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Genetics

Activation of a bacterial lipase by its chaperone

(extracellular enzyme/high-level expression/*Pseudomonas cepacia*)

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ABSTRACT **The gene *lipA* of *Pseudomonas cepacia* DSM 3959 encodes a prelipase from which a signal peptide is cleaved during secretion, producing a mature extracellular lipase. Expression of lipase in several heterologous hosts depends on the presence of another gene, *limA*, in cis or in trans. Lipase protein has been overproduced in *Escherichia coli* in the presence and absence of the lipase modulator gene *limA*. Therefore, *limA* is not required for the transcription of *lipA* or for the translation of the *lipA* mRNA. However, no lipase activity is observed in the absence of *limA*. *limA* has been overexpressed and encodes a 33-kDa protein, Lim. If lipase protein is denatured in 8 M urea and the urea is removed by dialysis, lipase activity is quantitatively recovered provided Lim protein is present during renaturation. Lip and Lim proteins form a complex precipitable either by an anti-lipase or anti-Lim antibody. The Lim protein has therefore the properties of a chaperone.**

A principle of molecular biology holds that the amino acid sequence determines the conformation of a protein or protein complex. There is good evidence from renaturation studies that this principle applies for many proteins—e.g., pancreatic ribonuclease (1), some viruses (2), and ribosomal subunits (3). However, recent experimental evidence has shown that the natural conformation of a protein or protein complex is not always only determined by the amino acid sequence. In some cases, accessory proteins, called molecular chaperones, are required to mediate the formation of the correct tertiary structure of another protein, or protein complex, but are not themselves components of the final functional structure (4). Chaperones do not cause covalent modifications of the target protein or protein complex.

The first chaperone to be described was nucleoplasmin, a nuclear protein that mediates the assembly of nucleosomes (5, 6). Numerous other chaperones have been identified, including the plastid ribulose-1,5-bisphosphate carboxylase (rubisco) subunit binding protein, GroEL and GroES, and many other heat shock proteins (4). Chaperones are now known to be involved in many kinds of protein folding including phage assembly, DNA replication, protein secretion, and refolding of proteins that have been denatured by natural processes (7). Propeptides of a number of bacterial extracellular enzymes have been shown to be essential for the proper folding of the mature protein; they can carry out this function when coded in trans (8–10), thus behaving as chaperones.

The extracellular lipase (triacylglycerol acylhydrolase, EC 3.1.1.3) of *Pseudomonas cepacia* DSM 3959 is encoded by *lipA* (11–13). Jørgensen *et al.* (12) discovered that a DNA sequence immediately downstream of *lipA* was required to confer a lipase-positive phenotype on heterologous hosts, and they showed that it could act in trans. They postulated that this sequence encoded a 344-amino acid lipase modulator protein, Lim, and called the gene *limA* (11–13). The putative Lim protein was not identified in these studies. Lim is not part of the active lipase enzyme, which is composed of a single polypeptide chain.

Here we show that the *limA* gene encodes Lim; Lim is not required for either transcription or translation of *lipA*. Lim causes the lipase to adopt an active state without detectable covalent modification. Lim forms a complex with lipase. Therefore, Lim has the properties of a molecular chaperone.

MATERIALS AND METHODS

Bacterial Strains, Plasmids, and Recombinant DNA Techniques. *Escherichia coli* TG1 (14) was the standard host. *E. coli* JA221 (15) and BL21(DE3) pLysS (16) were used for high-level protein expression. DNA and protein manipulations were performed essentially as described by Sambrook *et al.* (17). Plasmids used were pSJ150 (12), pJW2 (18), pET3a (19), pLysE, and pLysS (16).

Plasmid Constructions. (*i*) Plasmid pAHE2 was constructed by subcloning the 2264-kilobase (kb) *Nsi* I fragment including *lipA* and *limA* from pSJ150 (12) into the expression vector pJW2 (18). (*ii*) Plasmid pAHE10 was constructed by excising the *limA* gene from pAHE2. (*iii*) Plasmid pCBE6 was constructed by subcloning the 1.17-kb *Cla* I–*Sph* I fragment from pSJ150 into pET3a.

