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Human ABCA3, a product of a responsible gene for *abca3* for fatal surfactant deficiency in newborns, exhibits unique ATP hydrolysis activity and generates intracellular multilamellar vesicles☆

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Abstract

ABCA3 is highly expressed at the membrane of lamellar bodies in alveolar type II cells, in which pulmonary surfactant is stored. *ABCA3* gene mutations cause fatal surfactant deficiency in newborns. We established HEK293 cells stably expressing human ABCA3 and analyzed the function. Exogenously expressed ABCA3 is glycosylated and localized at the intracellular vesicle membrane. ABCA3 is efficiently photoaffinity labeled by 8-azido-[α^{32}P]ATP, but not by 8-azido-[γ^{32}P]ATP, when the membrane fraction is incubated in the presence of orthovanadate. Photoaffinity labeling of ABCA3 shows unique metal ion-dependence and is largely reduced by membrane pretreatment with 5% methyl-β-cyclodextrin, which depletes cholesterol. Electron micrographs show that HEK293/hABCA3 cells contain multivesicular, lamellar body-like structures, which do not exist in HEK293 host cells. Some fuzzy components such as lipids accumulate in the vesicles. These results suggest that ABCA3 shows ATPase activity, which is induced by lipids, and may be involved in the biogenesis of lamellar body-like structures.

Keywords: ABCA3; Cholesterol; Pulmonary surfactant; ATPase; ABC proteins; Lamellar body; Photoaffinity label

The lamellar body, in which pulmonary surfactant is stored, is a member of lysosome-related organelles also referred to as secretory lysosomes [1–3]. Lamellar bodies are secreted into the alveolar space by exocytosis. Secreted pulmonary surfactant coats the lumen of alveoli, where it reduces the surface tension at the alveolar air/liquid interface, thus preventing alveoli from collapsing and lowering the work of breathing. Pulmonary surfactant is composed of lipids (90%) and surfactant proteins (SP-A, SP-B, SP-C, and SP-D), which are densely packed into multilamellar structures. The most abundant lipid in pulmonary surfactant is phosphatidylcholine, especially dipalmitoylphosphatidylcholine. The mechanism by which lipids are packed into lamellar bodies is unknown.

The ATP-binding cassette transporter A3 (ABCA3) is predominantly expressed in lung [4,5] and localized to the limiting membrane of lamellar bodies in alveolar type II cells in humans and the rat [6,7]. Recently, it was revealed that *ABCA3* gene mutations cause fatal surfactant deficiency in newborns [8]. Some ABC transporters belonging to the ABCA subfamily are involved in the transmembrane transport of endogenous lipids such as ABCA1 [9], ABCA4 [10], and ABCA7 [11].

☆ *Abbreviations:* ABCA3, ATP-binding cassette transporter A3; MβCD, methyl-β-cyclodextrin; Endo H, Endoglycosidase H; PNGaseF, peptide *N*-glycosidase F; PBS, phosphate-buffered saline.

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ABCA3 might function as a transmembrane transporter of lipid components found in pulmonary surfactant. The exclusive expression of ABCA3 in the limiting membrane of lamellar bodies further supports the hypothesis that ABCA3 is involved in the formation and/or secretion of pulmonary surfactant. However, the functions of ABCA3 are still unknown. In this study, the function of ABCA3 and its interaction with ATP were examined using HEK293 cells stably expressing human ABCA3 (hABCA3).

Materials and methods

Materials. Polyclonal antibody was raised against the C-terminal 13 amino acids of ABCA3. 8-Azido-[α^{32}P]ATP, 8-azido-[γ^{32}P]ATP, and 8-azido-[α^{32}P]ADP were purchased from Affinity Labeling Technologies, pcDNA3.1/*myc*-HisB and monoclonal antibody C219 were purchased from Invitrogen and Signet Laboratories, respectively. All other chemicals were obtained from Sigma, Wako Pure Chemical Industries, and Nacalai Tesque.

