Conformational change of apolipoprotein A-I and HDL formation from model membranes under intracellular acidic conditions

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Abstract **The molecular mechanism by which nascent HDL forms via the interaction of apolipoprotein A-I (apoA-I) and transmembrane ABCA1 is poorly understood. Here, because ABCA1 has been reported to localize to acidic intracellular compartments, including the Golgi and endosome, we studied the interaction of apoA-I with model membranes under acidic conditions. Pure phosphatidylcholine liposomes were persistent against apoA-I at pH levels above 5.0, but were progressively transformed into reconstituted HDLs (rHDLs) by apoA-I at lower pH. Circular dichroism spectral measurements and 8-anilino-1-naphthalenesulfonic acid fluorescence measurements of lipid-free apoA-I ascribed this accelerated rHDL formation to the conformational change of the protein into a rather hydrophobic α-helical structure under acidic conditions. The addition of phosphatidylserine (PS) increased acidity at the bilayer surface and enabled the formation of discoidal rHDLs even at the pH of the endosome and slightly lower pH of the Golgi. These results suggest the following new scenario of nascent HDL formation: ABCA1, which colocalizes with apoA-I in acidic intracellular compartments, including the Golgi and endosome, increases acidity at the membrane surface on the luminal side by PS translocase activity and causes apoA-I to form nascent HDL.—**Fukuda, M., M. Nakano, M. Miyazaki, M. Tanaka, H. Saito, S. Kobayashi, M. Ueno, and T. Handa. **Conformational change of apolipoprotein A-I and HDL formation from model membranes under intracellular acidic conditions.** ***J. Lipid Res.*** **2008.** 49: **2419–2426.**

Supplementary key words endosome • Golgi • phosphatidylcholine • phosphatidylserine • reconstituted HDL • translocase activity

Apolipoprotein A-I (apoA-I, 28 kDa, 243 amino acids) is a major protein component of HDLs. HDL transports excess cholesterol from peripheral tissues to the liver, where cholesterol is metabolically converted to bile acids (1). This pathway, termed the reverse cholesterol transport pathway, has been a target of basic research for the development of new drugs for arteriosclerosis because of the correlation between high circulating levels of HDL and a lower risk of cardiovascular disease (2). In particular, the mechanism of HDL neogenesis has come under the spotlight. In recent years, it has been demonstrated that nascent HDL, called pre-β- or discoidal HDL, is formed by the interaction of apoA-I with transmembrane ABCA1 (3, 4). ApoA-I has a series of highly homologus 11- and 22-residue amphipathic class A and class Y α-helices (5). The secondary structural motif of the class A helix is characterized by clustered charge distribution with basic residues near the hydrophilic/hydrophobic interface and acidic residues clustered at the center of the polar face (6). In nascent HDL, apoA-I molecules surround the hydrophobic edge of the lipid bilayer like a belt (7, 8). The significance of ABCA1 in HDL biogenesis is demonstrated by mutations in the ABCA1 gene leading to Tangier disease, characterized by low plasma HDL levels (3). It is well established that ABCA1 transports phospholipids (PLs) and free (unesterified) cholesterol to lipid-free apoA-I, triggering the formation of nascent HDL (9); however, the molecular mechanism is poorly understood.

A number of mechanisms for ABCA1-mediated lipid efflux to apoA-I have been proposed, including apoA-I binding to ABCA1 directly or to ABCA1-disrupted bio-

This study was supported in part by Grants-in-aid for Scientific Research from the Japanese Ministry of Education, Culture, Sports, Science and Technology (17390011 and 20790032), research fellowships from the Japan Society for the Promotion of Science for Young Scientists (202796), and the program for the Promotion of Fundamental Studies in Health Science (04-8) of the National Institute of Biomedical Innovation.

Manuscript received 30 May 2008 and in revised form 11 July 2008.

Published, JLR Papers in Press, July 21, 2008.
DOI 10.1194/jlr.M800287-JLR200

Abbreviations: ANS, 8-anilino-1-naphthalenesulfonic acid; apoA-I, apolipoprotein A-I; CD, circular dichroism; LUV, large unilamellar vesicle; PC, phosphatidylcholine; PL, phospholipid; PS, phosphatidylserine; rHDL, reconstituted HDL; Rho-DOPE, dioleoyl-*sn*-glycero-3-phosphoethanolamine-*N*-(lissamine rhodamine B sulfonyl).

