Novel Zinc-binding Center and a Temperature Switch in the Bacillus stearothermophilus L1 Lipase*

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The bacterial thermoalkalophilic lipases optimally hydrolyze saturated fatty acids at elevated temperatures. They also have significant sequence homology with staphylococcal lipases, and both the thermoalkalophilic and staphylococcal lipases are grouped as the lipase family I.5. We report here the first crystal structure of the lipase family I.5, the structure of a thermoalkalophilic lipase from Bacillus stearothermophilus L1 (L1 lipase) determined at 2.0-Å resolution. The structure is in a closed conformation, and the active site is buried under a long lid helix. Unexpectedly, the structure exhibits a zinc-binding site in an extra domain that accounts for the larger molecular size of the family I.5 enzymes in comparison to other microbial lipases. The zinc-coordinated extra domain makes tight interactions with the loop extended from the C terminus of the lid helix, suggesting that the activation of the family I.5 lipases may be regulated by the strength of the interactions. The unusually long lid helix makes strong hydrophobic interactions with its neighbors. The structural information together with previous biochemical observations indicate that the temperature-mediated lid opening is triggered by the thermal dissociation of the hydrophobic interactions.

Lipases that hydrolyze emulsion of lipids with long-chain fatty acids are widely distributed in animals, plants, fungi, and bacteria. Of these, microbial lipases from fungi and bacteria have attracted special attention for various industrial applications because most microbial lipases can be extracellularly produced in large quantities, have broad substrate specificity, and are quite stable under non-natural conditions (1, 2).

The bacterial thermoalkalophilic lipases, which have important potential in applications for enzymatic processing of lipids at elevated temperatures and in an organic solvent phase (3), are found from various thermophilic bacterial strains including Bacillus stearothermophilus,1 Bacillus thermoleovorans (5), B. stearothermophilus, and Bacillus sp. TP10A.2 These thermoalkalophilic lipases have about 95% amino acid sequence identity among them and show a significant homology of 30–35% with the mature lipases from other Gram-positive bacteria Staphylococcus strains, some of which are pathogenic, and their lipases are involved in the pathogenic processes as reviewed previously (8). In contrast to the homology with staphylococcal lipases, the thermoalkalophilic lipases exhibit no sequence homology with other microbial lipases (2). They are also characterized by their molecular sizes of 40–45 kDa that are significantly larger than those of other microbial lipases (usually under 35 kDa). Thus, the thermoalkalophilic lipases and staphylococcal lipases were grouped together as one family of lipases named the lipase family I.5 (1) or Staphylococcus family (2). Although the thermoalkalophilic lipases and staphylococcal lipases are grouped as the same family, they show many differences in biochemical characteristics such as optimal temperature, optimal pH, and substrate specificity. The thermoalkalophilic lipases reach maximum activity at 60–75 °C and pH 8–10, whereas staphylococcal lipases show low optimal temperatures and pH optimum of 6–9 (8–10). In the case of Staphylococcus haemolyticus L62 lipase, the optimum temperature is 28 °C and retains more than 30% activity at 4 °C (10). Unlike the thermoalkalophilic lipases, some staphylococcal lipases show preference for acidic conditions (8).

Crystal structures of various lipases have been determined from mammalian, yeast, fungal, and bacterial origin (1). They include structures of four different bacterial lipases from Burkholderia glumae (11), Chromobacterium viscosum (12), Burkholderia cepacia (13, 14), and Pseudomonas aeruginosa (15). All of the four bacterial lipases belong to the Pseudomonas group (2) and have a temperature optimum of 30–50 °C. The core structure of these lipases consists of a central β-sheet surrounded by α-helices, exhibiting a common α/β-hydrolase fold (1). The active site involves a catalytic triad consisting of serine, aspartic (or glutamic) acid, and histidine residues. The active site is exposed to the solvent in the open conformation structures (B. glumae, B. cepacia, and P. aeruginosa), whereas the active site in the closed conformation structure (C. viscosum) is covered by a lid-like helix. The conformational change from the closed to open conformation is important for the activation of lipases (16). Despite the wealth of the structural information of Pseudomonas lipases, the available structures do not provide clues to the mechanism of enzyme characteristics of the thermoalkalophilic lipases and staphylococcal lipases because...
Pseudomonas lipases have no sequence homology with either the thermolipophilic lipases or staphylococcal lipases.

