C-Terminal Peptide of *Fusarium heterosporum* Lipase Is Necessary for Its Increasing Thermostability

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Saccharomyces cerevisiae bearing a lipase cDNA from *Fusarium heterosporum* produced two lipases, A and B. Lipase B was significantly more stable to temperature than lipase A, but their optimum temperatures were similar. Lipase B was composed of one polypeptide (301 amino acids), and lipase A was composed of two polypeptides (275 and 26 amino acids) generated by the cleavage between Arg275 and Asp276 with a trypsin-like protease. It was suggested that the C-terminal peptide (26 amino acids) tightened the lipase structure when bound to the catalytic domain (275 amino acids) through a peptide bond. The tight structure was loosened by cleavage of the C-terminal peptide, even though the peptide interacted noncovalently with the catalytic domain, possibly through charged amino acids, in which it is rich. Deletion of the C-terminal peptide greatly decreased the lipase production by the recombinant *S. cerevisiae*, although its transcriptional level was the same as that of cells carrying the wild-type gene. These facts suggested that the C-terminal peptide affected the lipase production in the post-transcriptional step.

Key words: C-terminal peptide, *Fusarium heterosporum*, lipase, stability.

A wide range of extracellular proteins are initially synthesized as preproteins bearing signal sequences that are cleaved off to yield mature products (*1*). In addition, hydrolases, growth factors, and hormones possess N-terminal prosequences, which are generally removed by proteolysis. Many proteases are synthesized as preproenzymes, and the N-terminal prosequences not only inhibit enzyme activities but also support the folding of the enzymes (*2-4*). However, there have been only a few studies on the prosequence in the C-terminal region: aqualysin from *Thermus aquaticus* (*5, 6*), IgA protease from *Neisseria gonorrhoeae* (*7*), and serine protease from *Serratia marcescens* (*8*).

Lipase [EC 3.1.1.3] is characterized by the ability to catalyze the hydrolysis of triglycerides at the interface between oil and water (*9-11*), and the structure of the catalytic triad is very similar to that of serine protease (*11-13*). Microbial lipases are classified into five groups, *Staphylococcus, Pseudomonas cepacia, Pseudomonas fluorescens, Geotrichum,* and *Rhizopus,* on the basis of their primary structures, and the enzymes in the same family show similar tertiary structure, reactivity, and production mechanism (*14*). Lipases homologous to *P. cepacia* lipase have folded active structures in the presence of a chaperone-like protein, which is the product of a gene lying downstream of the lipase gene (*15-18*). Lipase from *Rhizopus oryzae* is synthesized as a preproenzyme, and the N-terminal prosequence is important for the folding of the protein, as it is for many proteases (*19*).

We isolated *Fusarium heterosporum* producing a 1,3-specific lipase which is more solvent-tolerant than the lipases reported previously (*20, 21*). Its primary structure, which was estimated from the nucleotide sequence of the lipase cDNA (*22*), is homologous to those of the lipases from *Rhizomucor miehei* (*23*), *Rhizopus delemar* (*24*), *R. oryzae* (*19*), and others. It consists of a presequence (16 amino acids), a prosequence (16 amino acids), and a mature lipase (301 amino acids) (*22*). A high-level production system was constructed using *Saccharomyces cerevisiae* transformed with an expression plasmid, which was prepared by ligating the cDNA between the *GAP* (the gene coding glyceroaldehyde-3-phosphate dehydrogenase from *S. cerevisiae*) promoter and its terminator (*25-27*). The recombinant *S. cerevisiae* produced two kinds of lipases, A and B (*26*), and preliminary experiments showed that lipase B was more stable than lipase A. Thus the structures of lipases A and B were examined to clarify the relationship between the structure and stability.

In this study, we reveal that lipase A is generated by the cleavage of the C-terminal peptide of lipase B, and that the C-terminal peptide plays an important role in the tightening of the structure.

MATERIALS AND METHODS

Enzymes and Chemicals—Restriction endonucleases and the other enzymes for DNA manipulations were purchased from Takara Shuzo (Kyoto) and Toyobo (Osaka). Yeast extract and peptone were purchased from Wako Pure Chemical Industries (Osaka). DEAE-Sephadex A-50, SP-Sephadex C-50, Sephadex G-75, and molecular weight marker proteins were obtained from Pharmacia Biotech. (Uppsala, Sweden), and the oligonucleotides were obtained

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Abbreviation: *GAP*, glyceraldehyde 3-phosphate dehydrogenase.

from GIBCOBRL (Rockville, MD, USA). All other reagents were of special grade.