Transfection and establishment of HEK293 cells stably expressing hABCA3. HEK293 cells were cotransfected by pCMVhABCA3 and pcDNA3.1/*myc*-HisB with LipofectAMINE (Invitrogen) according to the manufacturer's instructions. Cells were selected by 1 mg/ml geneticin (G418) for 2 weeks. Single colonies were isolated, and the expression of hABCA3 was examined by Western blot analysis and immunofluorescent staining with anti-hABCA3 antibody. To establish HEK293 cell expressing human MDR1 (hMDR1), the cells were transfected with the hMDR1 expression vector pCAGGSP/MDR1 [12] and selected by 50–80 nM vinblastine for 2 weeks. Resistant cells were further selected by 400 nM vinblastine and 100 nM doxorubicin hydrochloride for 4 weeks. Mixed populations of vinblastine and doxorubicin resistant colonies were obtained, and the expression of hMDR1 was examined by Western blot analysis with monoclonal antibody C219.

Glycosylation of ABCA3. Endoglycosidase H (Endo H) and peptide *N*-glycosidase F (PNGaseF) (New England Biolabs, Beverly, MA) digestions were performed as described by the manufacturer. In brief, 20 μg of membrane proteins from HEK293 cells stably expressing ABCA3 was treated with 500 U Endo H or 0.3 U PNGaseF for 1 h at 37 °C. The deglycosylated proteins were separated by SDS–PAGE (7%) and analyzed by Western blot analysis by using anti-hABCA3 antibody.

Immunostaining and fluorescence microscopy. Cells were cultured on glass coverslips in Dulbecco's modified Eagle's medium supplemented with 10% (v/v) fetal bovine serum in 5% CO_2 at 37 °C. They were fixed with 4% paraformaldehyde and permeabilized with 0.4% Triton X-100 for 5 min. The cells and membranes were incubated overnight with anti-hABCA3 antibody, and then incubated with Alexa488-conjugated anti-rabbit IgG for 1 h. The cells and membranes were directly viewed with a 63× Plan-Neofluar or 100× Plan-Apochromat oil immersion objective using a Zeiss confocal microscope (LSM5 Pascal or LSM510).

Electron microscopy. HEK293 and HEK293/hABCA3 cells were cultured on plastic coverslips (Celldesk, LF1, Sumitomo Bakelite, Tokyo). For conventional electron microscopy, cells were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for 3 h. The cells were washed in the same buffer three times and were post-fixed in 1% OsO_4 in the buffer for 1 h. After washing in distilled water, the cells were incubated with 50% ethanol for 10 min and block stained with 2% uranyl acetate in 70% ethanol for 2 h. They were further dehydrated with a graded series of ethanol and were embedded in epoxy resin. Ultra-thin sections were doubly stained with uranyl acetate and lead citrate, and observed under a Hitachi H7600 electron microscope (Hitachi, Tokyo, Japan).

Vanadate-induced nucleotide trapping in ABCA3 with 8-azido-[α^{32}P]ATP, 8-azido-[γ^{32}P]ATP or 8-azido-[α^{32}P]ADP. The membrane fraction was prepared by nitrogen cavitation as previously described [13]. The membrane fraction (15–30 μg) was incubated with 10 μM 8-azido-[α^{32}P]ATP, 2 mM ouabain, 0.1 mM EGTA, and 40 mM Tris–HCl (pH 7.5) in a total volume of 6–10 μl for 10 min at 37 °C in the presence or absence of 400 μM orthovanadate and 3 mM $MgSO_4$. The reaction was stopped by adding 400 μl ice-cold TE buffer (40 mM Tris–HCl (pH 7.5), 0.1 mM EGTA) containing 1 mM $MgSO_4$. The supernatant containing unbound ATP was removed from the membrane pellet after centrifugation (14,000 rpm, 5 min, 2 °C), and this procedure was repeated once more. The pellets were resuspended in 8 μl of TE buffer containing 1 mM $MgSO_4$ and irradiated for 1 min (254 nm, 8.2 mW/cm^2) on ice. The samples were then electrophoresed on a 7% SDS–polyacrylamide gel, transferred to a PVDF membrane, and analyzed by Western blotting. After antibody was removed, the PVDF membrane was further analyzed by autoradiography.

MβCD-pretreatment of the membrane fraction. The membrane fraction (30 μg) was incubated in 20 μl of 40 mM Tris buffer containing no or 5% (w/v) MβCD for 30 min at 25 °C. The supernatant was removed after centrifugation (14,000 rpm, 5 min, room temperature), and the pellets were washed with 40 mM Tris buffer and subjected to vanadate-induced nucleotide trapping.