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membranes. We speculate that ABCA1 triggers the spontaneous formation of nascent HDL by changing the local environment of biomembranes around ABCA1 for the following reasons. *1*) It cannot be assumed from the thermodynamic point of view that nascent HDL is formed via a mechanism whereby ABCA1 transports hundreds of lipids to apoA-I one by one in HDL formation because higher energy is required to transport each PL from the membrane to the aqueous phase (~15 kcal/mol) than ATP hydrolysis (7.3 kcal/mol). *2*) ABCA1-mediated lipid efflux is not specific to apoA-I; other apolipoproteins (10, 11) and synthetic peptides (12, 13) with amphipathic helices can also efflux lipids from cells, indicating that physicochemical but not biochemical properties of the amphipathic helical structure are a prerequisite for the formation of nascent HDL. *3*) The lipid affinity of these amphipathic helical proteins positively correlates with its ability to remove cellular PLs (11, 14). *4*) The amount of apoA-I binding to cellular membranes is about 10-fold larger than that of apoA-I specifically binding to ABCA1 (15).

Newly synthesized apoA-I has been reported to be lipidated by ABCA1 in the Golgi (16). In addition, the trafficking of ABCA1, and possibly apoA-I, to the late endosome/lysosome compartment has been reported to occur (17–19) and to be responsible for a quantitatively significant percentage of total ABCA1-dependent cholesterol efflux (20). The pH in the Golgi and endosome is kept in the range of 6.2–6.6 (21–23) and ~5.5–6.3 (24, 25), respectively; therefore, it is interesting that apoA-I interacts with lipid membranes under these acidic conditions. Here, we report that pure phosphatidylcholine (PC) membranes, which are remarkably stable toward apoA-I at neutral pH and physiological temperature, are rapidly transformed into discoidal reconstituted HDLs (rHDLs) by apoA-I at below pH 5. This is because of the increased α-helicity and hydrophobicity of apoA-I at lower pH, and because the addition of phosphatidylserine (PS), which has been reported by a number of research groups (18, 26–29) to be redistributed to the exoplasmic/luminal side of the lipid bilayer by ABCA1, enables and accelerates the formation of discoidal rHDLs in the range of pH in the Golgi and endosome.

EXPERIMENTAL PROCEDURES

Materials

Egg yolk PC and bovine brain PS were purchased from Sigma Chemical Co. (St. Louis, Mo). Dioleoyl-*sn*-glycero-3-phosphoethanolamine-*N*-(lissamine rhodamine B sulfonyl) (Rho-DOPE) was purchased from Avanti Polar Lipids (Alabaster, AL). 8-Anilino-1-naphthalenesulfonic acid (ANS) was purchased from Molecular Probes (Eugene, OR). Purified apoA-I from human plasma (Cat. 0650-0311) was purchased from MorphoSys (Oxford, UK). ApoA-I was denatured in a 6 M guanidine hydrochloride solution and dialyzed against 10 mM Tris-HCl buffer (pH 7.4), containing 150 mM NaCl, 1 mM EDTA, and 0.01 g/ml NaN_3. The protein concentration was determined by the method of Lowry et al. (30), using BSA (Pierce) as a standard.

Liposome preparation

To prepare large unilamellar vesicles (LUVs) with specific lipid compositions, the required amounts of chloroform-methanol solutions of PC and PS were mixed in a round-bottomed glass flask. The organic solvent was removed by evaporation, and the residue was dried overnight under a vacuum. The dried lipids were dispersed in 10 mM buffer by vortexing. The following buffers were used: KCl-HCl buffer (pH 1.5–2.0); glycine-HCl buffer (pH 2.5–3.5); acetic acid-acetate buffer (pH 4.0–5.6); sodium phosphate buffer (pH 6.0–7.0); glycine-NaOH buffer (pH 9.0), containing 150 mM NaCl, 1 mM EDTA, and 0.01 g/ml NaN_3. After five rounds of freeze-thawing, the suspension was extruded through a 100 nm-pore polycarbonate filter. PC concentrations were determined using an enzymatic assay kit for choline from Wako (Osaka, Japan). The concentrations of total PLs (PC+PS) were determined using procedures described previously (31). The zeta potential (ζ) of each LUV was measured by Zetasizer Nano ZS (Malvern Instruments Ltd., UK) at 37°C. The surface potential (ϕ_0) was estimated from the zeta potential by the theory of Gouy-Chapman in a monovalent electrolyte solution (equation 1):

$$\phi(x) = \frac{2k_BT}{e}\ln\frac{\exp(e\phi_0/2k_BT)+1+\{\exp(e\phi_0/2k_BT)-1\}\exp(-\kappa x)}{\exp(e\phi_0/2k_BT)+1-\{\exp(e\phi_0/2k_BT)-1\}\exp(-\kappa x)} \quad (Eq.\ 1)$$

This formula can be rewritten as described in equation 2:

$$\phi_0 = \frac{2k_BT}{e}\ln\frac{\exp(-\kappa x)\{1+\exp(e\zeta/2k_BT)\}-1+\exp(e\zeta/2k_BT)}{\exp(-\kappa x)\{1+\exp(e\zeta/2k_BT)\}+1-\exp(e\zeta/2k_BT)} \quad (Eq.\ 2)$$

where e, k_B, T, x, and κ are the elementary charge (1.602×10^{-19} C), Boltzmann constant (1.381×10^{-23} J·K^{-1}), temperature (310 K), the distance from the surface to the shear plane of LUV (0.2 nm (32)), and the parameter of Debye-Huckel ($1{,}253 \times 10^{9}$ m^{-1}) at buffer containing 150 mM NaCl, respectively.