The thermolipophilic lipase from B. stearothermophilus L1 (L1 lipase) is one of the well-characterized lipases (17–19). The mature form of L1 lipase, which consists of 388 amino acids (43 kDa), is highly thermostable and has the unfolding temperature of 74 °C (18). In addition to the thermostability, L1 lipase exhibits a unique thermoactivity, by which the enzyme is most active at 68 °C for the hydrolysis of olive oil (18, 19). Other thermostable lipases from mesophiles show only low activity at temperatures above 60 °C (2, 20, 21). The thermostability and thermoactivity of L1 lipase are critically important for many industrial applications because the industrial lipase reactions often require high temperatures (50–80 °C) due to the high melting points of some lipids. L1 lipase shows high preference for saturated fatty acid (C₆–C₁₆) and natural solid lipid such as beef tallow and palm oil, which are usually bad substrates for mesophilic lipases.

We report here the crystal structure of the thermolipophilic lipase from B. stearothermophilus L1 (L1 lipase) determined by the multiple isomorphous replacement method. The crystal structure of L1 lipase reveals the detailed mechanisms of thermostability and thermoactivity that are interconnected to each other and provides a framework in understanding the function of the thermolipophilic and staphylococcal lipases. The L1 lipase structure shows a novel zinc-coordinated extra domain that is conserved among members of the lipase family I.5. The extra domain appears to control the lipase activation through interactions with the loop connected to the helical lid. The temperature-mediated dissociation of hydrophobic interactions between the helical lid and the enzyme active site and the rigidity in the packing of the active site residues explain the unique thermoactivity of L1 lipase.

**TABLE I**

| Structure and phasing statistics | Native | Samarium | Mercury |
|---------------------------------|--------|-----------|---------|
| Resolution limit (Å)            | 2.0    | 2.3       | 2.5     |
| Observations                    | 202,657| 106,333   | 102,816 |
| Unique reflections              | 63,643 | 40,625    | 32,184  |
| Completeness (%)                | 96.9 (95.2) | 93.9 (96.5) | 94.8 (93.7) |
| Multiplicity                    | 3.0    | 2.4       | 2.9     |
| Lorentz 1 (1/Å²)                | 20.2 (6.1) | 17.0 (6.3) | 15.4 (5.6) |
| Rmerge (%)                      | 5.9 (22.2) | 7.2 (21.8) | 10.2 (28.2) |
| Phasing power                   | 2.0/2.6 | 2.3/3.0   |         |

*Native and all heavy-atom derivative data were collected at room temperature.

**TABLE II**

| Refinement statistics            | Native |
|---------------------------------|--------|
| Resolution range (Å)            | 99–2.0 |
| R cryst (%)                     | 18.5   |
| Rfree (%)                       | 21.8   |
| Atoms in model                   | 6,086  |
| Protein (non-hydrogen)          |        |
| Metal atoms                      | 4      |
| Water molecules                  | 257    |
| Root mean square deviations from ideal |        |
| Bond lengths (Å)                | 0.006  |
| Bond angles (°)                 | 1.17   |
| Improper (°)                    | 0.74   |
| Dihedrals (°)                   | 23.6   |

**RESULTS AND DISCUSSION**

**Overall Structure**—The L1 lipase structure reveals a globular enzyme with approximate dimensions of 45 x 50 x 55 Å. A central β-sheet consisting of seven strands (β3–β9) is surrounded by α-helices and loops, resulting in an overall topology of the α/β hydrolase fold (Fig. 1a). One side of the central sheet asymmetric molecules are in the most favored region of the Ramachandran plot drawn by the program PROCHECK (27), and there is no residue in the disallowed region. The final model includes residues 1–388 in the first monomer, 4–388 in the second monomer, 2 calcium atoms, 2 zinc atoms, and 257 water molecules.