Results

Stable expression of human ABCA3 in HEK293 cells

To study the function of human ABCA3, a cell line (HEK293/hABCA3) stably expressing hABCA3 was established. A crude membrane fraction was analyzed by Western blotting, and two bands at about 190 and 150 kDa were detected by using the anti-ABCA3 anti-

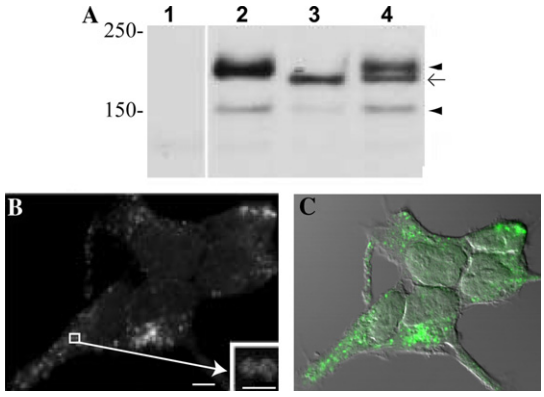

Fig. 1. (A) Western blot analysis and glycosylation of hABCA3 expressed in HEK293 cells. Membrane proteins (20 μg) prepared from HEK293 cells (lane 1) and HEK293/hABCA3 (lanes 2–4) were separated on 7% SDS–polyacrylamide gel. Membrane fractions were treated with PNGaseF (lane 3) or Endo H (lane 4), and Western blotting was done with anti-hABCA3 rabbit antibody. Immunoreactive bands of 190 and 150 kDa are indicated by the arrowheads. The deglycosylated 180 kDa ABCA3 is indicated by an arrow. (B) Immunofluorescence confocal microscopy analysis of HEK293/hABCA3 cells. Permeabilized cells were reacted with anti-hABCA3 antibody and anti-rabbit IgG-Alexa488. Scale bar, 5 μm. Inset: a magnified image of a part of the cell (marked by a square). Scale bar, 2 μm. (C) The overlaid image of ABCA3 fluorescence and differential interference contrast imaging.

body (Fig. 1A, lane 2, indicated by the arrowheads). No signal was detected in the membrane fraction prepared from HEK293 cells (Fig. 1A, lane 1). Glycosylation of ABCA3 was examined by the treatment with PNGaseF and Endo H. Endo H cleaves two proximal *N*-acetylglucosamine residues of the high mannose type but not of the complex type, while PNGaseF cleaves sugar chains of both types. Treatment with PNGaseF increased the electrophoretic mobility of 190 kDa ABCA3 to produce the 180 kDa protein (indicated by an arrow), which was the deglycosylated form. A large portion of the 190 kDa protein was insensitive to Endo H, but a portion of 190 kDa ABCA3 was sensitive to EndoH treatment resulting in the deglycosylated form. These results suggest that a large portion of ABCA3 contained complex-type sugar chains and was localized in the post-Golgi membrane. The 150 kDa protein was not affected by either glycosidase. Since the antibody was generated against the C-terminal 13 amino acids of hABCA3, the 150 kDa protein was not glycosylated and might have been a product of proteolytic cleavage at the N-terminus.

Subcellular localization of hABCA3 and the formation of vesicular structures in HEK293 cells

The subcellular localization of hABCA3 was analyzed by immunofluorescent confocal microscopy (Figs. 1B and C). ABCA3 was mainly localized at the intracellular vesicle membrane and showed a ring-like appearance (Fig. 1B, inset). Expression on the plasma membrane was rarely observed. ABCA3 immunoreactivity is mostly detected at the limiting membrane of lamellar bodies in the lung [6,7], and when EGFP-conjugated hABCA3 is expressed in human lung adenocarcinoma A549 cells, green fluorescence can be observed at the intracellular vesicle membrane [7]. The diameters of the vesicles observed in HEK293/hABCA3, at which ABCA3 was localized, were about 1 μm, corresponding with those of lamellar bodies.

Vanadate-induced nucleotide trapping in hABCA3

Next we examined if ABCA3 expressed in HEK293/hABCA3 cells was functional. Among the ABC proteins, MDR1 (ABCB1), MRP1 (ABCC1), and MRP2 (ABCC2), which transport various xenobiotics, efficiently trap Mg-ADP in the presence of orthovanadate, an analog of phosphate, and form a stable inhibitory intermediate during the ATP hydrolysis cycle. These intermediates can be specifically photoaffinity labeled in the membrane when 8-azido-[α^{32}P]ATP is used as an ATP analog [12,14–16]. For these proteins, vanadate-induced nucleotide trapping is stimulated by the addition of transport substrates, probably because they stimulate ATP hydrolysis.