Strains and Plasmids—*Escherichia coli* DH5 [*supE44 hsdR17 recA1 endA1 gyrA96 thi-1 relA1*] and plasmid pUC119 (*28*) were used for DNA manipulations. The *S. cerevisiae* expression vector, pYE22m, was kindly provided by Dr. S. Harashima with the permission of Suntory (Osaka) (*26*). The expression plasmid, pYGF2, bore the cDNA coding preprolipase between the *GAP* promoter and its terminator on pYE22m (*26*). The plasmid pFHL13 was constructed for expression of the lipase cDNA, and the cDNA coding preprolipase was inserted into pUC119 (*26*). *S. cerevisiae* SH2041 [*MATα ura3-1,2 leu2-3,112 trp1*] (generously given by Dr. S. Harashima) was used as a host strain for the production of mutant lipases, and SH2041 [pYGF2] was used for the production of wild-type lipase (*26*).

Culture Conditions of S. cerevisiae—*S. cerevisiae* was cultivated at 27°C for 3 d on a reciprocal shaker in an 18-mmφ test tube (working volume, 5 ml) or a 500-ml shaking flask (working volume, 100 ml) containing 3% yeast extract, 1% peptone, and 4% sucrose (pH 5.8).

Assay of Lipase—The lipase activity was measured by titrating fatty acids liberated from olive oil (Wako Pure Chemical Industries) with 50 mM KOH as described previously (*29*). The reaction was carried out at 30°C for 30 min with stirring at 500 rpm. One unit (U) of lipase activity was defined as the amount which liberated 1 μmol of fatty acid per min.

Purification of Lipase—The enzyme produced by *S. cerevisiae* was purified from the culture supernatant by ultrafiltration, DEAE-Sephadex A-50 chromatography, Sephadex G-75 gel filtration, and SP-Sephadex C-50 chromatography, as described previously (*26*). The SP-Sephadex C-50 column chromatography at the final step showed the presence of two lipase peaks (Fig. 1). The former and latter fractions were named lipases A and B, respectively. The activity was recovered in a yield of 42.4% of that in the culture supernatant. The specific activities and pIs of lipases A and B were 1,800 and 1,600 U/mg, and pI 4.7 and 5.0, respectively.
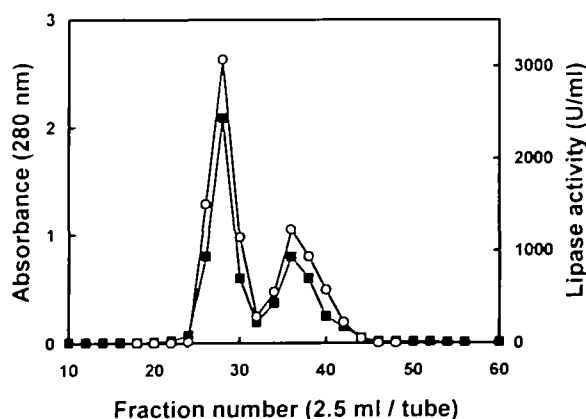

Fig. 1. **SP-Sephadex C-50 ion-exchange column chromatography of lipase produced by S. cerevisiae.** The lipase solution was applied to a SP-Sephadex C-50 column chromatography (1×17 cm) equilibrated with 10 mM sodium acetate buffer (pH 4.5), and elution was performed at a flow rate of 14 ml/h with an increasing gradient of 0–0.4 M NaCl in 200 ml of the same buffer. ○, lipase activity; ■, A_{280}.

Analysis of Lipase Structure—SDS-PAGE was done on a 12% separation gel under the conditions described by Hames (*30*). After the electrophoresis, proteins were stained with Coomassie brilliant blue. The N-terminal amino acid sequence of the enzyme was determined by automatic Edman degradation on a 473A gas-phase sequencer (Applied Biosystems, Foster, CA, USA). The molecular weight of the lipase was measured by a laser ionization mass spectrometer KOMPACT MALDI II (Shimadzu, Kyoto). The molecular mass of a protein of about 30 kDa can be measured within an error of ±1%.

