Introduction of a Lethal Redox Switch That Controls the Opening and Closing of the Hydrophobic Cavity in LolA*

Shoji Watanabe1, Yuki Oguchi1, Kazuki Takeda1‡, Kunio Miki1, and Hajime Tokuda1‡2

From the 1Institute of Molecular and Cellular Biosciences, University of Tokyo, Tokyo 113-0032, Japan, and the 2RIKEN Spring-8 Center, Harima Institute, Hyogo 679-5148, Japan, and the 3Department of Chemistry, Graduate School of Science, Kyoto University, Kyoto 606-8502, Japan

LolA plays a critical role in the outer membrane sorting of Escherichia coli lipoproteins because it carries a hydrophobic lipoprotein from the inner membrane through the hydrophilic periplasm to the outer membrane receptor LolB. LolA has an incomplete β-barrel structure composed of 11 β-strands with an α-helical lid forming a hydrophobic cavity inside. The accompanying study revealed that the hydrophobic cavity opens and closes upon the binding and release of lipoproteins, respectively. Ile93 in the α-helix and Phe140 in the β-strand are located close to each other in the hydrophobic cavity. These two residues were replaced by Cys to construct the I93C/F140C derivative. Expression of I93C/F140C immediately arrested growth whether wild-type LolA was present or not. However, this dominant negative phenotype was abolished by reducing agents, indicating that the intramolecular disulfide bonding between the two Cys residues is lethal. I93C/F140C was unstable, and its periplasmic level was lower than that of wild-type LolA or its single Cys derivative. Reduction of I93C/F140C was essential for the release of lipoproteins from the inner membrane. Moreover, treatment of I93C/F140C with divalent cross-linkers having different side chain lengths revealed that opening of the lid for a sufficient distance is required for the release activity. The binding of a fluorescent probe to the hydrophobic cavity of I93C/F140C also depended on reducing agents. Taken together, these results indicate that the two Cys residues introduced into LolA function as a redox switch, which regulates the opening and closing of the hydrophobic cavity.

LolA (1) accepts lipoproteins from LolCDE (2) in the inner membrane and then transports them to LolB (3) in the outer membrane, thereby playing a critical role in the outer membrane sorting of hydrophobic lipoproteins through the hydrophilic periplasm (4). There are at least 90 species of lipoproteins in Escherichia coli, most of which are sorted to the outer membrane by the Lol system (4, 5). The number of LolA molecules in a single cell was estimated to be several hundreds (1). Similar numbers were estimated for LolCDE and LolB molecules (2, 3). In contrast, ~106 molecules of the major outer membrane lipoprotein Lpp exist in a single cell (6, 7). However, the LolA-lipoprotein complex, an intermediate of the lipoprotein transfer reaction, was not detected in the periplasm under normal conditions, indicating that the outer membrane sorting of lipoproteins is highly efficient. It was found that Arg at position 43 of LolA decreases the strength of the hydrophobic interaction between LolA and lipoproteins, thereby enabling the efficient transfer of lipoproteins to LolB (8). In contrast, the LolA(R43L) mutant, in which Leu replaces Arg43, caused periplasmic accumulation of lipoproteins because the mutation significantly increased the strength of the hydrophobic interaction with lipoproteins (8, 9).

Both LolA and LolB comprise an incomplete β-barrel with an α-helical lid, which together form a hydrophobic cavity (10). The hydrophobic cavity of LolA is closed because of hydrogen bonding between Arg43 in the β-barrel and some residues in the lid (10). In contrast, the accompanying study revealed that most LolA(R43L) exists in an open form (11). Furthermore, the hydrophobic cavity of LolA was found to undergo opening and closing upon the binding and release of lipoproteins, respectively (11).

It has been suggested that the opening of the LolA lid is coupled to ATP hydrolysis by LolCDE (10), whereas lipoprotein transfer reactions after the formation of the LolA-lipoprotein complex proceed in the absence of energy input. For the formation of the LolA-lipoprotein complex, lipoproteins must be released from the inner membrane, transferred from LolCDE to LolA, and then properly placed in LolA. It is not known at present how the LolA lid opening is coupled to these events (12). To answer to this question and to gain detailed insights into molecular mechanisms, it seems useful if the opening and closing of the LolA lid can be regulated. Here, we report the introduction of a redox switch that can control the opening and closing of the hydrophobic cavity of LolA.

