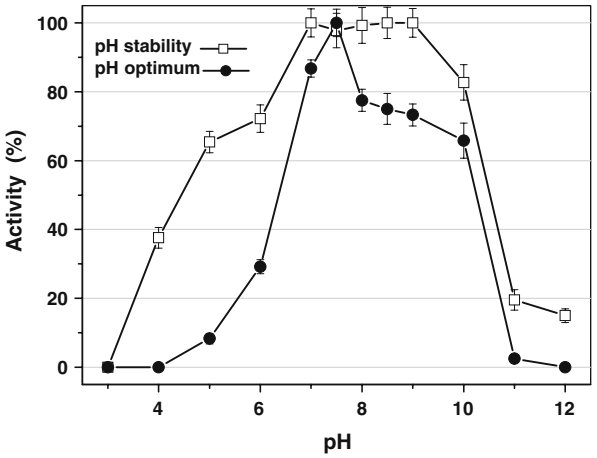

Fig. 3 Effect of pH in activity and stability of SOMP. The optimum pH (*filled circles*) was determined by assessing the enzyme activity at 50 °C in 100 mM buffers (pH 3.0–12.0). The pH stability (*open squares*) was determined by measuring the residual activity at 50 °C and pH 7.5 after aliquots of the enzyme samples were incubated in 100 mM buffer (pH 3.0–12.0) at 4 °C for 8 h. Buffers used as standard in both cases were: citric acid–sodium phosphate (pH 3.0–7.0), Tris–HCl (pH 7.5–9.0), $NaHCO_3$ (pH 9.6–11.0), and KCl–NaOH (pH 12.0). Each *point* represents the mean (n=3) ± standard deviation

Effects of Protease Inhibitors

SOMP activity was slightly affected by serine protease inhibitors PMSF, aprotinin, and pefabloc SC. Conversely, the activity was completely inhibited in presence of EDTA and EGTA, indicating that it might be a metalloprotease (Table 3).

Effects of Metal Ions, Detergents, and Oxidizing and Reducing Agents

The metal ions had varied effect on the enzyme activity. There was an increase in protease activity, approximately by 30%, with 2.5 mM Ca^{2+}, and the activity was found decreasing with more than 2.5 mM (Fig. 5a). Therefore, we investigated its effect on the thermal stability of SOMP at 2 mM. Calcium increased almost 36% of the residual activity than the case without the ion after incubating the enzyme at 50 °C for 1 h (Fig. 5b). However, the enzyme activity was not affected by Mg^{2+} and K^{+}; significantly inhibited by Co^{2+}, Zn^{2+}, and Cu^{2+}; and completely inhibited by Mn^{2+} and Fe^{2+} (Table 4). The enzyme remained highly stable in all tested nonionic detergents but remained partially stable in presence of anionic detergent SDS (Table 4). Interestingly, the activity was not only resistant but also significantly enhanced in the presence of oxidizing agent H_2O_2 in a concentration-dependent manner until 2%. But, in the presence of other oxidizing agent sodium perborate at 0.1%, the activity was marginally enhanced although intact activity remained stable (Table 4). Almost 65% of the initial activity found conserved in the presence of 0.5 mM β-mercaptoethanol showing that SOMP is partially stable in the presence of a reducing agent (Table 4).

Effect of Organic Solvents

The stability of SOMP in the presence of various organic solvents with log P values 5.6 to −1.37 was compared with other organic solvent-stable proteases (Table 5). It clearly shows that SOMP is highly stable in most of the solvents regardless of their log P values.