The proton concentration at the surface of LUV ($[H^+]_{surface}$) was calculated by Boltzmann distribution (equation 3):

$$[H^+]_{surface} = [H^+]_{bulk}\exp(-e\phi_0/k_BT) \quad (Eq.\ 3)$$

where $[H^+]_{bulk}$ is the proton concentration in bulk.

Circular dichroism spectral measurements

Circular dichroism (CD) spectra were recorded from 195 to 250 nm with a Jasco J-720 spectropolarimeter (Tokyo, Japan) at 37°C. Lipid-free apoA-I was diluted with buffer to a final concentration of 2 μM. A 0.1 cm-path-length cell was used to obtain the spectrum of apoA-I at each pH. The α-helix contents were calculated from the following equation using the mean residue molar ellipticity at 222 nm ($[\theta]_{222}$): percent α helix = $[(-[\theta]_{222} + 3{,}000)/(36{,}000 + 3{,}000)] \times 100$.

ANS fluorescence measurements

ANS fluorescence measurements were carried out at 37°C using a Hitachi F-4500 spectrofluorimeter. ANS and lipid-free apoA-I were mixed with buffer at 37°C to final concentrations of 250 μM and 1 μM, respectively. It was confirmed that the addition of ANS and apoA-I does not practically change the bulk pH. ANS fluorescence spectrum at each pH was recorded in the absence or presence of apoA-I from 400 to 600 nm with an excitation wavelength of 395 nm. Fluorescence intensities were normalized by the intensity at 490 nm observed in the presence of apoA-I at pH 7.0.

Kinetics of the microsolubilization of LUVs by apoA-I

The microsolubilization of LUVs by apoA-I was followed by a time-dependent decrease in right-angle light scattering. The reduction in light scattering is due to the transformation of LUVs ($d \sim$100 nm) to small discoidal rHDLs ($d \sim$10 nm). LUVs and apoA-I were mixed at 37°C to final concentrations of 100 μM and 1 μM, respectively. The change in the right-angle light scattering intensity was monitored on an F-4500 spectrofluorimeter for 10 min using excitation and emission wavelengths of 650 nm. The intensity ($I(t)$) was normalized by the initial intensity before the addition of apoA-I (I_0), which was corrected for the effect of volume change by the addition of apoA-I. The data were analyzed by an exponential decay curve to estimate the kinetic constant (k) and the plateau (C) of microsolubilization (equation 4):

$$I(t)/I_0 = (1 - C)\exp(-k\,t) + C \qquad (Eq.\ 4)$$

Gel filtration chromatography

LUVs (200 μM) containing 0.5 mol% Rho-DOPE were incubated with apoA-I (2 μM) at 37°C for 6 h. After the addition of NaBr to adjust specific gravity to 1.09, the mixture was ultracentrifuged (150,000 *g*) at 18°C for 15 h to separate the generated discoidal rHDLs from LUVs. The discoidal rHDL fractions were collected from the bottom and dialyzed against 10 mM Tris-HCl buffer (pH 7.4) containing 150 mM NaCl, 1 mM EDTA, and 0.01 g/ml NaN_3. The condensed samples were loaded on a Sepharose™ CL-6B (Amersham Biosciences) column equipped with a JASCO FP-6200 spectrofluorimeter, and the elution profiles were monitored by the fluorescence of Rho-DOPE with excitation/emission wavelengths of 550/590 nm, respectively. Running buffer was 10 mM Tris-Cl, 150 mM NaCl, 1 mM EDTA,