Site-Directed Mutagenesis and Construction of Plasmid for Production of Mutant Lipase—The primers, 5′ CCAT-GGAAGgccGACATGTCAGATG 3′, 5′ CATGGAAGAGGt-AgATcTCAGATGAGGAG 3′, and 5′ CTTGAGCATgcAG-CTGTGACAGTC 3′ (the small letters indicate the mutated nucleotides), were used for creating R275A, Δ275, and R−1A mutations, respectively. DNA manipulation was carried out as described by Sambrook *et al.* (*28*), and the site-directed mutagenesis was performed using pFHL13 (*26*) as a template plasmid and a mutation kit Mutant-K (Takara Shuzo). The mutation of ΔPRO was created by the deletion of a 51-bp *Pvu*II-*Pvu*II fragment, which was constructed by R−1A mutation. The R275A ΔPRO mutant was created by introducing the site-directed mutagenesis into ΔPRO mutation. A 1.14-kbp *Eco*RI-*Sal*I mutant fragment on pFHL13 was inserted into the *Eco*RI-*Sal*I gap of pYE22m, and the resulting plasmid was used for the production of the mutant lipase.

Transformation of S. cerevisiae—*S. cerevisiae* was transformed by the lithium acetate method developed by Ito *et al.* (*31*). The transformants were isolated by incubation at 30°C for 2 d on a plate of minimum medium composed of 6.7 mg/ml Yeast Nitrogen Base w/o amino acids (Difco, Detroit, MI, USA), 5 mg/ml glucose, 20 μg/ml uracil, 20 μg/ml L-histidine, 20 μg/ml L-leucine, and 15 mg/ml agar (pH 5.8).

Extraction of Total RNA from S. cerevisiae and Northern Blot Analysis—*S. cerevisiae* cells were collected by centrifugation of 3 ml of culture broth and washed with 3 ml of water. The cells were disrupted for 7 min with 0.3 g of glass beads (0.5 mm), 0.3 ml of RNA extract solution (0.5 M NaCl, 10 mM EDTA, 1% SDS, and 0.2 M Tris-HCl, pH 7.5), and 0.3 ml of phenol/CHCl₃ in a vortex mixer. Ethanol (1 ml) was added to the aqueous layer obtained by centrifugation, and the mixture was kept for 15 min at −80°C. The resulting precipitate was dissolved in 50 μl of water, and the RNA concentration was determined by measuring the absorbance at 260 nm. The total RNA (5 μg) was denatured in 2.2 M formaldehyde/50% formamide at 65°C, and electrophoresed on a 1% agarose gel containing 1.1 M formaldehyde and 20 mM MOPS (*32*). After the agarose gel electrophoresis, the RNAs were transferred to a nylon membrane (Hybond-N; Amersham, Buckinghamshire, UK) according to the supplier's protocol. The probe for Northern blot analysis was a 1.14-kbp *Eco*RI-*Sal*I fragment of pFHL13 (*22*), and the hybridization was carried out using an ECL direct nucleic acid labeling and detection system (Amersham).

RESULTS

Lipases A and B Produced by Recombinant S. cere-

visiae—The purified lipases A and B produced by *S. cerevisiae* were used to investigate their thermostabilities (Fig. 2A). Lipase A was stable for 30 min at up to 60°C and lipase B at up to 75°C. The half-lives of thermal inactivation of lipases A and B at 77°C were 0.36 and 81 min, respectively. However, the optimum temperatures of the two lipases were both 40°C (Fig. 2B).

The stabilities of the lipases to various solvents were investigated by measuring the remaining activity after incubation at 30°C for 16 h in mixtures containing 50% (v/v) concentration of methanol, ethanol, dimethylsulfoxide, acetone, acetonitrile, and *n*-hexane. Lipase B was very stable in the solvents tested, but lipase A was completely inactivated by the incubation in ethanol, acetone, and acetonitrile. When the activities were measured in the presence of 50% (v/v) of the above solvents, however, little difference was found between lipases A and B. Fatty acid specificities and K_m values for olive oil were also closely similar for the two lipases (data not shown). These results thus revealed that the enzymatic reactivities of the two lipases were the same, but their stabilities were different.