**Identification of Zinc Sites**—The zinc ion was identified by anomalous diffraction of L1 lipase crystal. Bijvoet data were collected using a frozen crystal of L1 lipase at the beamline BL18B of the Photon Factory (Tsukuba, Japan). The crystal was flash-frozen in liquid nitrogen after soaking in the buffer containing 100 mM sodium acetate (pH 4.6), 1.0 M sodium formate, and 20% glycerol. Under the frozen condition, cell constants of the crystal were a = 117.78 Å, b = 84.59 Å, c = 97.78 Å, and β = 99.32°, which are similar to those of native crystals. Before the data collection, we performed a fluorescence scanning to determine an experimental absorption edge of the crystal. To obtain sufficient Bijvoet mates, the data covering the entire reciprocal space (360° rotation) were collected. The diffraction data were processed with the program MOSFLM (28). Bijvoet data with over 99% coverage at 2.3-Å resolution were obtained for wavelengths slightly before (λ = 1.281 Å) and after (λ = 1.286 Å) the observed absorption edge (λ = 1.283 Å). Rmerge values for the data at λ = 1.281 Å and λ = 1.286 Å were 5.4 and 8.6%, respectively. The figures were drawn by using the programs MOLSCRIPT (29), RASTER3D (30), BOBSCRIPT (31), and RIBBONS (32).
is covered by helices a1 and a13, and the other by helices a2, a4, and a10. Two L1 lipase molecules in the crystallographic asymmetric unit are almost identical except for the C-terminal end of helix a6 and the loop following the helix (residues 193–204) where the two molecules exhibit alternative conformations. The flexibility of the 193–204 region seems to play a role in the enzyme activation (see below).

When the L1 lipase structure is compared with that of C. viscosum lipase, a Pseudomonas family enzyme, structural similarities mainly reside in the central sheet and some of flanking helices. However, there are significant structural differences in other regions because of numerous insertions and deletions throughout the structure (Fig. 1b). Most notably, L1 lipase exhibits an extra domain consisting of helices a3 and a5, strands b1 and b2, and connecting loops (Fig. 1b and Fig. 2). These insertions result in a significant increase in overall size of the enzyme compared with the Pseudomonas family enzymes (388 and 319 amino acids for L1 lipase and C. viscosum lipase, respectively). In addition to the extra domain, helices a6–a9 of the L1 lipase and connecting loops form a unique structure that cannot be aligned with the C. viscosum lipase structure. Helix a6 seems to function as the lid during enzyme activation (see below), and helices a7–a9 surround helix a6 forming a tightly packed structure above the active site of the enzyme.

**Active Site**—Based on the structural alignment of L1 lipase with other structurally known lipases, the catalytic triad residues of L1 lipase can be assigned to Ser-113, His-358, and Asp-317 (Fig. 2). The catalytic triad residues reside in the regions that are highly conserved throughout lipase families: a turn following strand b5, loop b9–a13, and loop a12–b8, respectively. The main- and side-chain conformations of these residues are well aligned with those of C. viscosum lipase. The identities of the active site residues are in agreement with the prediction by a recent modeling study (33). The active site residues are covered by a long helix (a6) and not accessible to solvent, indicating that the L1 lipase structure is in a closed conformation.