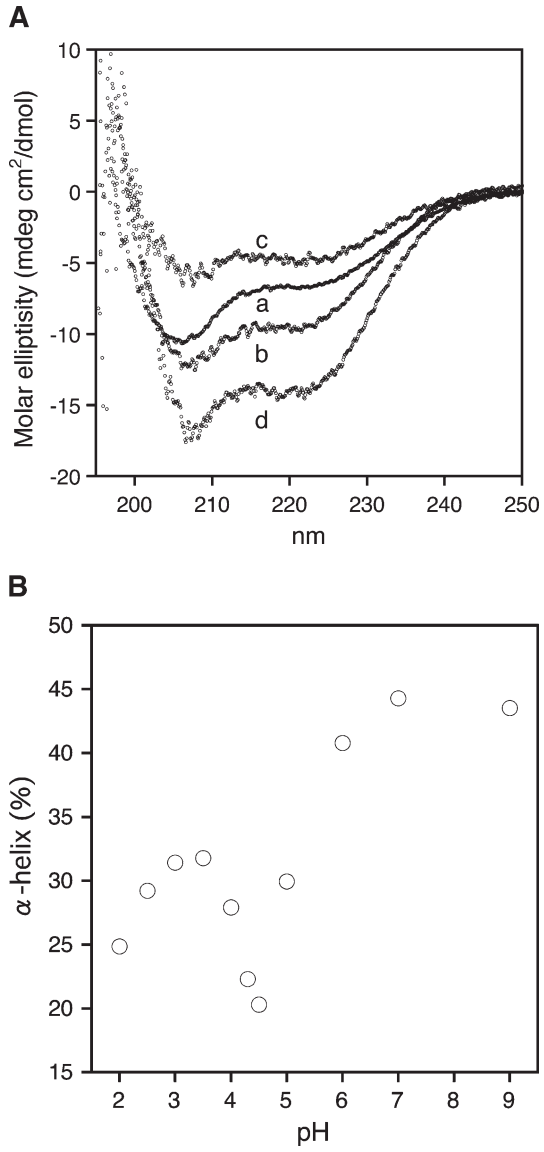

Fig. 1. Analysis of the secondary structures of lipid-free apolipoprotein A-I (apoA-I) under different pH conditions by circular dichroism (CD) spectroscopy. A: CD spectra of apoA-I at pH 2.0 (a), 3.5 (b), 4.5 (c), and 7.0 (d) at 37°C. The concentration of apoA-I was 2 μM. B: The α-helical content of apoA-I as a function of pH.

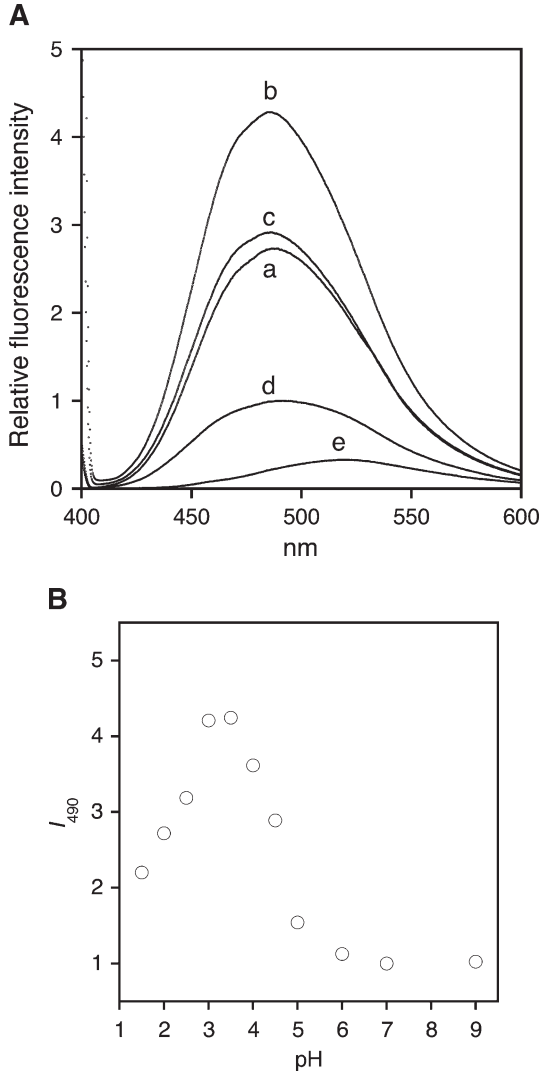

Fig. 2. Evaluation of hydrophobicity of apoA-I under different pH conditions by 8-anilino-1-naphthalenesulfonic acid (ANS) fluorescence measurements. A: ANS fluorescence spectra in the presence of apoA-I at pH 2.0 (a), 3.5 (b), 4.5 (c), and 7.0 (d) at 37°C. The concentrations of ANS and apoA-I were 250 μM and 1 μM, respectively. Fluorescence intensity was normalized by the intensity at 490 nm observed in the presence of apoA-I at pH 7.0. The spectra of free ANS (e) did not change over the pH range from 1.5 to 9.0. B: Relative fluorescence intensity of ANS at 490 nm (I_{490}) in the presence of apoA-I as a function of pH.

and 0.01 g/ml NaN_3, and the flow rate was 0.56 ml/min. The column was calibrated with standard proteins with known hydrophobic diameters: thyroglobulin (SIGMA), 17.0 nm; ferritin (SIGMA), 12.2 nm; catalase (Wako), 10.4 nm; and BSA (Pierce), 7.1 nm.