Induction of *E. coli* Cultures for High-Level Expression. Plasmids derived from pJW2 were transformed into *E. coli* JA221 and induced at 42°C as described by Wang *et al.* (18). Plasmids derived from pET3a were transformed into *E. coli* BL21(DE3)/pLysS and induced with isopropyl β-D-thiogalactoside (IPTG) as described by Studier (20).

Treatment and Preparation of Protein Samples. Culture samples were immediately cooled to 0°C. Cells were harvested and resuspended in Laemmli sample buffer (21). Acetone precipitates from culture supernatants were resuspended in Laemmli sample buffer. Protein samples were analyzed by SDS/PAGE as described by Laemmli (21). Immunoblot analysis was carried out as described by Towbin *et al.* (22). Alkaline phosphatase-conjugated goat anti-rabbit IgG (Sigma) was used as the secondary antibody. Antibodies raised in rabbits against lipase purified from *P. cepacia* were supplied by M. Schulein (Novo Nordisk).

Generation of Antibodies Against Lim. Lim was purified by using preparative SDS/polyacrylamide gels (23) and was used to raise anti-Lim antibodies in rabbits after intradermal and intramuscular injections.

Subcellular Fractionation. Extracellular, periplasmic, and intracellular fractions were isolated from *E. coli* and *P. cepacia* as described by Neu and Heppel (24).

Denaturation/Renaturation Procedures. Inclusion bodies were prepared and solubilized in 8 M urea (25, 26). Initial

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Abbreviation: IPTG, isopropyl β-D-thiogalactoside.

dialysis in 8 M urea was carried out for 12 hr at 4°C. Further dialyses using a lower concentration of urea (i.e., 6 M, 4 M, 2 M, none) were carried out for 3 hr at 4°C.

Immunoprecipitation of the Lip–Lim Complex. Immunoprecipitation was carried out based on published procedures (27, 28). The soluble fraction of the cell lysates (the supernatant after spinning at 6000 rpm for 15 min) obtained after induction of pAHE2, pAHE10, and pCBE6 was used as a source of lipase and Lim in these experiments.

Lipase Analysis. Lipase activity was detected on tributyrine plates and measured with a pH stat as described (12).

RESULTS

Inducible Expression of Lip and Lim Proteins. *LipA* and *limA* were expressed either together or independently by using efficient inducible expression systems. pAHE2 has the *lipA* and *limA* genes inserted in the temperature-inducible expression vector pJW2 under control of λ phage promotors P_R/P_L and T7 gene *10* ribosome binding site. pAHE10 was constructed by deleting two-thirds of *limA* from pAHE2. pCBE6 has *limA* inserted in pET3a, an IPTG-inducible expression vector under the bacteriophage T7 gene *10* promoter and ribosome binding site.

Heat induction of *E. coli* JA221 pAHE2 (*lipA* + *limA*) led to the production of a protein of 35 kDa in high amounts (Fig. 1*a*, lanes E–G). Heat induction of *E. coli* JA221 pAHE10 (*lipA* only) led to the production of the same protein in equivalent amounts (Fig. 1*a*, lanes J–L). In both cases the major expressed protein had the molecular mass predicted for prelipase, and it reacted in immunoblots using lipase antibody [Fig. 1*b*, lanes D–F (pAHE2) and I–K (pAHE10)]. Therefore, expression of *limA* is not a prerequisite for either transcription or translation in the expression of *lipA*. The majority (about 95%) of the lipase protein has a molecular mass expected for prelipase, both in the presence and absence of *limA* (Fig. 1*b*, lanes E and J). Cellular fractionation showed the bulk of the prelipase and lipase proteins to be in inclusion bodies whether expressed from pAHE2 or pAHE10. Some prelipase and lipase are present in the soluble fraction.