Despite the similarity of the catalytic residue conformations, the neighbors of the catalytic triad in L1 lipase exhibit important structural differences in comparison to those of C. viscosum lipase. In L1 lipase, there are characteristic substitutions in the residues close to the catalytic triad, resulting in a more rigid structure through tight side-chain packing. For example, Phe-16 (close to Ser-113), Ile-319 (close to His-358), Thr-269, and Met-325 (close to Asp-317) substitute for Leu-17, Leu-265, Gly-211, and Ser-271 of C. viscosum lipase, respectively (Fig. 3). Most lipases and serine proteases have the conserved Gly-X-Ser-X-Gly motif near the catalytic serine (Ser in the motif). The thermoalkalophilic lipases have a glycine to alanine substitution in the first glycine of the motif, which results in the new Ala-X-Ser-X-Gly motif (17). The side chain of the corresponding alanine (Ala-111) in L1 lipase is toward a hydrophobic core made of residues in the catalytic serine loop and stabilizes the loop conformation. The increased stability of the active site region by the tight packing and the stabilization of the catalytic serine loop should contribute to the thermostability of the L1 lipase. Staphylococcal lipases, which lack a significant thermostability, do not exhibit the glycine to alanine substitution in the catalytic serine motif. The substitutions to bulk residues around the catalytic Asp-317 of L1 lipase (Thr-269 and Met-325) also do not appear in staphylococcal lipases.

The tight packing of the active site residues and extra hydrophobic cores observed in the current structure of L1 lipase should contribute to the unique thermactivity of the enzyme as well as the overall stability of the enzyme at elevated temperatures. At low temperatures, the rigidity in the catalytic triad region prohibits the enzyme by the tight packing and calcium coordination with neighboring main- and side-chain atoms and water molecules, indicating that the density represents the calcium ion as predicted from a calcium-dependent thermostability of L1 lipase (17). The coordination includes interactions with two carboxyl oxygen atoms of Glu-360 and Asp-365, two main-chain carbonyl oxygen atoms of Gly-286 and Pro-366, and two water molecules (Fig. 2). The distances of the calcium coordination with four protein atoms range from 2.24 to 2.44 Å. Glu-360 is in an equivalent position to Asp-357 of Staphylococcus hyicus lipase whose mutation resulted in a decreased enzyme activity (9). The residue corre-
**Fig. 2. Sequence alignment of bacterial lipases.** Amino acid sequence of L1 lipase was aligned with other bacterial lipases. *B. thermocatenulatus* lipase 2 (BTL2) and *S. haemolyticus* lipase (ShaL) belong to the lipase family I.5 as L1 lipase, and *C. viscosum* lipase (CVL) is a *Pseudomonas* family lipase. Whereas the BTL2 and ShaL sequences are aligned using the sequence alignment program ClustalW (6), the alignment of *C. viscosum* lipase is based on the structural superposition with the L1 lipase structure. Secondary structural elements of the L1 lipase structure are indicated above the aligned sequences. Completely conserved residues are colored red, and residues with a conservation value above 8 and 6 as defined in the program ALSCRIPT (7) are colored green and red, respectively. Catalytic triad residues, zinc-coordinating residues, and calcium-coordinating residues of L1 lipase are indicated above the aligned sequences with inverted black triangles, pink circles, and cyan circles, respectively. Catalytic triad residues and calcium-coordinating residues of *C. viscosum* lipase (CVL) are indicated below the *C. viscosum* lipase sequence with black triangles and violet circles, respectively.

**Fig. 3. Side-chain packing of the catalytic triad region.** Characteristic substitutions in L1 lipase (Phe-16, Ile-319, Thr-269, and Met-325) near catalytic triad residues are presented as superposed with corresponding residues of *C. viscosum* (Leu-17, Leu-265, Gly-211, and Ser-271). Side chains of L1 lipase (purple) and *C. viscosum* lipase (cyan) are drawn in a ball-and-stick representation. Only the Ca trace of L1 lipase is presented because the two enzymes exhibit a good Ca trace alignment in the region. Side-chain conformations of catalytic triad residues (Ser-113, His-358, and Asp-317) also are well aligned between the two structures, and only those of L1 lipase are presented in the figure.