To examine vanadate-induced nucleotide trapping in hABCA3, the membrane fraction was prepared from HEK293/hABCA3 cells, and observed by confocal microscopy after immunofluorescent staining to confirm the presence of ABCA3. ABCA3 immunoreactivity was detected in vesicular structures, suggesting that they were preserved during membrane preparation (Fig. 2A).

Proteins of 190 and 150 kDa were specifically photoaffinity-labeled among the membrane proteins from HEK293/hABCA3 when incubated with 8-azido-[α^{32}P]ATP in the presence of orthovanadate and Mg^{2+}, and were irradiated after removing free nucleotides (Fig. 2B, the upper panel, lane 1). These labeled proteins were identified as ABCA3 by Western blot analysis (Fig. 2B, the lower panel). hABCA3 was only weakly photoaffinity-labeled in the absence of orthovanadate, and no protein was labeled in HEK293 host cells (data not shown). These results suggested that a stable inhibitory intermediate of ABCA3 was formed with orthovanadate during the ATP hydrolysis cycle.

To confirm that the photoaffinity-labeled ABCA3 trapped ADP after hydrolysis, the same experiment was performed using 8-azido-[γ^{32}P]ATP (Fig. 2B, lanes 3 and 4). ABCA3 was weakly photoaffinity-labeled by 8-azido-[γ^{32}P]ATP in the absence of orthovanadate. However, the photoaffinity-labeling was not enhanced by the addition of orthovanadate. When 8-azido-[α^{32}P]ADP was used as an ATP analog, hABCA3 was also photoaffinity-labeled in an orthovanadate-dependent manner (shown later in Fig. 3C). These results suggested that the stable inhibitory complex ABCA·3MgADP·Vi was formed in the membrane fraction after ATP hydrolysis, as was the case with transporter-type ABC proteins.

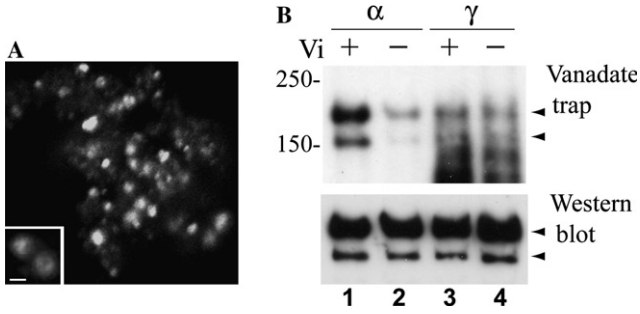

Fig. 2. Vanadate-induced nucleotide trapping (vanadate trap) in hABCA3. (A) An immunofluorescent confocal micrograph of crude membrane fraction prepared from HEK293/hABCA3. Inset, a magnified image. Scale bar, 1 μm. (B) Upper panel: vanadate trap with 8-azido-[α^{32}P]ATP or 8-azido-[γ^{32}P]ATP. Membranes (20 μg) were incubated with 10 μM 8-azido-[α^{32}P]ATP (α) or 8-azido-[γ^{32}P]ATP (γ) in the presence (+) or absence (−) of 400 μM orthovanadate at 37 °C for 10 min. Lower panel: Western blot analysis of the blot used for vanadate trap with anti-hABCA3 antibody. hABCA3 bands are indicated by the arrowheads.