EXPERIMENTAL PROCEDURES

Materials—TALON Co2+ affinity resin (Clontech) was used to purify His6-tagged proteins. FLAG M2 affinity gel, FLAG peptides, and 1,10-phenanthroline were purchased from Sigma. Cu2+ (phenanthroline)2 was prepared according to the reported method (13). Antibodies against LolA (1) and Lpp (14) were raised in rabbits as described. Tran[35S]label (a mixture of 70% [35S]Met and 20% [35S]Cys, 1000 Ci/mol) was obtained from MP Biochemicals. IgG sorb was purchased from Enzyme 

---

* This work was supported by grants from the Ministry of Education, Science, Sports and Culture of Japan (to H.T.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 Present address: RIKEN, Brain Science Institute, Saitama 351-0198, Japan.

2 To whom correspondence should be addressed: Institute of Molecular and Cellular Biosciences, University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-0032, Japan. Tel.: 81-3-5841-7830; Fax: 81-3-5841-8464; E-mail: htokuda@iam.u-tokyo.ac.jp.
Introduction of a Redox Switch into LolA

Center Inc. TCEP and three divergent maleimidyld cross-linkers, BMH, BMOE, and BMB, were purchased from Pierce. AMS was purchased from Molecular Probes.

**Bacterial Strains and Media—** *E. coli* K-12 strains XL1-Blue (Stratagene), DLP79–36 (15), TT016 (9), and TT015 (16) were used. The last two strains carried the chromosomal *lolA* gene under the control of the lacose promoter-operator. *E. coli* B strain BL21(DE3) (17) was used for high level expression of LolA-His<sub>6</sub>. The cells were grown on LB broth (Difco) or 2× YT broth (17). When required, chloramphenicol and spectinomycin were added at 25 and 50 μg/ml, respectively.

**Construction of Plasmids Carrying Genes for LolA Cys Mutants—** Ile<sup>200</sup> and Phe<sup>140</sup> of FLAG-tagged LolA were mutated to Cys by means of PCR using *Pfu* ultra polymerase (Stratagene) with pSW77 (PBAD-*lolA*-FLAG) (18) as a template and the following oligonucleotide pairs; IC-F (5′-gtgatatcgcctgttcgctAG-TGTCgccgaccgatccacggacgac3′) and IC-R (5′-gtgctgctgatgcgcttcgcccACAGcagttacagatcc3′) for I93C; and FC-F (5′-gtgatctcgccaatctacagTGTgcgcgctgttcgctagcagtac3′) and FC-R (5′-atgatctcgccaatctacagTGTgcgcgctgttcgctagcagtac3′) for F140C, in which the uppercase letters denote Cys. Derivatives of pSW77 were amplified in XL1-Blue, and then chloramphenicol-resistant transformants were selected on LB agar plates supplemented with 25 μg/ml chloramphenicol. The mutations were confirmed by sequencing of both strands of the respective plasmids. The mutant plasmids thus obtained were named pSW1 and pSWF and encoded I93C-FLAG and F140C-FLAG, respectively, under PBAD. A mutant plasmid, pSWIF, carrying both the I93C and F140C mutations was constructed by PCR with pSW1 as a template and a pair of primers, FC-F and FC-R.

Plasmids allowing higher level expression of His<sub>6</sub>-tagged LolA were constructed for fluorescent bis-ANS binding assays. To construct pYORIF encoding His<sub>6</sub>-tagged LolA(R43L/I93C/F140C), two Cys residues were introduced into pYOR43L encoding LolA(R43L)-His<sub>6</sub> (11), as mentioned for the construction of pSWIF.