Corresponding to Asp-354 of *S. hyicus* lipase (Asp-357 in L1 lipase), another residue predicted to bind the calcium ion (9), is not involved in the calcium binding of L1 lipase. In *Pseudomonas* group lipases, the calcium ion was found to form hydrogen bonds with the region of the active histidine, and loss of the calcium ion results in enzyme inactivation (34). Although the calcium ion of L1 lipase also stabilizes the conformation of the active His-358 loop, the calcium ion of L1 lipase is a little more displaced from the active His-358 compared with that of *Pseudomonas* group lipases. In addition, helix a8 of *Pseudomonas* group lipases, which covers the calcium-binding site, is missing in L1 lipase. These differences make the calcium-binding site of L1 lipase more accessible to solvent, suggesting that the calcium binding in L1 lipase may not be as critical as in *Pseudomonas* group lipases. In agreement with the structural observation, the abstraction of calcium ion by EDTA results in a modest decrease in the unfolding temperature of the L1 lipase (by 8–10°C) and no significant loss of enzyme activity (17).

**Novel Zinc Site and the Extra Domain**—In addition to the calcium site, L1 lipase exhibits another putative metal-binding site. A strong (~12σ height) and isolated electron density map was located in the 2F_0 − F_0 electron density map. The density is tetragonally coordinated by the O-61 atom of Asp-61 (helix a2), the N-ε2 atom of His-81 (helix a3), the N-ε1 atom of His-87 (loop a3-b2), and the O-δ2 atom of Asp-238 (loop a8-a9) (Fig. 4a). The coordination distances are 2.14, 2.13, 2.26, and 2.07 Å, respectively. Considering the intensity of the electron density and its coordination geometry, we propose that the density is a zinc ion. To analyze the nature of the metal site, we characterized anomalous diffraction of the L1 lipase crystal (Fig. 4b). First, we determined the absorption edge of the crystal, which revealed that the crystal has an absorption edge close to the theoretical value for zinc (λ = 1.283 Å). The Bijvoet difference Fourier map calculated from the data at a wavelength (λ = 1.281 Å) slightly before the edge clearly shows the zinc site, whereas the map at a wavelength (λ = 1.286 Å) slightly after the edge does not show a peak at the site, indicating that the metal site is indeed occupied by a zinc ion. There are no other metals that have absorption edges significantly close to the
Both maps are drawn at a contour level of 3.4 Å.

An incubation of the enzyme with a strong zinc chelator, N,N,N',N'-tetrakis(2-pyridylmethyl)ethylenediamine (TPEN) (35), decreases the thermotolerance of the enzyme significantly (by about 20 °C) as discussed above, and an incubation of the enzyme with a strong zinc chelator, N,N,N',N'-tetrakis(2-pyridylmethyl)ethylenediamine (TPEN) (35), decreases the thermotolerance of the enzyme significantly (by about 20 °C), indicating the existence of the tightly bound zinc ion in the enzyme.3

The unusual zinc-binding site of L1 lipase is the first example among all known lipases. The novel zinc site stabilizes the characteristic extra domain consisting of helices α3 and α5, strands b1 and b2, and associated loops by forming coordination bonds with residues from both the extra domain (His-81 and His-87) and the core domain of the protein (Asp-61 and Asp-238). The extra domain and zinc-coordinating residues are well conserved in other members of the lipase family I.5 (Fig. 2).

The thermoacidophilic archaeal ferredoxin from Sulfolobus species also contains a novel zinc-binding site at an extra domain that was attributed as an important factor for the thermostability of the protein (36). In Sulfolobus ferredoxins, the zinc-coordinated extra domain stabilizes two β-sheets of the core domain.

The zinc-coordinated extra domain of L1 lipase provides tight interactions with the core domain as well as the characteristic cluster of helices α8 and α9 and loops around the lid helix (α6). These interactions may contribute to the thermostability of the enzyme as in the Sulfolobus ferredoxins. In addition to the stabilizing effects, the zinc-coordinated extra domain of L1 lipase appears to play an important role in the regulation of the lid opening. The region of the zinc-binding site makes tight contacts with a long loop (residues 194–221) that is extended from the C terminus of the lid helix (Fig. 1). The opening of the lid helix during enzyme activation is likely to involve a conformational transition of the loop region, and the lid opening may be affected by the strength of the loop interactions with the zinc-coordinated extra domain. The zinc-coordinated extra domain is conserved also in Staphylococcus lipases that do not exhibit thermostability. However, the C terminus of the lid helix and the following loop are significantly shortened in Staphylococcus lipases (Fig. 2), supporting that the tight interactions of the loop with the extra domain may be important in the thermostability and activity regulation.