Structures of Lipases A and B—The molecular weights of lipases A and B estimated by SDS-PAGE were 31 and 34 kDa, respectively. An additional band of less than 10 kDa was detected in the lipase A preparation (Fig. 3). Laser ionization mass spectrometry indicated that lipase A consisted of two peptides, 29,068 and 3,103 Da, and that

lipase B consisted of one peptide, 32,322 Da.

The N-terminal amino acid sequences determined by the protein sequencer are shown in Table I. The sequence of lipase B agreed with that of mature lipase estimated from the cDNA. However, equal amounts of two amino acids were detected in each cycle of the lipase A preparation, leading to two N-terminal sequences: one was Ala-Val-Thr---, which was identical to that of the mature lipase, and the other was Asp-Met-Ser---, which coincided with the sequence starting from the position 276. When the 31-kDa band of lipase A after SDS-PAGE was put on the sequencer, only Ala-Val-Thr--- was detected. These results showed that lipase B consisted of one polypeptide (301 amino acids) and that lipase A consisted of two polypeptides (275 and 26 amino acids). Because the polypeptide of 275 amino acids contained the catalytic triad, it was named the catalytic domain. The molecular weight determined by the laser ionization mass spectrometer approximately agreed with that calculated from the amino acid sequence (lipase A, 29,513 and 3,195 Da; lipase B, 32,690 Da). These results supported the conjecture that lipase A was generated by the cleavage of the peptide bond between Arg275 and Asp276, but that the C-terminal peptide interacted noncovalently with the catalytic domain. The two polypeptides of lipase A are not separated from each other by the purification procedures employed but are separated by SDS-PAGE.

Role of C-Terminal Peptide—We constructed two mutant lipases to investigate the role of the 26 amino acids of the C-terminal peptide (Fig. 4). One mutant lipase, R275A, of which the C-terminal peptide was not cleaved, was constructed by converting Arg275 into alanine. The expression plasmid of R275A mutant lipase was named pYGF2R275A. The other one, Δ275, which did not have the
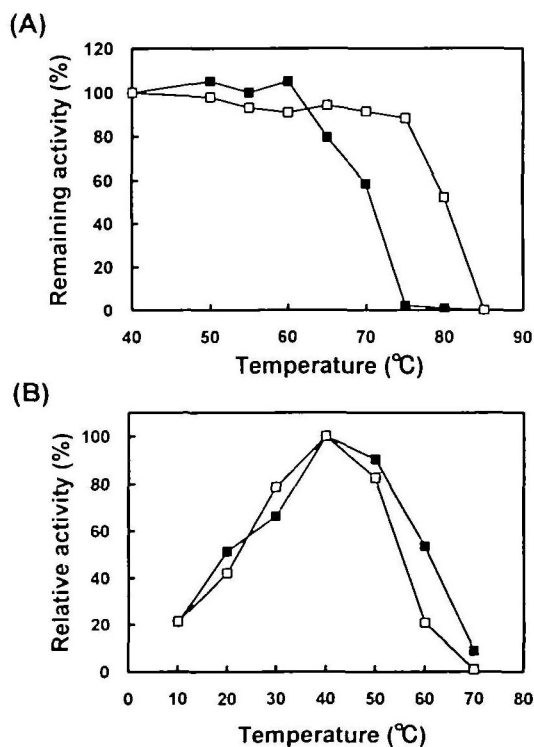

(A)

(B)

Fig. 2. **The thermostabilities and optimum temperatures of lipases A and B.** (A) Effect of temperature on the stability. The enzyme (0.8 mg/ml) was kept at the indicated temperature for 30 min in 50 mM acetate buffer (pH 5.6), then placed on ice for 10 min. The remaining activity was expressed relative to that before incubation. (B) Effect of temperature on the activity. The activity at the indicated temperature was expressed as a percentage of the maximum activity. ■, lipase A; □, lipase B.
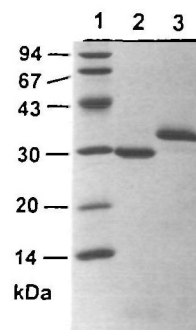

Fig. 3. **SDS-PAGE of lipases A and B.** Lane 1, molecular weight markers; lane 2, lipase A; lane 3, lipase B.