**Purification of FLAG-tagged LolA Proteins—** A reported method (18) was slightly modified. Briefly, TT016 cells harboring pSW77, pSWI, pSWF, or pSWIF were grown on 2× YT broth containing 25 μg/ml chloramphenicol at 37 °C. When the culture OD<sub>660 nm</sub> reached 0.8, the cells were induced by the addition of 0.2% arabinose and 10 mM 2-mercaptoethanol for 2 h at 37 °C, followed by conversion to spheroplasts. Periplasmic fractions were obtained as spheroplast supernatants and dialyzed against 20 mM Tris-HCl (pH 8.0) containing 5 mM 2-mercaptoethanol at 12 h for 4 °C. After centrifugation at 100,000 × g for 60 min to remove aggregated materials, the periplasmic fractions were applied to a MonoQ column, which had been equilibrated with 20 mM Tris-HCl (pH 8.0) and 5 mM 2-mercaptoethanol. The column was developed with a linear gradient of NaCl (0–1.0 M). The fractions containing LolA-FLAG were examined by Western blotting using anti-FLAG antibodies and then dialyzed against 20 mM Tris-HCl (pH 8.0) for 6 h at 4 °C. The fractions were centrifuged at 100,000 × g for 60 min and further purified on a FLAG M2 affinity column. The column was eluted with 20 mM Tris-HCl (pH 8.0) containing 100 μg/ml FLAG peptides. FLAG-tagged LolA proteins were dialyzed against 20 mM Tris-HCl (pH 8.0) containing 10% glycerol for 6 h at 4 °C.

**Pulse-Chase Labeling of LolA(I93C/F140C)—** TT016 cells harboring pSW77 or pSWIF were grown on 5 ml of M63–0.2% maltose minimal medium supplemented with 0.5% NaCl and 0.1 mM IPTG at 37 °C. When the culture OD<sub>660 nm</sub> reached 1.0, the cells were induced for 5 min by the addition of 0.2% arabinose in the presence and absence of 2 mM TCEP (pH 7.5), a reducing agent containing no SH group. The cells in 5 ml of culture were collected by centrifugation and then resuspended in 1 ml of M63–0.2% maltose minimal medium supplemented with 0.5% NaCl. The cell suspensions were pulse-labeled with 10 μCi of Tran<sup>[35S]</sup>label for 1 min and then chased with 12 mM nonradioactive Met and Cys for the indicated times. The reaction was terminated by the addition of trichloroacetic acid. Trichloroacetic acid-precipitated proteins were analyzed by SDS-PAGE and fluorography after immunoprecipitation with anti-LolA antibodies.

**Modification of LolA Cys Mutants with SH-specific Reagents—** FLAG-tagged Cys derivatives of LolA (3 μg) were incubated with 2 mM TCEP at 95 °C for 5 min and then chilled on ice for 1 min. LolA derivatives were fully active after these treatments. The reduced derivatives were oxidized with 5 mM Cu<sup>2+</sup>(phenanthroline), and then kept reduced in the presence of 2 mM 2-mercaptoethanol or TCEP or modified with 2 mM divalent cross-linker, BMOE, BMB, or BMH, at 25 °C for 2 h. These reagents were removed with a Microbiospin column (Bio-Rad). To examine the state of SH groups, LolA derivatives (100 ng) modified with the above reagents were further incubated with 2 mM AMS at room temperature for 2 h. The reaction was terminated by the addition of trichloroacetic acid. Trichloroacetic acid-precipitated proteins were analyzed by nonreducing SDS-PAGE (14%) and Western blotting with antibodies against FLAG peptides.

**Release of L10P from Spheroplasts—** The release of L10P from spheroplasts was examined as reported (18). Briefly, *E. coli* DLP79–36 cells harboring pYL10P (PBAD-L10P) (19) were grown on M63–0.2% maltose minimal medium supplemented with 0.5% NaCl at 37 °C. When the culture OD<sub>660 nm</sub> reached 1.0, the cells were induced with 0.2% arabinose for 5 min and then converted to spheroplasts. The spheroplasts were collected by centrifugation at 16,000 × g for 2 min after the addition of 20 mM MgCl<sub>2</sub> and then resuspended in M63–0.2% maltose minimal medium supplemented with 0.5% NaCl, 250 mM succrose, and 10 mM MgCl<sub>2</sub>.