Temperature Switch in the Lid Helix—The helical lid of L1 lipase (helix α6, 19 amino acids) is significantly longer than that of the closed-form structure of C. viscosum lipase (12 amino acids) (Fig. 5a). The inner face of the lid helix of the L1 lipase points toward the active site and consists of mainly hydrophobic residues. In particular, a characteristic array of phenylalanines (Phe-176, Phe-180, and Phe-181) makes strong hydrophobic interactions with the active site region and the groove formed by neighboring helices and loops (Fig. 5b). Side chains of the three phenylalanines interact with Trp-234 of helix α8, Val-171 and Leu-170 of loop β6-α6, Phe-16 of loop β3-α1, and Leu-56 of loop β4-α2. Other hydrophobic residues in the lid helix (Leu-183, Val-187, Leu-188, Ala-190, Ala-191, Ala-192, and Ala-194) also form strong interactions with neighboring hydrophobic residues (Phe-16, Trp-19, and Phe-27 of loop β3-α1; Tyr-199 and Tyr-204 of loop α6-α7; Phe-221 and Phe-225 of helix α7; Phe-290 of helix α11; Ile-319 of loop α2-β4; and Val-364 of loop β9-α13) (Fig. 5b). In the closed-form structure of C. viscosum lipase, there is a small opening between the lid helix and the active site in the C-terminal region of the helix. However, the extended lid helix of L1 lipase completely blocks the opening with tight hydrophobic interactions. In addition, the lid helix is surrounded by extra helices (α7–α9) that are not present in C. viscosum lipase. The lid helix of Staphylococcus lipase also does not have the extended C-terminal region, which is consistent with the lack of thermostability in the enzymes (Fig. 2). The tight hydrophobic interactions and extra helices near the lid helix of the L1 lipase seem to contribute to the stability of the lid helix.

The enzyme activity of B. subtilis lipase can be completely inhibited by a 10-min incubation with 1 mM phenylmethylsulfonyl fluoride at 30 °C. However, L1 lipase retains 90 and 77% of the control enzyme activity after 10 and 30 min of incubation under the same conditions (17). About 80% of the control activity remains even after a 10-min incubation at 55 °C with 1 mM phenylmethylsulfonyl fluoride (17). These observations are consistent with the strong interaction of the lid helix with the catalytic site in L1 lipase, which may protect the catalytic serine from the phenylmethylsulfonyl fluoride modification at low temperatures. The lid helix may move from the active site only when the temperature is increased to a certain value, providing a temperature switch in the activation of the L1 lipase. The tight association of the lid helix at temperatures below the activation temperature may be important for protection of the hydrophobic active site region from nonspecific aggregation of the enzyme.

In the lid helix of the C. viscosum lipase, there are two adjustable loops flanking the helix that allow a lateral movement of the helix as shown in the open-form structures of B. cepacia and P. aeruginosa lipases (Fig. 5a). However, the N terminus of the lid helix of L1 lipase is connected to the core of the protein with a tight turn, missing the N-terminal adjustable loop observed in C. viscosum lipase. Instead, the loop C-terminal to the lid helix (residues 194–221) in L1 lipase is unusually extended and makes interactions with the zinc-coordinated extra domain (see below). Interestingly, the N-terminal half of the helix has residues with bulky side chains (Phe-

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3 H.-K. Kim and T.-K. Oh, unpublished data.
The C-terminal half is composed of residues with short side chains (Ala-186, Val-187, Ala-190, Ala-191, Ala-192, Val-193, and Ala-194) (Fig. 2). The existence of an adjustable loop only at one end of the helix and the bipartite distribution of hydrophobic residues in the lid helix indicate that the lid opening is likely to occur mainly in the C-terminal side. The start region of the C-terminal loop (residues 193–204) has alternative conformations between two independent lipase molecules in the asymmetric unit, supporting the possible lid opening in the C-terminal side (Fig. 5a). Together with the strong hydrophobic interactions of the lid helix, the unidirectional opening mechanism of the lid also may contribute to the stability of the lid helix in the closed conformation, providing another control in the temperature-mediated activation of L1 lipase.