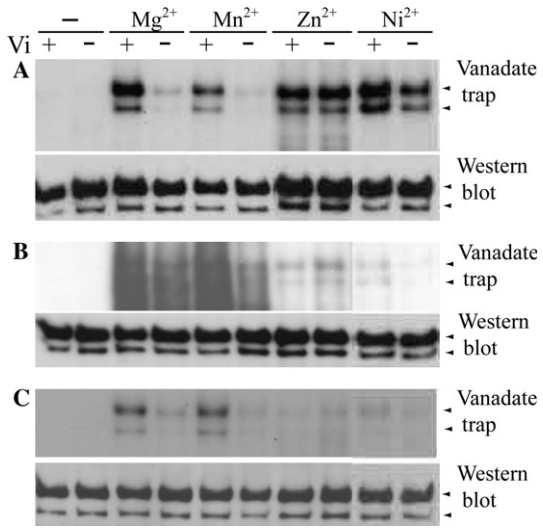

Fig. 3. Effect of metal ions on photoaffinity labeling by 8-azido-[α^{32}P]ATP (A), 8-azido-[γ^{32}P]ATP (B) or 8-azido-[α^{32}P]ADP (C). Upper panels (vanadate trap): membranes were incubated with 10 μM 8-azido-[α^{32}P]ATP (A), 8-azido-[γ^{32}P]ATP (B) or 8-azido-[α^{32}P]ADP (C), and 3 mM $MgSO_4$, $MnCl_2$, $ZnCl_2$, or $NiCl_2$, in the presence (+) or absence (−) of 400 μM orthovanadate at 37 °C for 10 min. Lower panels: Western blot analysis of the blot used for vanadate trap with anti-hABCA3 antibody. hABCA3 bands are indicated by the arrowheads.

Effect of metal ions on vanadate-induced nucleotide trapping in hABCA3

The divalent cation dependence of vanadate-induced nucleotide trapping varies among ABC proteins. Although trapping occurs with Mg^{2+}, Mn^{2+} or other divalent cations in MDR1 and MRP1, it occurs in the presence of Ni^{2+} but not Mg^{2+} in MRP6 (ABCC6) [17]. We examined the dependence of ABCA3 photoaffinity labeling on the divalent cations Mg^{2+}, Mn^{2+}, Zn^{2+}, and Ni^{2+} (Fig. 3A).

In the presence of Mn^{2+}, ABCA3 was photoaffinity-labeled in a vanadate-dependent manner as occurred in the presence of Mg^{2+}, but the efficiency was lower. In the presence of Zn^{2+}, ABCA3 was photoaffinity-labeled as strongly as in the presence of Mg^{2+}. However, labeling in the presence of Zn^{2+} was not dependent on orthovanadate. Labeling in the presence of Ni^{2+} was stronger than in the presence of Mg^{2+}. In addition, labeling occurred even in the absence of orthovanadate.

Since efficient photoaffinity-labeling was observed even in the absence of orthovanadate using Zn^{2+} and Ni^{2+}, we examined whether labeling occurs after hydrolysis. When the reaction was carried out using 8-azido-[γ^{32}P]ATP, ABCA3 was rarely photoaffinity-labeled, suggesting that 8-azido-[γ^{32}P]ATP was hydrolyzed and γ-phosphate was released (Fig. 3B). However, it was also rarely photoaffinity-labeled with 8-azido-[α^{32}P]ADP in the presence of Zn^{2+} and Ni^{2+} (Fig. 3C), although photoaffinity labeling was observed in the presence of Mg^{2+} and Mn^{2+}. These results suggest that the stable complexes ABCA3ZnADP and ABCA3NiADP formed only during ATP hydrolysis.

MβCD-pretreatment suppressed nucleotide trapping in hABCA3

Since efficient vanadate-induced nucleotide trapping in ABCA3 was observed without exogenously added substrates, we speculated that endogenous substrate(s) of ABCA3 were present in the membrane fraction. Lipids and surfactant proteins are densely packed in lamellar bodies, and therefore phospholipids and/or cholesterol are likely to be substrates of ABCA3. To examine the validity of this hypothesis, the membrane fraction was treated with 5% MβCD, which depletes cholesterol from the membrane [18–20], at 25 °C for 30 min before reaction with 8-azido-[α^{32}P]ATP. Vanadate-induced nucleotide trapping in hABCA3 was strongly suppressed by the treatment with 5% MβCD (Fig. 4A).

We also examined the effect of MβCD-pretreatment on vanadate-induced nucleotide trapping in MDR1, whose endogenous substrate was suggested to be cholesterol [21]. Vanadate-induced nucleotide trapping in MDR1 in the absence of exogenously added substrate was suppressed by treatment with 5% MβCD (Fig. 4B). In the presence of verapamil, a transport substrate of MDR1, photoaffinity labeling occurred efficiently, even after treatment with 5% MβCD (Fig. 4B). These results suggest that substrate recognition and ATP hydrolysis by MDR1 are not impaired by treatment with 5% MβCD. Therefore, reduced vanadate-induced nucleotide trapping in ABCA3 and MDR1 by treatment with 5% MβCD was due to the depletion of endogenous substrates, most likely cholesterol, from the membrane.