RESULTS

CD and α-helical content

The secondary structures of lipid-free apoA-I at different pH conditions were analyzed by CD spectroscopy (**Fig. 1A**). The CD spectra of apoA-I were drastically altered by the change of pH. The α-helix contents were calculated from the mean residue molar ellipticity at 222 nm and plotted as a function of pH (Fig. 1B). The α-helical content of apoA-I was ~44% at pH 7.0, which is in good agreement with previous reports (33, 34), and was approximately constant over the pH range between 7.0 and 9.0; however, apoA-I helicity decreased under acidic pH conditions. The most intensive decrease in the α-helical content was observed in the pH range from 6.0 to 5.0, where the isoelectric point of apoA-I exists (35). The helicity reached minimum at pH 4.5 (~20%), whereas it increased under more acidic conditions, peaking at pH 3.5 (~32%) and decreasing again under more acidic conditions.

ANS fluorescence

ANS fluorescence measurements were performed to elucidate the effect of pH on the exposure of hydrophobic regions in apoA-I to an aqueous environment (33). In buffer alone, ANS represented pH-independent weak fluorescence over the pH range from 1.5 to 9.0, with a λ_{max} of ~520 nm. Addition of apoA-I resulted in a blue shift (λ_{max} ~490 nm) and increased intensity (**Fig. 2A**).

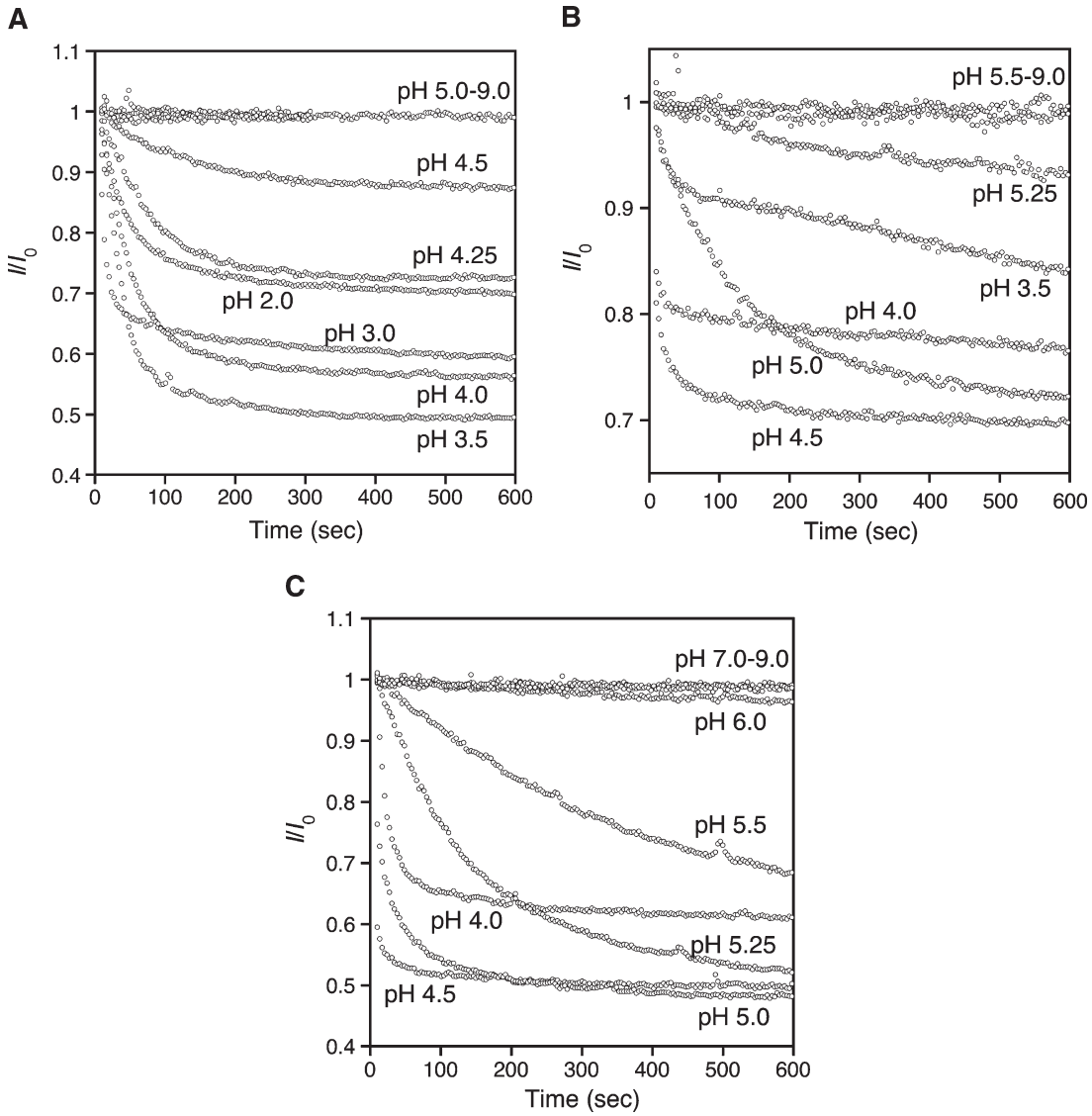

Fig. 3. Reduction in light-scattering intensity of pure phosphatidylcholine (PC) (A), phosphatidylcholine/phosphatidylserine (PC/PS) 8/2 (B), and 6/4 (C) large unilamellar vesicles (LUVs) by apoA-I at 37°C and different pH conditions. The concentrations of phospholipids and apoA-I were 100 μM and 1 μM, respectively. Data were analyzed by an exponential decay curve (equation 4).