The spheroplast suspension (300 μl) was mixed with 750 μl of M63–0.2% maltose minimal medium supplemented with 0.5% NaCl, 250 mM succrose, 10 mM MgCl<sub>2</sub>, and 10 μCi of Tran<sup>[35S]</sup>label and then subjected to 1 min pulse-labeling, followed by a 10-min chase with a 12 mM nonradioactive Met and Cys mixture. LolA proteins (2 μg/ml) treated with various Cys-specific reagents were then added to induce the release of L10P.

---

The abbreviations used are: TCEP, tris-(2-carboxyethyl)phosphine hydrochloride; bis-ANS, 4,4′-dianilino-1,1′-binaphthyl-5,5′-disulfonic acid; IPTG, isopropyl-β-D-thiogalactopyranoside; AMS, 4-acetamido-4′-maleimidylstiblene-2,2′-disulfonic acid; BMH, bis-maleimidohexane; BMOE, bis-maleimidoethane; BMB, 1,4-bis-maleimidobutane.
To terminate the reaction, the mixture was chilled in ice water, followed by centrifugation at 16,000 × g for 2 min. The spheroplasts and supernatant thus obtained were subjected to trichloroacetic acid precipitation and then immunoprecipitation with anti-Lpp antibodies as reported (1). 35S-Labeled L10P was analyzed by SDS-PAGE and fluorography.

**RESULTS**

**Construction of Cys Mutants**—The accompanying study revealed that the hydrophobic cavity of LolA undergoes opening and closing upon the binding and release of lipoproteins, respectively (11). To examine the functional importance of the opening and closing of the LolA cavity in more detail, we attempted to introduce a switch that can regulate the open/closed state of the cavity. For this, we used Ile93 in the α2 helix and Phe140 in the β10 strand. The crystal structure of LolA(R43L) revealed that the Cβ-Cβ distances between Ile93 and Phe140 are 7.0 Å for the closed form and 10.9 Å for the open form (Fig. 1A). It has been reported that two Cys residues form a disulfide bond when the Cβ-Cβ distance is within 3–5 Å (21). This is applicable to two Cys residues located in the same domain (22). If a Cys is present in a mobile domain, disulfide bonds can be formed between two Cys residues at more than 10 Å apart (23). Thus, we constructed a LolA mutant in which Cys replaced either Ile93 or Phe140, or both.

TT016 (lacPO-loLA lpp-) or TT015 (lacPO-loLA lpp-) cells harboring a plasmid that carried the gene for wild-type LolA or a Cys mutant of it were grown in the presence of 0.1 mM IPTG and 0.2% arabinose (Fig. 1, B and C). LolA and its Cys derivatives were expressed as FLAG-tagged proteins. All of the five Lol proteins are essential for E. coli growth (16, 24, 25). Two single Cys mutants, I93C and F140C, did not inhibit the growth of the two strains. Furthermore, these single Cys mutants were functional and complemented the absence of wild-type LolA (data not shown). In marked contrast, a double Cys mutant, I93C/F140C, caused very severe inhibition of the growth of both strains even in the presence of LolA expressed from the chromosome. On the other hand, the I93G mutant, which was previously characterized as a strong dominant negative mutant (18), arrested the growth of TT016, but not that of TT015, in the presence of chromosomally encoded LolA. Because mislocalization of Lpp in the inner membrane causes a very toxic covalent linkage between the mislocalized Lpp and peptidoglycan (26), the defect of lipoprotein sorting caused by I93G is severe in Lpp cells but only marginal in Lpp cells. In contrast, I93C/F140C arrested the growth of not only Lpp cells but also Lpp cells, indicating that this is the strongest dominant negative derivative among the more than 20 LolA mutants previously isolated (8, 9, 18).