E. coli BL21(DE3) pLysS pCBE6 (*limA* only) was induced with IPTG, and a major protein of 33 kDa was observed on SDS/polyacrylamide gels (Fig. 1*a*, lanes O–Q). This is smaller than the 36.5-kDa protein expected from the DNA sequence of *limA*. However, the protein was found to have the N-terminal amino acid sequence Thr-Ala-Arg-Gly-Gly-Arg-Ala-Pro-Leu-Ala-Arg-Arg-Ala-Val-Val-Tyr-Gly-Ala-Val-Gly as predicted from the DNA sequence for the 36.5-kDa protein. Cellular fractionation shows that Lim is the major protein in the soluble fraction of induced *E. coli* cell extracts.

Gel-purified Lim expressed after induction of *E. coli* pCBE6 was used to raise polyclonal antibodies in rabbits. The antiserum reacted with Lim expressed after induction from pCBE6 in *E. coli* (see Fig. 3*b*, lane K). Some samples from *E. coli* pCBE6 and *E. coli* pAHE2 showed two bands detected by the Lim antiserum (Fig. 2*b*, lane A). The upper band has the same electrophoretic mobility as a protein from the cytoplasmic extract of *P. cepacia* (Fig. 2*b*, lane B). The lower band does not appear when Lim is expressed in *Bacillus subtilis*. We presume it is caused by limited proteolysis in *E. coli*. Extracts of *P. cepacia* were treated (*Materials and Methods*) to obtain fraction I containing both intracellular and inner membrane material, fraction II containing outer membrane and periplasmic material, and fraction III containing extracellular material. Prelipase predominated in fraction I; both prelipase and lipase were found in fraction II, while only lipase was found in fraction III (Fig. 2*a*, lanes B, C, and D respectively). Lim was detected in fractions I and II (Fig. 2*b*, lanes B and C, respectively).

Mechanism of Action of Lim. Lipase activity was observed after heat induction when *lipA* and *limA* were coexpressed in *E. coli* from the inducible expression vector pAHE2 (data not shown). However no lipase activity was observed on plates or in extracts when *lipA* alone was expressed from the inducible plasmid pAHE10. Since a similar amount of lipase protein was synthesized in *E. coli* pAHE10 as in pAHE2 (Fig. 1*a*, lanes F and K), these results indicate that Lim must be activating the lipase protein in some way posttranslationally.

Activation of Lipase by Lim *in Vitro*. Lim might cause the lipase to adopt an active conformation. However, when extracts of *E. coli* JA221 pAHE10 and *E. coli* BL21(DE3) pLysS pCBE6 were mixed, no lipase activity was detected after incubation at different temperatures (4°C, 15°C, and 37°C) for up to 24 hr.

Most proteins are denatured in high concentrations of urea; some will renature if the concentration of urea is slowly