The intensity was approximately constant over the pH range from 6.0 to 9.0, but markedly increased when pH fell below 5.0, peaking at pH 3.5, but falling under more acidic conditions (Fig. 2B). The exposure of hydrophobic interfaces of apoA-I at lower pH was presumably caused by the conformational change of apoA-I and progressive neutralization of basic residues (pK_a of Asp and Glu is about 3.9 and 4.4, respectively).

Microsolubilization of LUVs by apoA-I

The effect of pH on the spontaneous solubilization of pure PC LUVs by apoA-I was monitored by right-angle light scattering at 37°C. PC LUVs were persistent against apoA-I over the pH range from 5.0 to 9.0, but were progressively solubilized by apoA-I at lower pH (**Fig. 3A**). The half time ($t_{1/2} = (\ln 2)/k$) of the solubilization was amazingly short, in the order of minutes. Maximal solubilization, which was represented by the lowest plateau value of light scattering, was attained at pH 3.5. With a further decrease in pH, the plateau value of the scattering increased. The plateau (C) was determined by fitting the solubilization profile with equation 4 and was plotted as a function of pH (**Fig. 4**). The maximum microsolubilization of pure egg PC LUVs by apoA-I occurred at pH 3.5, where the α-helical content and the hydrophobicity of apoA-I were maximized. This result suggests that the hydrophobic α-helical structure of apoA-I formed under acidic conditions triggers discoidal rHDL formation.

To elucidate the effect of PS, similar experiments were conducted with PC/PS LUVs. Compared with PC LUVs, PC/PS LUVs solubilized at higher pH. In addition, maximal solubilization was also achieved under higher pH conditions, i.e., pH 4.5 and 5.0 in 20% and 40% PS, respectively (Fig. 3B, C). A further decrease in pH increased the plateau value of the scattering, as in PC LUVs.

To estimate maximal pH (pH_{onset}), at which apoA-I starts to solubilize the membranes, plateau C was plotted as a function of pH (Fig. 4). The pH_{onset} of PC/PS LUVs with 0%, 20%, and 40% PS was estimated at 4.7, 5.4, and 6.1, respectively, showing an upper shift with increasing PS fraction.

Acidic PLs are known to decrease the surface potential of liposomes, thereby decreasing the surface pH of liposomes. To calculate $pH_{surface}$ ($= -\log[H^+]_{surface}$) using equations 2 and 3, the zeta potential of each LUV was measured at different pH. The zeta potential of PC LUVs was practically zero and $pH_{surface}$ was almost equal to pH of bulk (pH_{bulk}) at each pH. On the other hand, $pH_{surface}$ of PC/PS LUVs with 20% and 40% PS was reduced by about 0.3 and 0.6, respectively, from pH_{bulk} at each pH (**Fig. 5**). The $pH_{surface}$ values of these LUVs at pH_{onset} ($pH_{surface,\ onset}$) were calculated to be about 5.0 and 5.5 for 20% and 40% PS, respectively, similar to the $pH_{surface,\ onset}$ value for PC LUV (4.7). These results imply that the accelerated microsolubilization of PS-containing membranes is attributable mainly to the decrease in surface pH by PS, which changes the conformation and polarity of surface apoA-I. The disagreement between the $pH_{surface,\ onset}$ values of three kinds of LUVs suggests that other factors, such as increased electrostatic apoA-I-membrane interaction and modification of membrane structures by PS, may contributed to facilitated microsolubilization.

Gel filtration chromatography

To confirm the formation of discoidal rHDLs with the microsolubilization of LUVs, the products were separated from LUVs and their size was analyzed by gel filtration chromatography (**Fig. 6**). As expected, the product from pure PC LUVs/apoA-I mixture incubated at pH 7.0

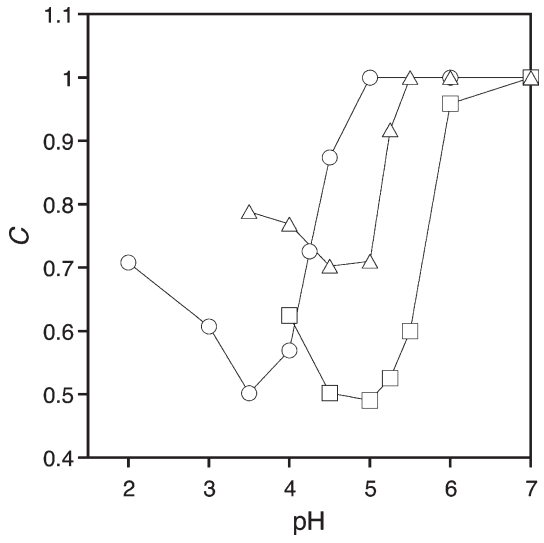

Fig. 4. The plateau (C) for microsolubilization of pure PC (circles), PC/PS 8/2 (triangles), and 6/4 (squares) LUVs by apoA-I as a function of pH. The plateau (C) was determined as shown in Fig. 3.