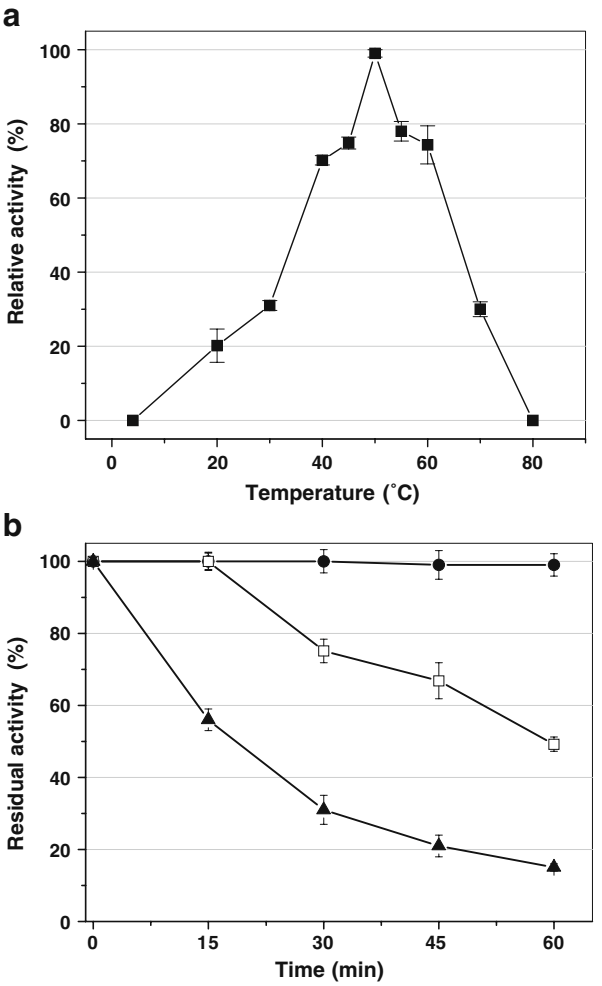

Fig. 4 Effect of temperature in SOMP activity and stability. **a** Temperature optimum was determined by assaying the activity at various temperatures for 20 min at pH 7.5. **b** Thermal stability of SOMP was assessed by measuring the residual activity after enzyme samples were incubated at 45 °C (*filled circles*), 50 °C (*open squares*), or 55 °C (*filled triangles*) for 15–60 min. Each *point* represents the mean (n=3) ± standard deviation

Kinetic Parameter

K_m and V_{max} values of SOMP determined with substrate azocasein were 0.74 mg/mL and 3,876 U/mg, respectively.

Discussion

Enzymes which are active and stable in the presence of oxidants, surfactants, denaturants, and organic solvents as well as in broad pH and temperature range are important from an industrial perspective. In this study, we have presented purification and biochemical characteristics of an organic solvent-, detergent-, and oxidant-stable metalloprotease, designated as SOMP, from *S. olivochromogenes*. SOMP was purified to homogeneity by ammonium sulfate fractionation, followed by sequential chromatographic steps on Poros HQ and Sepharose CL-6B. In the electrophoretic analysis, the enzyme appeared as a single

Table 2 Biochemical properties of SOMP and other solvent- and/or detergent-stable proteases.

Enzyme source	MW (kDa)	Optimal		pH stability			T stability			Ref.
		pH	*T*	pH	Time	RA %	*T*	Time	RA%	
S. olivochromogenes	51	7.5	50	7–9	8 h	100	45	1 h	100	This work
B. mojavensis A21 (BM1)	29	8–10	60	7–10	1 h	100	50	1 h	>55	[1]
B. mojavensis A21 (BM2)	15.5	10	60	8–9	1 h	100	50	1 h	>55	[1]
B. mojavensis A21	20	8.5	60	7–12	1 h	100	<40	3 h	100	[2]
S. clavuligerus	49–50	10	70	8–11	6 h	>90	50	1 h	~100	[3, 20]
B. cereus	28	10	60	8–11	1 h	>80	60	1 h	>92	[32]
P. aeruginosa	34	8	60	6–10	1 h	81	40	2.5 h	85	[33]
B. cereus	45.6	7	45	6–9	1 h	85	50	2 h	55	[34]
B. pumilus CBS	34.6	9–10.6	65	9–10.6	1 h	>90	50	1 h	66	[21]
B. cereus SV1	35.5	8	55	7.5–10	15 min	>80	60	1 h	7.9	[35]
S. tendae	21	6	70	4–9	1 h	100	60	0.5 h	70	[36]