Due to the strong hydrophobic interactions of the lid helix with the catalytic triad region, the lid may not be opened at low temperatures even in the presence of lipid substrates. The increased kinetic energy at high temperatures should allow the movement of the lid by effective solvation of the residues involved in the hydrophobic interactions (37). Hydrophobic interactions play an important role in the temperature-mediated structural switches of other proteins also. The temperature- and redox-mediated activation of Hsp33 involves a reversible dissociation of two domains attached by hydrophobic interactions as well as zinc ligation (38). The temperature-mediated monomer-to-trimer transition of HSF1 also involves reversible dissociation of hydrophobic interactions in leucine zipper helices (39). Thus, the reversible dissociation of hydrophobic interactions seems to be a common mechanism utilized in proteins for the temperature-mediated control of protein activities.

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REFERENCES
1. Jaeger, K. E., Dijkstra, B. W., and Reetz, M. T. (1999) *Annu. Rev. Microbiol.* 53, 315–351
2. Schmidt-Dannert, C., Rua, L., and Schmid, R. D. (1998) *Methods Enzymol.* 284, 184–220
3. Rúa, M. L., Atuni, H., Schmidt-Dannert, D., and Schmid, R. D. (1998) *Appl. Microbiol. Biotechnol.* 49, 405–410
4. Schmidt-Dannert, C., Satajer, H., Stockeitn, W., and Schmid, R. D. (1994) *Biochim. Biophys. Acta* 1214, 43–53
5. Cho, A.-R., Yoo, S.-K., and Kim, E.-J. (2000) *FEBS Microbiol. Lett.* 186, 235–238
6. Thompson, J. D., Higgins, D. G., and Gibson, T. J. (1994) *Nucleic Acids Res.* 22, 4673–4680
7. Barton, G. J. (1993) *Protein Eng.* 6, 37–40
8. Rosenstein, R., and Gotz, F. (2000) *Biochim. Biophys. Acta* 155, 1005–1014
9. Simons, J.-W. F. A., Kampen, M. D., Ubarretxena-Belandia, I., Cox, R. C., Santos, C. M. A., Egmond, M. R., and Verheij, H. M. (1999) *Biochemistry* 38, 29–36
10. Oh, B.-C., Kim, H.-K., Lee, J.-K., Kang, S.-C., and Oh, T.-K. (1999) *FEBS Microbiol. Lett.* 179, 385–392
11. Nobel, M. E. M., Cleasby, A., Johnson, L. N., Egmond, M. R., and Frenken, L. G. J. (1998) *FRS Lett.* 321, 125–128
12. Lang, D., Hofmann, B., Haalck, L., Hecht, H.-J., Spener, F., Schmid, R. D., and Schomburg, D. (1996) *J. Mol. Biol.* 259, 704–717
13. Kim, K. S., Song, H. K., Shin, D. H., Hwang, K. Y., and Suh, S. W. (1997) *Structure* 5, 173–185
14. Schrag, J. D., Li, Y., Cygler, M., Lang, D., Burgdorf, T., Hecht, H.-J., Schmid, R., Schomburg, D., Rydel, T. J., Oliver, J. D., Stickland, L. C., Dunaway, M. M., Larson, S. B., Day, J., and McPherson, A. (1997) *Structure* 5, 187–201