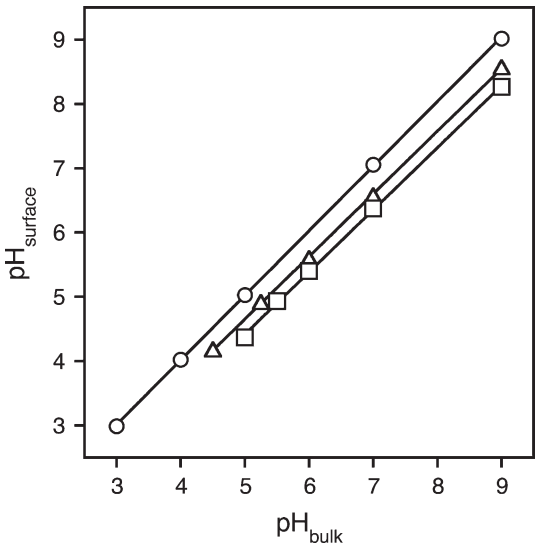

Fig. 5. The pH at the surface ($pH_{surface}$) of pure PC (circles), PC/PS 8/2 (triangles), and 6/4 (squares) LUVs as a function of the pH of bulk (pH_{bulk}). The $pH_{surface}$ was calculated using equation 2 and equation 3 from zeta potential measurements. The plots were approximated by linear function (solid lines).

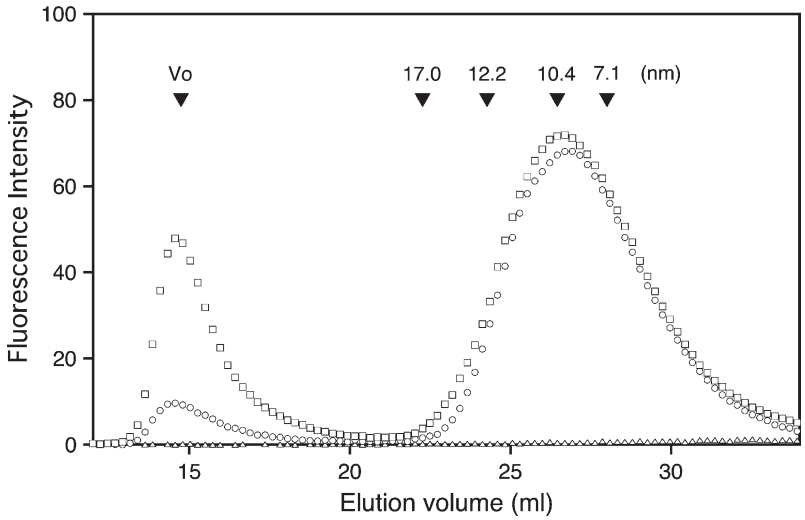

Fig. 6. Gel filtration chromatography profiles of the products from LUVs/apoA-I mixtures. The products from pure PC LUVs/apoA-I mixtures incubated at pH 4.0 (circles) and pH 7.0 (triangles) and PC/PS (8/2) LUVs/apoA-I mixtures incubated at pH 5.0 (squares) were separated from the remaining LUVs by ultracentrifugation and then analyzed. Arrows indicate elution volumes of gel filtration standards: 17.0 nm, thyroglobulin; 12.2 nm, ferritin; 10.4 nm, catalase; 7.1 nm, BSA.

showed no peaks, suggesting no rHDL formation. The products from pure PC and PC/PS LUVs/apoA-I mixtures incubated under acidic conditions were eluted in two fractions. The peaks at a retention volume of 26.5 ml corresponded to the molecular size of ~10 nm, consistent with the previously reported diameter of discoidal rHDLs (36). The peaks at the void volume represent the presence of larger aggregates, which could be remaining lipid particles associated with apoA-I.

DISCUSSION

Studies of the interaction between apoA-I and model membranes are important for elucidating the mechanism of the formation of nascent HDL by ABCA1. Many previous studies using artificial membranes have shown that heterogeneous membranes, owing to phase separation, can be transformed into discoidal rHDLs by apoA-I (37–40). These data imply the possible involvement of local phase separation in the formation of nascent HDL. Meanwhile, Surewicz et al. (41) found that model membranes containing excess acidic PLs (e.g., phosphatidylglycerol or PS) are also microsolubilized by apoA-I. The authors ascribed the phenomenon to electrostatic interactions between the anionic lipid head group and cationic sites of apoA-I and/or to increased head group separation caused by electrostatic repulsion among anionic PLs; however, model membranes with lipid compositions relevant to biological membranes are little microsolubilized by apoA-I at neutral pH and physiological temperature.