RA residual activity, *T* temperature (°C), *time* incubation period, *S Streptomyces*, *B Bacillus*, *P Pseudomonas*

band corresponding to 51 kDa, which gives the characteristics of the protease from *Streptomyces clavuligerus* [20]. The V_{max} value of SOMP measured with azocasein is 3,876 U/mg, which is between those of detergent-stable proteases BPN′ (2,871 U/mg) and SAPB (12,870 U/mg) measured with the same substrate [21]. Additionally, the V_{max}/K_m of SOMP is almost 72-fold higher than that of FP84, a fibrinolytic protease purified from *Streptomyces* sp. CS684, determined with the same substrate azocasein [14], indicating that SOMP is more efficient than FP84 in azocasein hydrolysis. The enzyme activity was almost completely inhibited by EDTA and EGTA, and it remained less sensitive with serine protease inhibitors PMSF, aprotinin, and pefabloc SC, suggesting that it might be a metalloprotease. SOMP is highly active and stable between pH 7.0 and 10.0, indicating that it is an alkaline protease. The effect of pH and temperature of SOMP, as illustrated in Table 2, shows that all parameters are within the range of detergent and/or organic solvent-stable proteases. SOMP remained highly stable in presence of nonionic detergents Triton

Table 3 Effect of various enzyme inhibitors on SOMP stability.

Inhibitor	Concentration	Residual activity (%)
EDTA	1 mM	13.9±2.1
	2 mM	3.6±1.3
EGTA	1 mM	26.5±1.5
	2 mM	6.3±2.5
PMSF	5 mM	98.1±2.5
	10 mM	97.4±3.0
Aprotinin	2 μg/mL	87.2±4.5
Pefabloc SC	4 mM	75.7±5.3
None		100±1.5

Enzyme samples were pre-incubated with various protease inhibitors for 1 h at room temperature before determining the residual activity. Enzyme activity measured in the absence of any inhibitor was taken as 100%. Results are presented as means ± standard deviation (n=3)