TABLE I. **N-Terminal amino acid sequences of lipases determined by the protein sequencer.**

Lipase	N-Terminal amino acid sequence
Lipase A	Ala-Val-Thr-Val-Thr-Thr-Gln-Asp- Asp-Met-Ser-Asp-Glu-Glu-Leu-Glu-
Lipase A, 31 kDa[a]	Ala-Val-Thr-Val-Thr-Thr-Gln-Asp-
Lipase B	Ala-Val-Thr-Val-Thr-Thr-Gln-Asp-
cDNA, from 1[b]	Ala-Val-Thr-Val-Thr-Thr-Gln-Asp-
cDNA, from 276[c]	Asp-Met-Ser-Asp-Glu-Glu-Leu-Glu-

[a]The 31-kDa polypeptide obtained by SDS-PAGE of lipase A. [b]N-Terminal amino acid sequence of mature lipase estimated from the nucleotide sequence of the lipase cDNA. [c]Amino acid sequence starting from position 276.

C-terminal peptide, was constructed by converting Asp276 into the amber codon; the expression plasmid of Δ275 mutant lipase was pYGF2Δ275. *S. cerevisiae* carrying each mutant lipase gene was cultivated at 27°C for 3 d, then the lipase activity in the culture supernatant was measured. *S. cerevisiae* [pYGF2R275A] produced 63 U/ml of lipase, almost the same amount as the wild-type enzyme producer, *S. cerevisiae* [pYGF2] (54 U/ml); but *S. cerevisiae* [pYGF2Δ275] produced only a small amount of lipase activity (2 U/ml).

Culture supernatants of *S. cerevisiae* strains were concentrated and applied to SDS-PAGE (Fig. 5A). *S. cerevisiae* [pYGF2] produced three lipases of 36, 34, and 31 kDa (lane 2), whereas *S. cerevisiae* [pYGF2R275A] produced two lipases of 36 and 34 kDa (lane 3). However, these proteins were not detected in the culture supernatant of *S. cerevisiae* [pYGF2Δ275] (lane 4). The protein of 36 kDa possessed 16 amino acids of the prosequence, because the N-terminal amino acid sequence was Gly-Pro-Val---, when the band after SDS-PAGE was put on the sequencer. The proteins of 34 and 31 kDa showed the same mobilities as lipases A and B, respectively. It was therefore confirmed that *S. cerevisiae* [pYGF2R275A] produced only the lipase of which the C-terminal peptide was not cleaved. *S. cerevisiae* [pYGF2] produced lipase A, but the band of the C-terminal peptide was not detected (lane 2). We then applied a larger amount of the culture supernatant to SDS-PAGE, but we were unable to detect the band of the C-terminal peptide because of the interference by contaminants.

To determine the amount of mRNA transcribed from the Δ275 gene, Northern blot analysis was carried out using lipase cDNA as a probe (Fig. 5B). No hybridization band was detected in the total RNA from *S. cerevisiae* [pYE22m] carrying no lipase cDNA (lane 1). However, one band was detected in the total RNA from *S. cerevisiae* strains carrying pYGF2, pYGF2R275A, and pYGF2Δ275 (lanes 2, 3, and 4, respectively). The intensities of the bands were almost the same, suggesting that the Δ275 gene was transcribed at a similar level to the wild-type and R275A genes. Thus the C-terminal peptide was found to

play an important role in the post-transcriptional process.

The thermostabilities of R275A and Δ275 lipases were examined using the culture supernatants of *S. cerevisiae* strains carrying pYGF2R275A and pYGF2Δ275, respectively. The thermostability of the lipase from *S. cerevisiae* [pYGF2R275A] was the same as that of lipase B. On the other hand, the lipase from *S. cerevisiae* [pYGF2Δ275] was less stable than lipase A, having a half-life of thermal inactivation at 67°C of 3.5 min, one-eleventh of that of purified lipase A (39 min). However, the difference in thermostability between lipase A and Δ275 lipase was very small compared with that between lipases A and B.