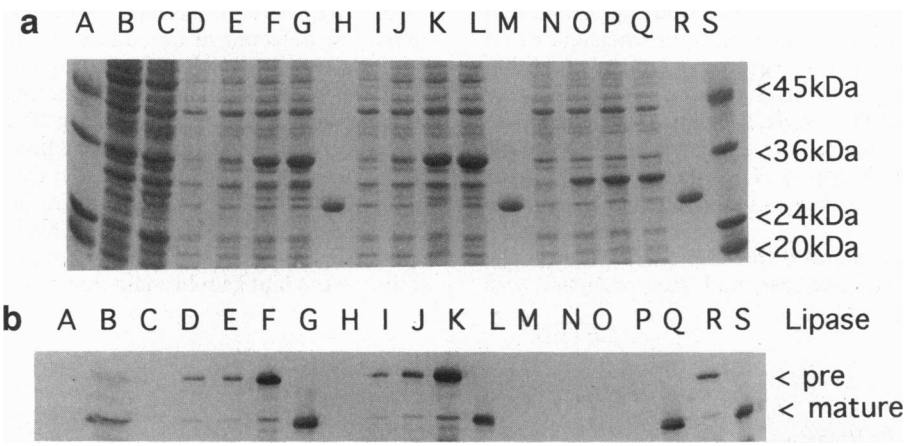

FIG. 1. Analysis of proteins expressed in *E. coli* JA221. *E. coli* cultures were induced and samples were prepared as described. (*a*) SDS/PAGE. Lanes: A and S, molecular weight markers; B, pJW2 (vector); C, pSJ150 (original lipase construct in pUC); D, pAHE2 at 30°C for 1 hr; E, pAHE2 at 42°C for 1 hr; F, pAHE2 at 42°C for 1.5 hr; G, pAHE2 at 42°C for 2 hr; H, M, and R, purified lipase from *P. cepacia*; I, pAHE10 at 30°C for 1 hr; J, pAHE10 at 42°C for 1 hr; K, pAHE10 at 42°C for 1.5 hr; L, pAHE10 at 42°C for 2 hr; N, pCBE6 without IPTG for 1 hr; O, pCBE6 with IPTG for 1 hr; P, pCBE6 with IPTG for 1.5 hr; Q, pCBE6 with IPTG for 2 hr. (*b*) Immunoblot analysis using lipase antibody. Lanes: A, pJW2 (vector); B, pSJ150; C, pAHE2 at 30°C for 1 hr; D, pAHE2 at 42°C for 1 hr; E, pAHE2 at 42°C for 1.5 hr; F, pAHE2 at 42°C for 2 hr; G, L, Q, and S, purified lipase (10 units) from *P. cepacia*; H, pAHE10 at 30°C for 1 hr; I, pAHE10 at 42°C for 1 hr; J, pAHE10 at 42°C for 1.5 hr; K, pAHE10 at 42°C for 2 hr; M, pCBE6 without IPTG for 1 hr; N, pCBE6 with IPTG for 1 hr; O, pCBE6 with IPTG for 1.5 hr; P, pCBE6 with IPTG for 2 hr; R, pAHE2 and pAHE10 at 42°C for 1.5 hr.

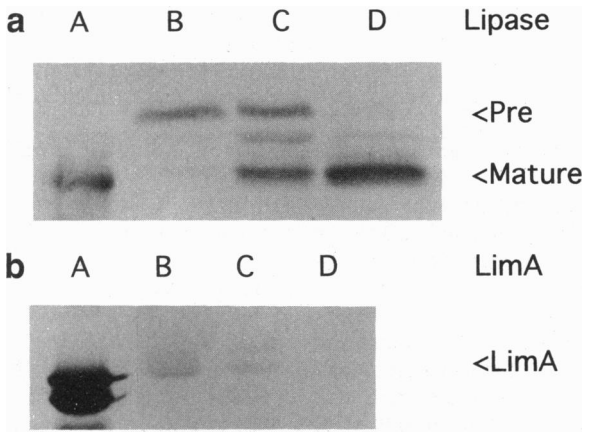

FIG. 2. Cellular localization of lipase and Lim in *P. cepacia*. Immunoblot analysis of lipase and Lim in fraction I (cytoplasm and inner cell membrane), fraction II (periplasmic and outer membrane), and fraction III (extracellular fraction) of *P. cepacia* induced with oleyl alcohol. Equal amounts of protein were loaded onto each lane corresponding to 0.01%, 17%, and 21%, respectively. (*a*) Lipase immunoblot. Lanes: A, purified lipase from *P. cepacia*; B, intracellular fraction; C, periplasmic fraction; D, extracellular fraction. (*b*) Lim immunoblot. Lanes: A, lim expressed from pCBE6; B, intracellular fraction; C, periplasmic fraction; D, extracellular fraction.

reduced (1–3, 8). We tested the effect *in vitro* of a urea denaturation–renaturation process on the lipase in the presence and absence of the Lim protein.