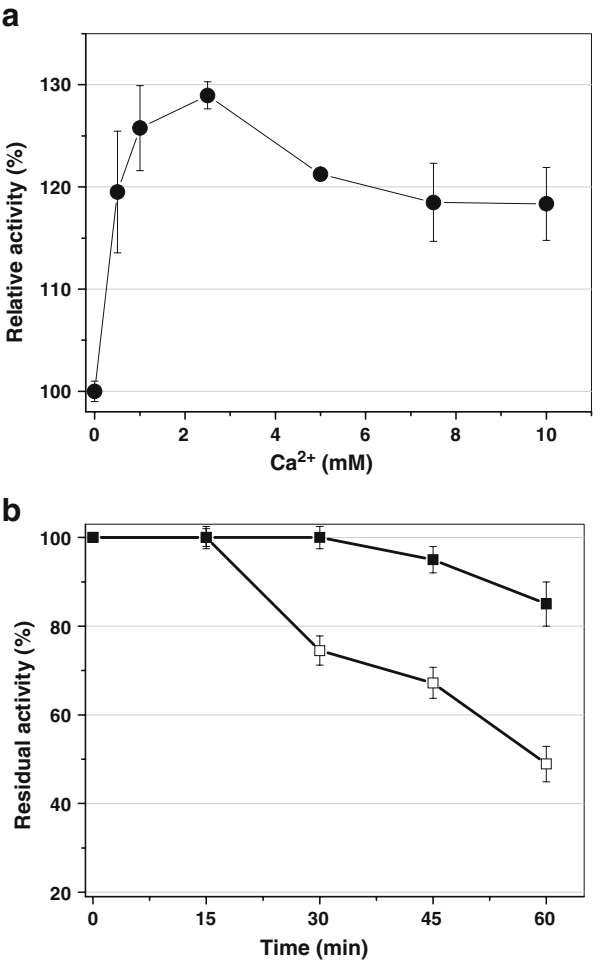

Fig. 5 Effects of calcium on activity and thermal stability of SOMP. **a** Effect of calcium was determined with the enzyme assay in the presence of various concentrations of calcium under the standard assay protocol. **b** The effect of calcium on the thermal stability of SOMP was assessed by measuring the residual activity after aliquots of enzyme samples were incubated at 50 °C in the presence (*filled squares*) or absence (*open squares*) of 2 mM calcium for 15–60 min. Each *point* represents the mean (n=3) ± standard deviation

X-100, Tween-20, and Tween-80. More than 50% of original activity remained stable even in presence of anionic detergent SDS. This feature of detergent stability may make SOMP useful in detergent industry. SDS stability of SOMP is comparable with metalloprotease CP-1 and CP-2 of *Serratia rubidaea* strain [22], while it is slightly lower than surfactant-stable alkaline serine proteases from *Bacillus mojavensis* A21 [1, 2]. SOMP, when compared to serine proteases from *Bacillus mojavensis* A21, is less stable with reducing agents but more stable with oxidizing agents [1, 2]. Interestingly, oxidizing agent H_2O_2 significantly enhanced SOMP activity in a concentration-dependent manner and the maximum activity was at 2%, which is comparable with detergent-stable protease from *Bacillus pumilus* CBS [21]. This feature further substantiates its potential biotechnological applications such as a detergent additive. Although the underlying mechanism is not clear, it is most likely that oxidizing agent disintegrates the substrate azocasein into small fragments. Enzyme can hydrolyze the disintegrated substrate faster than the integrated one, thus increased concentration of oxidizing agents enhances the activity up to a certain limit. Beyond the optimum concentration, the enzyme conformation may be altered as in the case of excess H^+, which inactivates the enzyme. Calcium not only increased the activity but

Table 4 Effect of metal ions on enzyme activity and detergents, and oxidizing and reducing agents on the enzyme stability.

Additive	Concentration	Activity (%)
None		100±1.1
Metal ions		
Ca^{2+}	5 mM	121±2.7
Mg^{2+}	5 mM	99.8±4.4
Co^{2+}	5 mM	33.2±5.3
Cu^{2+}	5 mM	14.1±1.6
Zn^{2+}	5 mM	10±0.25
K^{+}	5 mM	99.4±1.3
Mn^{2+}	5 mM	0
Fe^{2+}	5 mM	0
Detergents		
Triton X-100	1%	100±0.2
Tween-20	1%	101±0.35
Tween-80	1%	102±0.5
SDS	0.10%	62±3.3
	0.50%	51±2.5
Oxidizing agents		
H_2O_2	0.1%	104.2±1.8
	0.25%	122.7±5.7
	0.5%	136.9±5.6
	1%	147±3.4
	2%	175±6.5
	3.5%	159±5
	5%	120±4.8
Sodium perborate	0.1%	102.8±1.8
	0.5%	47.5±4.8
Reducing agent		
β-Mercaptoethanol	0.5 mM	65±2.5
	1 mM	43.9±2.3

Results are presented as means ± standard deviation (n=3)

also increased thermal stability of SOMP as in metalloendopeptidase from *Streptomyces septatus* TH-2 [23]. Ca^{2+} is a well-known stabilizer of many alkaline proteases, protecting them from conformational changes such as against influences of higher pH and temperature [13]. A previous study regarding Ca^{2+} binding sites on some bacterial proteases showed that they contain a number of co-coordinating Asp and Glu residues [24]. We believe that SOMP may contain such coordinating residues, based on its thermostability enhancement caused by Ca^{2+}.