15. Nardini, M., Lang, D. A., Liebeton, K., Jaeger, K. E., and Dijkstra, B. W. (2000) *J. Biol. Chem.* 275, 31219–31225
16. Grochulski, P., Li, Y., Schrag, J. D., Bouthillier, F., Smith, P., Harrison, D., Rubin, B., and Cygler, M. (1993) *J. Biol. Chem.* 268, 12843–12847
17. Kim, H.-K., Park, S.-Y., Lee, J.-K., and Oh, T.-K. (1998) *Biosci. Biotechnol. Biochem.* 62, 66–71
18. Kim, M.-H., Kim, H.-K., Lee, J.-K., Park, S.-Y., and Oh, T.-K. (2000) *Biosci. Biotechnol. Biochem.* 64, 280–286
19. Kim, M.-H., Kim, H.-K., Oh, B.-C., and Oh, T.-K. (2000) *J. Microbiol. Biotechnol.* 10, 764–769
20. Chung, G. H., Lee, Y. P., Yoo, O. J., and Rhee, J. S. (1991) *Appl. Microbiol. Biotechnol.* 35, 257–241
21. Sugihara, A., Ueshima, M., Shimada, Y., Tsunasawa, S., and Tominaga, Y.
22. Jeong, S. T., Kim, H. K., Kim, S. J., Pan, J. G., Oh, T. K., and Ryu, S. E. (2001) Acta Crystallogr. Sect. D Biol. Crystallogr. 57, 1300–1302
23. de La Fortelle, E., and Bricogne, G. (1997) Methods Enzymol. 276, 472–494
24. Abrahams, J. P., and Leslie, A. G. (1996) Acta Crystallogr. D Sect. Biol. Crystallogr. 52, 30–42
25. Jones, T. A., Zou, J. Y., Cowan, S. W., and Kjeldgaard, M. (1991) Acta Crystallogr. Sect. A 47, 110–119
26. Brünger, A. T., Adams, P. D., Clore, G. M., DeLano, W. L., Gros, P., Grosse-Kunstleve, R. W., Jiang, J. S., Kuszewski, J., Nilges, M., Pannu, N. S., Read, R. J., Rice, L. M., Simonson, T., and Warren, G. L. (1998) Acta Crystallogr. Sect. D Biol. Crystallogr. 54, 905–921
27. Laskowski, R. A., MacArthur, M. W., Moss, D. S., and Thornton, J. M. (1993) J. Appl. Crystallogr. 26, 283–291
28. Leslie, A. G. W. (1999) Acta Crystallogr. Sect. D Biol. Crystallogr. 55, 1696–1702
29. Kraulis, P. (1991) J. Appl. Crystallogr. 24, 946–950
30. Merritt, E. A., and Bacon, D. J. (1997) Methods Enzymol. 277, 505–524
31. Esnouf, R. M. (1997) J. Mol. Graphics Mod. 15, 132–136
32. Carson, M. (1997) Methods Enzymol. 277, 493–505
33. Sinhaikul, S., Sookkheo, B., Phutrakul, S., Wu, Y. T., Pan, F. M., and Chen, S. T. (2001) Biochem. Biophys. Res. Commun. 283, 868–875
34. Deveer, A. M. (1992) Ph.D. thesis, Mechanism of Activation of Lipolytic Enzymes, Rijksuniversiteit, Utrecht
35. Jakob, U., Eer, M., and Bardwell, J. C. A. (2000) J. Biol. Chem. 275, 38302–38310
36. Fujii, T., Hata, Y., Wakagi, T., Tanaka, N., and Oshima, T. (1996) Nat. Struct. Biol. 3, 834–837
37. Callies, A., and Karplus, M. (1995) J. Mol. Biol. 252, 672–708
38. Kim, S.-J., Jeong, D.-G., Chi, S.-W., Lee, J.-S., and Ryu, S.-E. (2001) Nat. Struct. Biol. 8, 459–466
39. Rabindran, S. K., Haroun, R. I., Closs, J., Wisniewski, J., and Wu, C. (1993) Science 259, 230–234