It remains unclear whether nascent HDL is generated intracellularly and/or at the cell surface (42). Numerous experiments using modified ABCA1 suggest that ABCA1 localizes not only to plasma membranes but also to the Golgi complex (42). A recent study has shown that ABCA1 present at the cell surface amounts to 30–40% of total cellular ABCA1 (15). In addition, it has been reported that newly synthesized apoA-I in hepatocytes is lipidated in the endoplasmic reticulum and Golgi and that the lipidation of apoA-I in the Golgi requires ABCA1 (16). It has been shown that a remarkably hypertrophic Golgi complex is seen in Tangier disease fibroblasts (43, 44). Several research groups have reported that brefeldin A, which disrupts the Golgi apparatus, strongly blocks cholesterol and PL efflux to apoA-I (45, 46) and other apolipoproteins, including apoA-II, A-IV, C-I, C-II, and C-III (46). These results indicate that the Golgi plays an important role in the formation of nascent HDL by ABCA1. On another front, GFP-ABCA1 fusion proteins were found in vesicle trafficking among early and late endosomes, lysosomes, and plasma membranes (17). Smith et al. (18) and Takahashi and Smith (19) have reported the uptake and resecretion of labeled apoA-I and colocalization of the protein with GFP-ABCA1 in endosomes. It has been shown that cells expressing a mutant ABCA1 (ABCA1delPEST), which show impaired ABCA1 internalization, are less able to efflux cholesterol in late endosomes and lysosomes to apoA-I (20). Recently, Hassan et al. (47) reported that the endocytosis of ABCA1 induced by apoA-I and apoA-I associating with the endosomes is rapidly resecreted as nascent HDL, suggesting that the endocytotic pathway plays a central role in the formation of nascent HDL. It is known that the pH in the Golgi, trans-Golgi network, and early and late endosomes is in the range of ~6.2–6.6 (21–23), ~6.0 (21, 48), 5.5–6.3 (24, 25), and ~5.5 (24, 25), respectively; therefore, we aimed to study the interaction between apoA-I and lipid membranes at acidic pH.

Under acidic conditions, apoA-I increased its α-helical content and hydrophobicity and promoted the formation of discodal rHDL. Tall, Shipley, and Small (49) have reported that thermal denaturation of apoA-I is irreversible below pH 6.5, owing to protein aggregation following the denaturation. This is consistent with our present results, in which the hydrophobicity of apoA-I gradually increases below pH 6.0 (Fig. 2B). It is conceivable that the formation of the hydrophobic α-helical structure of apoA-I raises the affinity to lipid membranes and enables discoidal rHDL formation. The promoted transformation of dimyristoyl-

phosphatidylcholine liposomes into discoidal rHDL at phase transition temperature with decreasing pH has been reported for apolipophorin III (50, 51) and the apoE3 22 kDa fragment (52). Our experiments showed for the first time that even egg PC membranes, which are remarkably stable toward apoA-I at neutral pH, are rapidly transformed into discoidal rHDL at physiological temperature by the reduction of pH. These results imply that the promoted ability to form discoidal rHDL under acidic conditions is a characteristic common to apolipoproteins that are able to form discoidal rHDL.

Numerous research groups have reported the PS translocase activity of ABCA1. The increase of cell-surface PS has been demonstrated in ABCA1-expressing cells by fluorescent annexin V binding or by using fluorescent-labeled PS (18, 26–29). Decreased annexin V binding in Tangier fibroblasts has also been reported (29). In addition, Albrecht et al. (26) have reported that a novel missense mutation in ABCA1 is found in patients with Scott syndrome, which is a bleeding disorder characterized by a failure to expose PS to the outer leaflet of the platelet plasma membrane, and that overexpression of wild-type ABCA1 in Scott syndrome lymphocytes complements PS exposure at the cell surface. On the other hand, Williamson et al. (53) have reported that a lack of ABCA1 does not affect PS externalization. Thus, the PS translocase activity of ABCA1 remains controversial; therefore, we examined the effect of PS on the formation of rHDL by apoA-I. The results showed that PS-containing LUVs were transformed to discoidal rHDL at the pH of the endosome or the slightly lower pH of the Golgi, which was not the case for egg PC LUVs. The promoted formation of discoidal rHDL from PS-containing LUVs was attributed to increased acidity at the membrane surface. Most PS in the endosome/Golgi membranes is located in the cytoplasmic leaflet; therefore, ABCA1-mediated PS translocation into the luminal leaflet in the endosome/Golgi membranes would trigger the intracellular lipidation of apoA-I. This mechanism, which differs from that of the extracellular lipidation at higher pH, would function as a countermeasure to excess accumulation of intracellular cholesterol.

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