Effect of Prosequence—We constructed three mutant lipases: R−1A (the prosequence is not cleaved, because Arg−1 is converted to Ala), ΔPRO (the prosequence is deleted), and R275AΔPRO (the prosequence is deleted, but the C-terminal peptide is not cleaved) (Fig. 4). The expression plasmids of mutant lipases were named pYGF2R−1A, pYGF2ΔPRO, and pYGF2R275AΔPRO, respectively. *S. cerevisiae* strains carrying these mutant genes were cultivated, and the culture supernatants were analyzed by SDS-PAGE (Fig. 5A). *S. cerevisiae* [pYGF2ΔPRO] produced 34- and 31-kDa lipases (lane 6), and *S. cerevisiae* [pYGF2R275AΔPRO] produced only 34-kDa lipase (lane 7). The mobilities of the 34- and 31-kDa lipases coincided with those of lipases B and A, respectively (compare lanes 2 and 3). *S. cerevisiae* [pYGF2R−1A] produced 36- and 32-kDa lipases (lane 5), and these lipases were not detected in the culture supernatants of the strains carrying pYGF2ΔPRO and pYGF2R275AΔPRO. Furthermore, the 36-kDa lipase was also produced by the strains carrying pYGF2 and pYGF2R275A (lanes 2 and 3). These results confirmed that *S. cerevisiae* [pYGF2R−1A] produced lipases possessing the prosequence and that the strains carrying pYGF2ΔPRO and pYGF2R275AΔPRO produced lipases without it. When *S. cerevisiae* strains carrying the
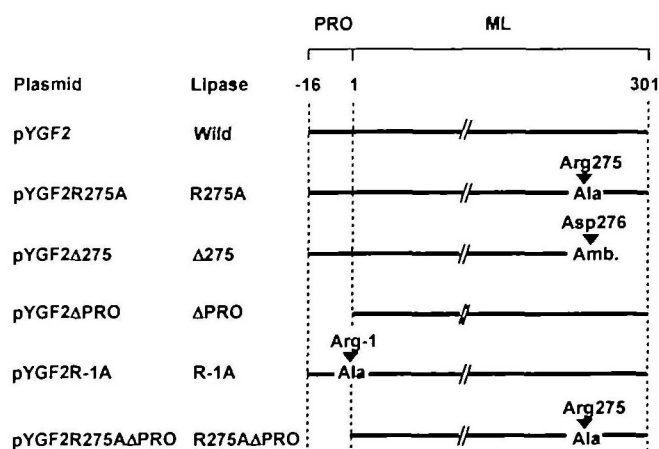

Fig. 4. Schematic structures of lipase mutants. Amino acid residues are numbered from the N-terminus of mature lipase, and the prosequence is indicated by negative numbers. PRO, prosequence; ML, mature lipase; Amb, amber codon.
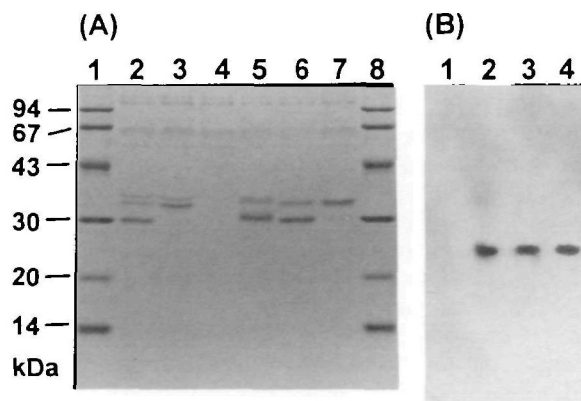

Fig. 5. **SDS-PAGE of lipase mutants and Northern blot analysis.** (A) SDS-PAGE of lipase mutants. Culture supernatants of *S. cerevisiae* were concentrated 50 times by ultrafiltration, and the resulting concentrates (0.3 μl) were subjected to the electrophoresis: Lanes 1 and 8, molecular weight markers; lane 2, *S. cerevisiae* [pYGF2]; lane 3, *S. cerevisiae* [pYGF2R275A]; lane 4, *S. cerevisiae* [pYGF2Δ275]; lane 5, *S. cerevisiae* [pYGF2R−1A]; lane 6, *S. cerevisiae* [pYGF2ΔPRO]; lane 7, *S. cerevisiae* [pYGF2R275AΔPRO]. (B) Northern blot analysis. Total RNA was extracted from *S. cerevisiae* strains, and lipase cDNA was used as a probe: Lane 1, *S. cerevisiae* [pYE22m]; lane 2, *S. cerevisiae* [pYGF2]; lane 3, *S. cerevisiae* [pYGF2R275A]; lane 4, *S. cerevisiae* [pYGF2Δ275].

genes of mutant lipases (ΔPRO, R275AΔPRO, and R−1A) were cultivated for 3 d, they produced 54.3, 51.7, and 64.7 U/ml of lipase in the supernatants, respectively. Because their production levels were about the same, the prosequence coding region was found not to affect the transcriptional, translational, and post-translational steps.