We next examined the growth of TT016 cells expressing LolA (Fig. 1D) or I93C/F140C (Fig. 1E) in the presence of various concentrations of 2-mercaptoethanol. Their growth was unaffected by 2-mercaptoethanol when wild-type LolA was expressed (Fig. 1D). In contrast, the growth of cells expressing I93C/F140C absolutely required 2-mercaptoethanol (Fig. 1E).
or another reducing agent such as dithiothreitol or TCEP (data not shown). These results indicate that the lethality of I93C/F140C is caused by the formation of a disulfide bond.

Oxidized and Reduced Forms of LolA(I93C/F140C)—TT016 cells were grown in the presence of IPTG to express wild-type LolA from the chromosome. When the culture OD$_{660}$nm reached 0.6, arabinose was added to induce LolA or the three Cys derivatives from plasmids in the presence and absence of 2-mercaptoethanol. Only the expression of I93C/F140C in the absence of 2-mercaptoethanol inhibited growth. Whole cell proteins were precipitated with trichloroacetic acid and then analyzed by SDS-PAGE and fluorescent flow cytometry in the presence of 2-mercaptoethanol and then purified (Fig. 3A). Irrespective of the presence or absence of 2-mercaptoethanol in the growth medium, wild-type LolA, I93C and F140C migrated to the same positions as single bands. In contrast, two bands were detected with I93C/F140C. The slower migrating molecule most likely represents the oxidized form of I93C/F140C whose structure had become more compact because of an internal disulfide bond. No materials were detected with anti-FLAG peptide antibodies (Fig. 3). Reduced and AMS-treated I93C/F140C was slower than that of wild-type LolA. The periplasm is a highly oxidative environment, and a small amount of the oxidized form of I93C/F140C was detected even in the presence of a reducing agent (Fig. 2A, lower panel). The decreased expression of I93C/F140C in the presence of TCEP seems to be caused by the presence of the unstable oxidized form.

**Divalent Cross-linking of Purified LolA(I93C/F140C)—I93C/F140C and the other LolA proteins were overexpressed in the presence of 2-mercaptoethanol and then purified (Fig. 3A). In contrast to the results of in vivo analysis (Fig. 2A), the oxidized form of the purified I93C/F140C comprised the major fraction because of oxidation during its purification, whereas only the reduced form was detected for wild-type LolA and the two single Cys mutants (Fig. 3A). Furthermore, no proteins were detected in other parts of the SDS-PAGE stained with Coomassie Brilliant Blue.

Purified proteins were reduced by TCEP and then either oxidized with Cu$^{2+}$ (phenanthroline)$_3$, kept reduced in the presence of TCEP, or treated with SH-specific AMS (Fig. 3B). Non-reducing SDS-PAGE revealed that the purified I93C/F140C molecules after these treatments migrated to three different positions as single bands (lanes 10–12). On the other hand, the mobility of wild-type LolA was not affected by these treatments. AMS (molecular mass = 536 Da) modifies free SH groups and causes slower migration on SDS-PAGE. The two single Cys mutants migrated slightly more slowly than the reduced form after AMS treatment, indicating that I93C and F140C were each modified with one AMS molecule. The migration of AMS-treated I93C/F140C was slower than that of the AMS-treated single Cys mutants. The difference between the migration positions of the reduced and AMS-treated I93C/F140C was about 1 kDa, indicating that two AMS molecules are attached to I93C/F140C.

The purified I93C/F140C was reduced with TCEP and then modified with divalent cross-linkers with different side chain lengths (Fig. 3C). Oxidized, reduced, and cross-linked I93C/F140C molecules were further treated with AMS and then analyzed by nonreducing SDS-PAGE (Fig. 3D). Cross-linked I93C/F140C migrated slightly slower than oxidized I93C/F140C and faster than I93C/F140C modified with two AMS molecules. The mobility of I93C/F140C modified with divalent cross-link-
ers remained unchanged on further treatment with AMS (Fig. 3E). Taken together, these results indicate that I93C/F140C molecules were completely modified with the respective divalent cross-linkers.