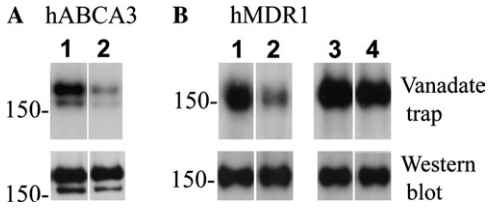

Fig. 4. Effect of MβCD treatment on vanadate-induced nucleotide trapping in hABCA3 and hMDR1. (A) Membranes prepared from HEK293/hABCA3 pretreated without (lane 1) or with 5% MβCD (lane 2) at 25 °C for 30 min. Upper panel: vanadate trap with 10 μM 8-azido-[α^{32}P]ATP at 37 °C for 10 min. Lower panel: Western blot analysis of the blot used for vanadate trap with anti-hABCA3 antibody. (B) Membranes prepared from HEK293/hMDR1 pretreated without (lanes 1 and 3) or with 5% MβCD (lanes 2 and 4) at 25 °C for 30 min. Upper panels: vanadate trap with 10 μM of 8-azido-[α^{32}P]ATP in the absence (lanes 1 and 2) or presence (lanes 3 and 4) of 25 μM verapamil at 37 °C for 10 min. Lower panels: Western blot analysis of the blot used for vanadate trap with anti-MDR1 antibody C219.

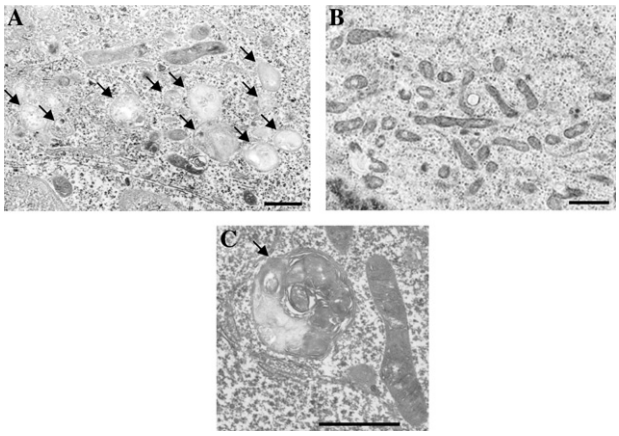

Fig. 5. Electron micrograph of HEK293/hABCA3 (A,C) and HEK293 host cells (B). Lamellar body-like structures are indicated by the arrows. Scale bars, 1 μm.

Generation of lamellar body-like structures in HEK293/hABCA3

Finally, HEK293/hABCA3 cells were observed by electron microscopy and compared with HEK293 host cells to confirm that the vesicular structures were generated by exogenously expressed ABCA3, In HEK293/hABCA3 cells, many unique vesicles 0.6–1 μm in diameter were observed (Figs. 5A and C). They appeared to be multilamellar and contained fuzzy components. These lamellar vesicular structures were scarcely observed in HEK293 host cells (Fig. 5B).

Discussion

Because *ABCA3* gene mutations cause fatal surfactant deficiency in newborns [8], the function of ABCA3 is crucial for the formation and/or secretion of pulmonary surfactant. However, the function of ABCA3 is still unknown. In this study, we showed that exogenously expressed ABCA3 generates vesicular structures in HEK293 cells. Electron micrographs show that HEK293/hABCA3 cells contain multivesicular, lamellar body-like structures, which do not exist in HEK293 host cells. Some fuzzy components such as lipids accumulate in the vesicles. These results suggest that ABCA3 may be involved in the generation of lamellar body-like structures.