When samples of a cell-free extract of *E. coli* JA221 pAHE2 were denatured in 8 M urea and renatured by dialyzing against decreasing concentrations of urea, lipase activity was recovered nearly quantitatively (Table 1). In contrast cell-free extracts from *E. coli* JA221 pAHE10 (which produces lipase protein, no lipase activity, and no Lim protein) gave no lipase activity after denaturation and renaturation (Table 1). Extracts prepared from *E. coli* JA221 pAHE10 to which equimolar amounts of Lim protein [provided as an extract of *E. coli* BL21(DE3) pLysS pCBE6] were added gave lipase activity after denaturation and renaturation (Table 1). Addition of increasing amounts of Lim resulted in increasing lipase activities (Table 1). At higher amounts of Lim, the recovery of lipase activity decreased (data not shown). No lipase activity was observed when lipase and Lim were renatured separately and then mixed or when an extract of cells not expressing *limA* was used as a control. Addition of extra Lim from *E. coli* BL21(DE3) pLysS pCBE6 to extracts of *E. coli* JA221 pAHE2 during renaturation increased the amount of lipase above the value of the sample prior to denaturation (Table 1). The effect of Lim on the renaturation of lipase activity was not dependent on ATP (data not shown).

Immunoblot analysis showed that the distribution of the lipase protein between prelipase and mature lipase was

Table 1. *In vitro* renaturation of lipase in the presence of Lim

Sample	Lipase units: Prior to denaturation	Lipase units: After renaturation
pAHE2 alone	7.5	5.3
+ 10 μl of Lim	7.5	9.3
+ 20 μl of Lim	7.5	12.3
+ 30 μl of Lim	7.5	13.2
+ non-Lim lysate	7.5	5.0
pAHE10 alone	0.0	0.0
+ 10 μl of Lim	0.0	6.5
+ 20 μl of Lim	0.0	8.3
+ 30 μl of Lim	0.0	16.0
+ non-Lim lysate	0.0	0.0

unchanged after renaturation *in vitro* in the presence of Lim (Fig. 3*a*). Therefore, Lim does not cause large covalent modifications of lipase or prelipase. Neither is Lim consumed during the denaturation–renaturation procedure (Fig. 3*b*).

All lipase activity from *P. cepacia* is extracellular (Table 2). The intracellular fraction contains only inactive prelipase, and the extracellular fraction contains only mature lipase, as detected by immunoblots and assays (Fig. 2). When Lim was provided as an extract of *E. coli* BL21(DE3) pLysS pCBE6, mature extracellular lipase from *P. cepacia* could be reactivated quantitatively after denaturation/renaturation only in the presence of Lim. No lipase activity was observed when intracellular prelipase was used in a similar experiment with or without Lim (Table 3).

The activation of lipase but not prelipase from *P. cepacia* by Lim during renaturation experiments is consistent with the results when using lipase proteins produced in *E. coli*. The *E. coli* lipase samples are composed of ≈5% mature lipase and 95% prelipase (Table 2). The amount of lipase activity observed before denaturation (pAHE2 only) and after renaturation (pAHE2 and pAHE10) is equivalent to ≈5% of that expected from the total amount of lipase protein seen on SDS/PAGE.

Immunoprecipitation of a Lip–Lim Complex. The observation that Lim is required for the activation of the lipase suggested that the lipase and Lim proteins must interact with each other at some stage during and/or after the synthesis of lipase. If lipase and Lim interact strongly, such an interaction might be detected in immunoprecipitates. Lipase and Lim antibodies were used to form immunoprecipitates of lipase and Lim in various combinations. The immunoprecipitates were analyzed by immunoblotting (Fig. 4). Lipase antibody precipitated Lim in the presence of lipase (Fig. 4*b*, lane G) but not in its absence (Fig. 4*b*, lane J); the Lim antibody precipitated lipase in the presence of Lim (Fig. 4*a*, lane I) but not in its absence (Fig. 4*a*, lane H). This is strong evidence that the lipase and Lim proteins physically interact with each other. Note that Lim brought down both lipase and prelipase