Lipoprotein Releasing Activity of Cross-linked LolA(I93C/F140C)—The release of 35S-labeled lipoprotein L10P (19) from spheroplasts was examined with I93C/F140C that had been modified with three divalent cross-linkers (Fig. 4A). The activities of reduced and oxidized I93C/F140C molecules were also examined. The amount of L10P remaining in spheroplasts or released into the supernatant was then determined (Fig. 4B). The activity of wild-type LolA was not affected by any treatment. On the other hand, oxidation with Cu2+/H11001(phenanthroline)3 completely inactivated I93C/F140C, whereas reduced I93C/F140C was as active as wild-type LolA. The activity of I93C/F140C treated with divalent cross-linkers was affected by the length of the space arms (Fig. 4B). Cross-linking with the shortest BMOE caused near complete inhibition, whereas I93C/F140C treated with BMH was as active as the reduced form, indicating that the LolA function is inhibited when the distance between the α2 helix and the β10 strand is short. On the other hand, if the fixed distance is greater, cross-linked I93C/F140C was able to release L10P. These results, taken together, indicate that the opening of the LolA lid is essential for lipoprotein binding activity.

Introduction of a Redox Switch into the Hydrophobic Cavity of LolA(R43L)—Arg at position 43 of LolA plays an important role in maintaining the closed conformation of free LolA (11). The crystal structure of LolA(R43L), in which Leu replaced Arg at position 43, revealed that the majority of this derivative exists in the open conformation. It was then found that the fluorescent probe bis-ANS binds to the hydrophobic cavity of LolA(R43L) or liganded wild-type LolA (11). Based on these observations, we introduced a redox switch comprising I93C/F140C into LolA(R43L) derivative. The increase in the bis-ANS fluorescence induced by R43L/I93C/F140 was completely dependent on the reduction of the switch (Fig. 5). On the other hand, neither oxidation nor reduction affected the binding of bis-ANS when the redox switch was not introduced into LolA(R43L) (Fig. 5, inset). Taken together, these results indicate...
Introduction of a Redox Switch into LolA

Crystallographic and biochemical studies revealed that the hydrophobic cavity of LolA undergoes opening and closing upon the binding and release of lipoproteins, respectively (11). To address the functional importance of opening of the hydrophobic cavity in more detail, we tried to introduce a redox switch to LolA. We expected that two Cys residues replacing Ile93 and Phe140 in the \( \beta \)-barrel might form a disulfide bond and then function as a switch for the hydrophobic cavity, although the \( CB-\beta B \) distance for these residues was more than 7 Å. To our surprise, the LolA(I93C/F140C) derivative thus constructed was found to be the strongest dominant negative mutant so far isolated in the absence but not the presence of a reducing agent (Fig. 1). As far as we know, this is the first example of an \( E. \ coli \) strain of which the growth absolutely depends on a reducing agent. Pulse-chase labeling of LolA(I93C/F140C) revealed that its turnover rate is considerably faster than that of wild-type LolA, and therefore it exists at a low level even in the presence of a reducing agent (Fig. 2). Interestingly, the dominant negative property of LolA(I93C/F140C) was partly suppressed on the overexpression of LolCDE, suggesting that oxidized I93C/F140C inhibits the binding of wild-type LolA to LolCDE. It was recently revealed that free LolA and liganded LolA have distinct properties, i.e. free LolA does not interfere with the lipoprotein transfer from liganded LolA to LBB (28), but liganded LolA is unable to catalyze the lipoprotein release reaction from the inner membrane (11), suggesting that the interaction with LolCDE is limited to the closed form of LolA and that with LBB is restricted to the liganded form of LolA. The oxidized I93C/F140C seems to have higher affinity for LolCDE than wild-type LolA has and thereby exhibits the dominant negative property.

The ability of I93C/F140C to release lipoproteins absolutely required the reduction of the redox switch (Fig. 4A), indicating that opening of the cavity is essential for the lipoprotein releasing activity. Moreover, treatment of I93C/F140C with divalent cross-linkers having different side chain lengths revealed that opening of the lid for a sufficient distance (more than 10 Å) is essential for the function (Fig. 4B). It seems therefore likely that the open conformation of LolA(R43L) (Fig. 4A, right panel) represents the conformation of liganded LolA. Nevertheless, the free form of LolA(R43L) does not inhibit the lipoprotein transfer from liganded wild-type LolA to LBB (28). These results suggest that an additional conformation change occurs in LolA upon the lipoprotein binding, which then enables the interaction with LBB. Elucidation of the exact conformation of liganded LolA must await crystallographic study.