Organic solvents affect the enzyme activity by directly interacting with the essential water surrounding the enzyme molecule, which depends upon the polarity of the solvent. Highly polar solvents vigorously absorb the essential water from the enzyme, thereby losing the catalytic property of the enzyme [5]. The toxicity of the organic solvents is measured by their log P value. In general, solvents with log P values below 4 are toxic

Table 5 Effect of organic solvents on the stability of SOMP and other proteases.

Organic solvent	Log *P*	This work	[17]	[6]	[37]	[18]	[33]	[3]
Decane	5.6	92	142	103	102	nd	nd	nd
Octane	4.9	118	158	103	nd	nd	nd	nd
Heptane	4.0	132	125	102	90	nd	nd	nd
Hexane	3.5	115	96	103	75	110	nd	nd
Xylene	3.1	82	98	nd	97	103	nd	65
Toulene	2.5	115	98	100	92	nd	nd	nd
Benzene	2.0	95	92	127	98	68	nd	17
Dichloromethane	1.25	112	nd	nd	nd	nd	nd	nd
Diethylether	0.87	100	nd	nd	nd	nd	nd	nd
Ethylacetate	0.71	107	nd	nd	nd	nd	nd	nd
Isopropanol	0.074	23	nd	23	nd	102	9.7	nd
Acetone	−0.21	58	nd	17	nd	nd	nd	79
Ethanol	−0.24	25	36	52	112	97	40	24
Acetonitrile	−0.39	21	nd	nd	nd	101	nd	nd
Methanol	−0.76	90	35	nd	nd	105	100	nd
DMSO	−1.37	109	nd	100	nd	nd	100	nd
None	–	100	100	100	100	100	16	100

Number in square bracket represents the reference number and 'nd' means that the data is not available

since their degrees of partitioning into the aqueous layer are higher [17]. Enzymes stable in such toxic conditions may be greatly helpful in bioremediation and biocatalysis in the presence of an organic phase [25]. SOMP is highly stable in range of solvents but its stability does not correlate with the log *P* values. It is in agreement with previous reports [6, 26]. SOMP activity was not only stable but also enhanced by octane, heptanes, hexane, toluene, dichloromethane, ethylacetate, and DMSO by various extents, which is highly encouraging. Ogino et al. [27] described that the disulfide bond existing between Cys-270 and Cys-297 in PST-01, an organic solvent-stable protease derived from organic solvent-tolerant *Pseudomonas aeruginosa*, was essential for the enzyme activity. Similarly, the disulfide bond between Cys-30 and Cys-58 played an important role in the organic solvent stability of the protease. In the light of this finding, we presume that such disulfide bonds might exist in SOMP, which make it resistant against various organic solvents. It has been shown that solvents such as DMSO, *N*,*N*-dimethyl formamide, methanol were beneficial in enzymatic organic synthesis [3, 6, 23, 28–30]. Several proteases such as thermolysin, chymotrypsin, and papain have been used as biocatalysts in organic-based peptide synthesis; however, these proteases were not satisfactorily stable in the presence of an organic solvent [30]. Since SOMP is highly stable in the presence of highly polar solvents like DMSO and methanol used in organic synthesis, it can be a promising biocatalyst for organic solvent-based enzymatic synthesis. Additionally, although solvent- and/or detergent-stable proteolytic enzymes have been extensively reported from various bacteria and fungi, these enzymes from actinomycetes are rare. Characteristics of SOMP such as solvent, oxidant, and detergent stability along with pH and temperature profiles are different from those of stable proteases reported from actinomycetes strains [3, 20, 31] and from other sources (Tables 2 and 5); therefore, it might be a novel proteolytic enzyme.

Conclusion

An alkaline metalloprotease was purified from culture medium of *S. olivochromogenes*. It is highly stable in the presence of oxidants, detergents, and organic solvents. SOMP could be a novel protease based on its producing source and peculiar stability characteristics. The alkaline protease may be useful in a wide range of biotechnological applications especially in organic solvent-based enzymatic synthesis and detergent formulation.

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