The 34- and 31-kDa lipases produced by S. cerevisiae [pYGF2ΔPRO] and the 36- and 32-kDa lipases produced by S. cerevisiae [pYGF2R−1A] were purified as described under "MATERIALS AND METHODS." The C-terminal peptide was not detected by SDS-PAGE of the crude enzyme solution (Fig. 5A), but it was detected in the purified lipases showing 31- and 32-kDa of mobilities. Their specific activities were about the same as those of lipases A and B, although those of the 34- and 36-kDa lipases were only a little lower than those of the 31- and 32-kDa lipases. The thermostabilities of the 34- and 36-kDa lipases agreed with those of lipase B, and the stabilities of the 31- and 32-kDa lipases agreed with those of lipase A (data not shown). Because the prosequence did not affect the thermostability, it was suggested that the sequence did not participate in the tightening of the structure.

DISCUSSION

We have described the structure-stability relationship of two lipases, A and B, produced by S. cerevisiae bearing F. heterosporum lipase cDNA. Lipase B was composed of one polypeptide (301 amino acids), whereas lipase A consisted of two polypeptides (a catalytic domain of 275 amino acids and a C-terminal peptide of 26 amino acids) generated by the cleavage between Arg275 and Asp276. It was suggested that the C-terminal peptide exerted an important role in the tightening of the structure when bound to the catalytic domain through a peptide bond.

Lipase B, of which the C-terminal peptide was not cleaved, was more stable than lipase A carrying the noncovalently bound peptide. The stabilization of lipase B may be rationalized as follows. The C-terminal peptide when bound to the catalytic domain through a peptide bond strengthens the tertiary structure of the enzyme by functioning as a clamp. The function of the clamp is lost after the cleavage of the C-terminal peptide, even though the peptide binds noncovalently to the catalytic domain.

The Δ275 gene was transcribed at a similar level to the wild-type gene, but only small amount of lipase was detected in the culture supernatant of S. cerevisiae [pYGF2Δ275]. These results suggested two possibilities: (i) the C-terminal peptide was important for the secretion; (ii) the C-terminal peptide played an important role in the folding of lipase. The former possibility is unlikely, however, because no lipase activity was detected in the cell lysate of S. cerevisiae [pYGF2Δ275].

Carboxypeptidase Y and many proteases have prosequences which participate in the folding and the inhibition of the enzymes (3, 4). Many charged amino acids are present in the prosequences and play an important role in the folding (3). The C-terminal peptide of F. heterosporum lipase also has many charged amino acids. Only 18.9% of amino acids in the catalytic domain were charged (the net charge was +6), but 11 of the 26 amino acids in the C-terminal peptide (42.3%) were charged (3 Asp, 5 Glu and 3 Lys; net charge, −5). This suggested that lipase B was

stabilized by fitting the C-terminal peptide into the desired region of the catalytic domain through their electrostatic interaction.

R. oryzae lipase is homologous to F. heterosporum lipase, and its precursor also contains the prosequence. It was suggested that the prosequence of 97 amino acids of R. oryzae lipase is necessary for the folding of the polypeptide, and the prolipase is more thermostable than the mature lipase (19). F. heterosporum produces a lipase precursor bearing a prosequence of 16 amino acid residues, which is shorter than that of R. oryzae lipase. In addition, the mature lipase consists of 301 amino acid residues, and the C-terminus is longer by 32 amino acids than that of R. oryzae (269 amino acids). Experiments with a mutant lipase containing no prosequence (ΔPRO) showed that the prosequence of F. heterosporum did not influence the stability or production of lipase. In contrast, the C-terminal peptide was necessary for the tightening of lipase structure. The results showed that the function of the C-terminal peptide of F. heterosporum lipase was same as that of the prosequence of R. oryzae lipase. It was therefore suggested that the C-terminal peptide participated in the folding of the lipase, as do the prosequences of carboxypeptidase Y (3, 4), many proteases (5-8), and R. oryzae lipase (19). Hence, the Δ275 lipase lacking the C-terminal peptide may be easily decomposed by a protease inside or outside the cell, because the folding rate is accelerated.

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