Construction of a LolA derivative with a switch that turns the LolA function on and off is expected to be useful for the detailed analyses of the lipoprotein transfer reaction. The structure of

---

**FIGURE 4. Release of lipoproteins by cross-linked LolA(I93C/F140C).**

A, L10P was labeled with Tran\(^{35}\)S in spheroplasts prepared from \( E. \ coli \) DLP79–36 cells harboring pJYL10P. The release of L10P was started at 30 °C by the addition of LolA or I93C/F140C, which had been treated with 2-mercaptoethanol (2Me), Cu\(^{2+}\) (phenanthroline), (Phe), or the indicated cross-linkers, as described in the legend to Fig. 3D. The reaction mixture was fractionated at the specified times into a supernatant (S) and a pellet (P) and then analyzed by SDS-PAGE and fluorography after immunoprecipitation with antibodies against Lpp. B, the results shown in A were determined with an ATTO densitograph, and the percentages of L10P released into the supernatants were plotted as a function of time. WT, wild type.

---

**FIGURE 5. The I93C/F140C switch regulates the bis-ANS binding to the hydrophobic cavity of LolA(R43L).** R43L (inset) or the R43L/I93C/F140C derivative was dialyzed against 20 mM sodium phosphate (pH 7.2) to remove dithiothreitol and then oxidized with 2 mM Cu\(^{2+}\) (phenanthroline)\(_3\) at room temperature for 1 h. After dialysis against 20 mM sodium phosphate, the oxidized LolA derivatives were reduced with 2 mM dithiothreitol or not reduced. The fluorescence emission spectrum of 5 \( \mu \)M bis-ANS was then measured in the presence of 2.5 \( \mu \)M oxidized and reduced LolA derivatives.
LolB is very similar to that of LolA (10), and its hydrophobic cavity also binds bis-ANS.5 The lipoprotein transfer from LolA to LolB takes place in the absence of energy input through the two similar hydrophobic cavities. The LolB function is likely to be regulated when a similar redox switch is introduced. The switch will be a useful tool for determining the molecular events of the transfer reaction in detail.

Acknowledgment—We thank Rika Ishihara for technical support.

REFERENCES
1. Matsuyama, S., Tajima, T., and Tokuda, H. (1995) EMBO J. 14, 3365–3372
2. Yakushi, T., Masuda, K., Narita, S., Matsuyama, S., and Tokuda, H. (2000) Nat. Cell Biol. 2, 212–218
3. Matsuyama, S., Yokota, N., and Tokuda, H. (1997) EMBO J. 16, 6947–6955
4. Tokuda, H., and Matsuyama, S. (2004) Biochim. Biophys. Acta 1693, 5–13
5. Narita, S., Matsuyama, S., and Tokuda, H. (2004) Arch. Microbiol. 182, 1–6
6. Braun, V. (1975) Biochim. Biophys. Acta 415, 335–377
7. Mizushima, S. (1984) Mol. Cell. Biochem. 60, 5–15
8. Taniguchi, N., Matsuyama, S., and Tokuda, H. (2005) J. Biol. Chem. 280, 34481–34488
9. Miyamoto, A., Matsuyama, S., and Tokuda, H. (2001) Biochem. Biophys. Res. Commun. 287, 1125–1128
10. Takeda, K., Miyatake, H., Yokota, N., Matsuyama, S., Tokuda, H., and Miki, K. (2003) EMBO J. 22, 3199–3209
11. Oguchi, Y., Takeda, K., Watanabe, S., Yokota, N., Miki, K., and Tokuda, H.
5 Y. Oguchi and H. Tokuda, unpublished observation.

Introduction of a Redox Switch into LolA

SEPTEMBER 12, 2008 • VOLUME 283 • NUMBER 37
JOURNAL OF BIOLOGICAL CHEMISTRY