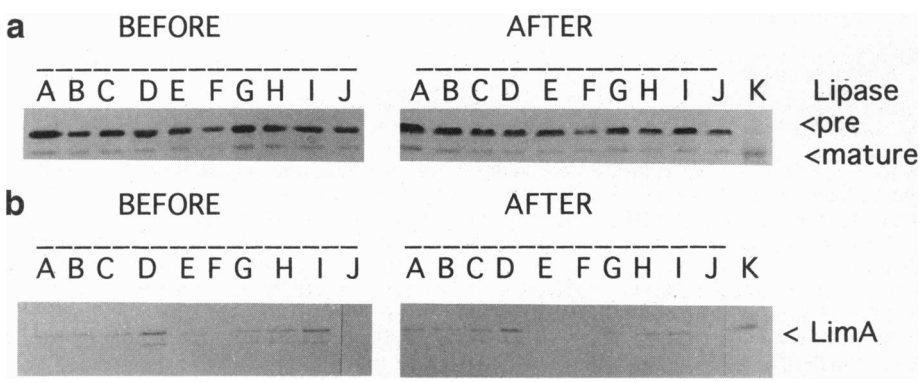

FIG. 3. Immunoblot analysis of Lip (*a*) and Lim (*b*) before and after denaturation/renaturation. (*a*) Lipase immunoblot. Lanes: A, pAHE2 (Lip + Lim); B, pAHE2 + 5 μl of Lim (pCBE6); C, pAHE2 + 10 μl of Lim; D, pAHE2 + 15 μl of Lim; E, pAHE2 + BL21 pLysS pET3a (vector); F, pAHE10 (Lip); G, pAHE10 + 5 μl of Lim; H, pAHE10 + 10 μl of Lim; I, pAHE10 + 15 μl of Lim; J, pAHE10 + BL21 pLysS pET3a; K, cell-free extract of lipase from *P. cepacia*. (*b*) Lim immunoblot. Lanes are as above except for lane K which contained *E. coli* cell-free extract of Lim.

Table 2. Localization and activity of lipase in *E. coli* and *P. cepacia*

	Gene			Lipase	Protein, %	
	lipA	*limA*	Lim	activity	Prelipase	Lipase
E. coli plasmid						
pAHE2	+	+	—	+	95	5
pAHE10	+	−	—	−	95	5
pCBE6	−	+	—	−	−	−
P. cepacia fraction						
Intracellular	—	—	+	−	100	ND
Extracellular	—	—	−	+	ND	100

and that in these experiments Lim appeared to be able to form complexes with both active lipase encoded by pAHE2 (Fig. 4*a*, lane D) and inactive lipase encoded by pAHE10 (Fig. 4*a*, lane I). In control reactions Lim antibody did not precipitate lipase in the absence of Lim (Fig. 4*a*, lane K), and lipase antibody did not precipitate Lim in the absence of lipase (Fig. 4*b*, lane J).

DISCUSSION

Jørgensen *et al.* (11–13) speculated that *limA* might be required for (*i*) elongation of either the lipA transcript or LipA protein, (*ii*) stabilization of the mRNA, (*iii*) stabilization of the LipA polypeptide, or (*iv*) secretion, either by maintaining the lipase in a suitable conformation for secretion or by participating directly in secretion at the membrane. They were not able to detect the Lim protein.

We have overexpressed *limA* and identified the Lim protein. Our results show that Lim does not affect either transcription or translation in the production of prelipase protein. Large amounts of inactive lipase are produced in *E. coli* JA221 pAHE10, though this strain does not have *limA* (Fig. 1*a*, lanes J–L). The lipase expressed in *E. coli* is predominantly (95%) found at the molecular weight expected for prelipase. The lipase is not substantially covalently modified by Lim (Fig. 1*b*): the distribution of lipase proteins (prelipase/lipase) on immunoblots is the same in samples produced *in vivo* in the presence of *limA* (*E. coli* JA221 pAHE2) or in its absence (*E. coli* JA221 pAHE10). However, active lipase is expressed only in the presence of Lim. These observations suggested Lim might affect the conformation of lipase either during or after translation.