ABCA3 is strongly photoaffinity labeled by 8-azido-[α^{32}P]ATP when the membrane fraction is incubated in the presence of Mg^{2+} and orthovanadate. Photoaffinity labeling by 8-azido-[γ^{32}P]ATP is weak and is not stimulated by the addition of orthovanadate, indicating that ABCA3 shows strong ATPase activity in the isolated membrane. Vanadate-induced nucleotide trapping in ABCA3 was strongly suppressed by pretreatment of the membrane with MβCD, which depletes cholesterol [18–20]. The basal vanadate-induced nucleotide trapping in MDR1, whose endogenous substrate is suggested to be cholesterol [21], is also inhibited by pretreatment of the membrane with MβCD. The reduction of photoaffinity labeling of ABCA3 and MDR1 is most likely due to cholesterol depletion from the membrane. These results suggest that cholesterol may be a transport substrate for ABCA3. Alternatively, MβCD might deplete phospholipids together with cholesterol. Because dipalmitoylphosphatidylcholine, most abundant lipid in pulmonary surfactant, has high affinity to cholesterol, it is possible that phospholipids with saturated fatty acid chains are also depleted from HEK293 cell membrane together with cholesterol. This might cause the suppression of vanadate-induced nucleotide trapping in ABCA3.

The dependence of photoaffinity labeling of ABCA3 on divalent cations showed a specific and unusual pattern. The most striking feature of ABCA3 was the strong photoaffinity labeling in the presence of Zn^{2+} and Ni^{2+}, which was not dependent on orthovanadate. Since ABCA3 was scarcely photoaffinity labeled by 8-azido-[γ^{32}P]ATP, 8-azido-ATP was hydrolyzed and γ-phosphate was released before a stable complex was formed. The state of the inhibitory complex was further examined using 8-azido-[α^{32}P]ADP. ABCA3 was photoaffinity labeled by 8-azido-[α^{32}P]ADP in the presence of Mg^{2+} and Mn^{2+} in a vanadate-dependent manner. However, it rarely occurred in the presence of Zn^{2+} or Ni^{2+}. These results suggest that a stable ABCA3·MeADP·Vi complex, where Me is Mg^{2+} or Mn^{2+}, can be formed in two ways. In one way, it is formed after ATP hydrolysis in the presence of orthovanadate, and in the other way ADP takes part in the stable ABCA3·MeADP·Vi complex without ATP hydrolysis. In contrast, in the presence of Zn^{2+} and Ni^{2+}, stable complexes form even without vanadate, and ADP cannot take part in the complex without ATP hydrolysis. Conformational changes caused by ATP hydrolysis are likely required to form this stable complex. The relevance of this phenomenon to the physiological role of ABCA3 remains to be solved.

hABCA3 was detected as 190 and 150 kDa proteins by immunoblot analysis using antibody generated against the C-terminal 13 amino acids of hABCA3 when stably expressed in HEK293 cells. It was previously reported that hABCA3 was detected as a single band 150-kDa protein in the crude membrane fraction of human lung tissue using the same antibody [6]. This can be explained by the cleavage of hABCA3 at its N-terminus, producing the mature form of the 150-kDa protein. The 150-kDa protein in HEK293/hABCA3 might also be a product of proteolytic cleavage. There are two consensus amino acid sequences (asparadine-124 and asparadine-140), which can be modified by N-linked oligosaccharides, in the first extracytosolic domain.

Unlike ABCA1, there is no consensus sequence for glycosylation in other extracytosolic loops. Lysosome-related organelles including the lamellar body have proteolytic enzymes in their lumen, and therefore hABCA3 may be cleaved at its lumenal loop after the second N-linked glycosylation site (asparadine-140) in vesicular structures as was reported for the yeast vacuolar ABC transporter Ycf1p [22,23].

Recently, we showed that human ABCA7 expressed in HEK293 cells is localized at the plasma membrane, and the first extracellular domain is exposed to the extracellular space [24,25] as is the case for ABCA1 [25,26]. ABCA7 showed apoA-I-dependent cholesterol and phospholipid release [11,24] as ABCA1 [9,26]. On the other hand, ABCA4 associates with intracellular vesicles when expressed in COS-1 cells [27]. ABCA4 is expressed in photoreceptors and transports retinal-phosphatidylethanolamine complexes across the photoreceptor disk membrane of rods [10,28,29]. ABCA3 is not targeted to the plasma membrane either, but to the membrane of intracellular vesicular structures when stably expressed in HEK293 cells. This is consistent with previous observations in which ABCA3 was mainly localized at intracellular vesicles when transiently expressed in the human adenocarcinoma cell lines A549 or NCI H441 [7]. The size (0.6–1μm in diameter) of the vesicular structures is also comparable with that of lamellar bodies in alveolar type II cells. In conclusion, ABCA3 may be involved in dynamics of cholesterol and possibly also phospholipid, and the biogenesis of lamellar body-like structures.

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