The *in vitro* denaturation/renaturation experiments show that Lim does significantly affect the conformation of lipase during renaturation from urea. Renaturation from urea and other denaturants such as guanidinium-hydrochloride has been widely used as a method that allows proteins to adopt their lowest free-energy conformation (1–3, 8, 10). When Lim is present during renaturation, lipase activity is recovered. In the absence of Lim, no activity is recovered. The distribution of lipase proteins (prelipase and lipase) as seen in immunoblots of SDS/PAGE (Fig. 3) does not change in any significant way after denaturation/renaturation in the presence or absence of Lim. Therefore, we conclude that Lim does not cause cleavage of the lipase signal peptide or cause any other large covalent modification during renaturation. It seems as if Lim is only affecting the conformation finally adopted by the lipase. This *in vitro* observation suggests that Lim *in vivo* might interact with lipase during translation in such a way as to cause the nascent polypeptide to take up its active conformation. In other words, the evidence suggests that Lim is behaving as a molecular chaperone. No homology was found between Lim and any of the molecular chaperone sequences in the National Biomedical Research Foundation and Protein Identification Resource (release 32) or GenBank (release 72) sequence data bases.

Table 3. *In vitro* renaturation of prelipase and lipase from *P. cepacia* in the presence and absence of Lim

	Lipase units	
Cell fraction of *P. cepacia*	Prior to denaturation	After renaturation
Intracellular* (prelipase)		
20 μl alone	0.0	0.0
+ 10 μl of Lim	0.0	0.0
+ 20 μl of Lim	0.0	0.0
+ 30 μl of Lim	0.0	0.0
+ non-Lim lysate	0.0	0.0
Extracellular (mature lipase)		
20 μl alone	1.5	0.0
+ 10 μl of Lim	1.5	0.4
+ 20 μl of Lim	1.5	0.8
+ 30 μl of Lim	1.5	1.3
+ non-Lim lysate	1.5	0.0

*Cytoplasm + inner cell membrane.

The fact that Lim forms a complex with both prelipase and mature lipase, both active and inactive, as detected by immunoprecipitation (Fig. 4) suggests that Lim must have a complex role in the production of active extracellular lipase. Lipases are unusual molecules, normally dissolved in aqueous environments but also interacting with the hydrophobic surfaces of their lipid substrates, that are often in the form of micelles. Lipases have special structural properties that

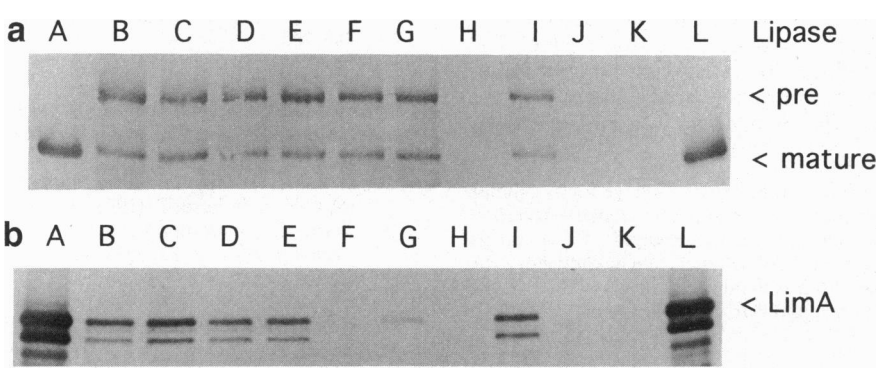

FIG. 4. Immunoprecipitation of the Lipase–Lim complex. (*a*) Lipase immunoblot. Lanes: A and L, lipase standard; B, lipase immune complex (lipase IC) + pAHE 2; C, lipase IC + pAHE2 + pCBE6; D, Lim IC + pAHE2; E, Lim IC + pAHE2 + pCBE6; F, lipase IC + pAHE10; G, lipase IC + pAHE10 + pCBE6; H, Lim IC + pAHE 10; I, Lim IC + pAHE 10 + pCBE6; J, lipase IC + pCBE6; K, Lim IC + pAHE10. (*b*) Lim immunoblot. Lanes: A and L, Lim standard; B, lipase immune complex (lipase IC) + pAHE2; C, lipase IC + pAHE2 + pCBE6; D, Lim IC + pAHE2; E, Lim IC + pAHE2 + pCBE6; F, lipase IC + pAHE10; G, lipase IC + pAHE10 + pCBE6; H, Lim IC + pAHE10; I, Lim IC + pAHE10 + pCBE6; J, lipase IC + pCBE6; K, Lim IC + pAHE10. The lipase IC is a mixture of rabbit anti-lipase and goat anti-rabbit IgG. The Lim IC is a mixture of rabbit anti-Lim and goat anti-rabbit IgG.

facilitate these interactions (29). It is possible that Lim in some way interacts with prelipase to preserve a hydrophobic patch or perhaps to prevent the lipase from aggregating. Lipase activity might be lethal if expressed intracellularly or in passage through the lipid membranes; in addition to causing the prelipase to adopt an active conformation during translation, Lim may act to inhibit lipase activity and to facilitate translocation across one or both cell membranes. It has previously been noted that Lim could be a membrane-associated protein with amino acids 14–34 forming a transmembrane helix (12). Lim affects the conformation of lipase and forms a strong complex with both prelipase and lipase (Fig. 4), and Lim has been detected in two fractions of *P. cepacia* containing (*i*) cytoplasmic and inner membranes and (*ii*) periplasmic and outer membrane material (Fig. 2). Thus, it seems likely that Lim is involved in the translocation of prelipase/lipase across one or both membranes. Other proteins that act as molecular chaperones have been shown to be involved in translocation in different ways across the cell membranes—SecY, SecE, SecD, SecF, SecA (30–33), and PapD (34) of *E. coli*; PrsA of *B. subtilis* (35); and PrtM of *Lactococcus lactis* (36). Lim is not homologous to these proteins and does not have features to suggest it is a lipoprotein analagous to PrsA or PrtM.

The structure of the *lipA-limA* operon, with only 3 base pairs between the STOP codon of *lipA* and the START codon of *limA* (ref. 12 and this paper), suggests that there is translational coupling and synthesis of equimolar amounts of prelipase and Lim. In six other *Pseudomonas* isolates [sp. KW1-56 (37), sp.109 (38), *glumea* (39, 40), *P. aeruginosa* PAO1 (41), *P. aeruginosa TE3285* (42), and 75-10A (S.T.J., unpublished observations)], the lipase genes are associated with genes that resemble *limA*. The *lip* and *lim* gene pairs have homology ranging from 95% to 30%. The structures of the *lipA-limA* (12), *lip-act* (37), *lipL-limL* (38), *lipA-ORF2* (39, 40), *lipA-lipH* (41), *lipA-lipB* (42), and *lipD-limD* operons in these seven systems all suggest strong translational coupling and the need for a 1:1 complex in the interaction between lipase and Lim. Preliminary evidence from immunoprecipitation in which lipase and Lim have been titrated against each other shows that lipase and Lim form a 1:1 complex (data not shown). Whole-cell extracts of *E. coli* pAHE2 following induction do not show equimolar amounts of lipase and Lim; this might arise if Lim were more sensitive to proteolysis in *E. coli* or for some other reason that does not reflect the natural situation in *P. cepacia*.

Finally, it is noteworthy that the DNA sequence of *lipA* and *limA*, with 3 base pairs between the two genes, suggests that the target protein, prelipase, and its "private chaperone," Lim, may once have been encoded by a single gene. The question is then why evolution dictated the folding of proteases to be guided by an intramolecular chaperone, the propeptide, while some lipases need an intermolecular private chaperone.

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