Synthetic amphipathic helical peptides promote lipid efflux from cells by an ABCA1-dependent and an ABCA1-independent pathway

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Abstract In order to examine the necessary structural features for a protein to promote lipid efflux by the ABCA1 transporter, synthetic peptides were tested on ABCA1-transfected cells (ABCA1 cells) and on control cells. L-37pA, an L amino acid peptide that contains two class-A amphipathic helices linked by proline, showed a 4-fold increase in cholesterol and phospholipid efflux from ABCA1 cells compared to control cells. The same peptide synthesized with a mixture of L and D amino acids was less effective than L-37pA in solubilizing dimyristoyl phosphatidylcholine vesicles and in effluxing lipids. In contrast, the 37pA peptide synthesized with all D amino acids (D-37pA) was as effective as L-37pA. Unlike apoA-I, L-37pA and D-37pA were also capable, although at a reduced rate, of causing lipid efflux independent of ABCA1 from control cells, Tangier disease cells, and paraformaldehyde fixed ABCA1 cells. The ability of peptides to bind to cells correlated with their lipid affinity. In summary, the amphipathic helix was found to be a key structural motif for peptide-mediated lipid efflux from ABCA1, but there was no stereoselective requirement. In addition, unlike apoA-I, synthetic peptides can also efflux lipid by a passive, energy-independent pathway that does not involve ABCA1 but does depend upon their lipid affinity.—Remaley, A. T., F. Thomas, J. A. Stonik, S. J. Demosky, S. E. Bark, E. B. Neufeld, A. V. Bocharov, T. G. Vishnyakova, A. P. Patterson, T. L. Eggerman, S. Santamarina-Fojo, and H. B. Brewer. Synthetic amphipathic helical peptides promote lipid efflux from cells by an ABCA1-dependent and an ABCA1-independent pathway. J. Lipid Res. 2003. 44: 828–836.

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ABCA1, a member of the ATP binding cassette transporter family (1), is expressed by many cell types (2) and is involved in the removal or efflux of excess lipids from cells to extracellular apolipoproteins (3). Mutations in the ABCA1 transporter lead to Tangier disease (4–8), which is characterized by the accumulation of excess cellular cholesterol, low levels of HDL, and an increased risk for cardiovascular disease (9). Fibroblasts from Tangier disease patients are defective in the initial step of cholesterol and phospholipid efflux to extracellular apolipoproteins (10, 11).

Apolipoprotein A-I (apoA-I), the principal apolipoprotein on HDL, has been shown to promote lipid efflux from ABCA1-transfected cells (12–14); however, the nature of the interaction between apoA-I and ABCA1 is not fully understood. By cross-linking experiments, apoA-I was found to form a close complex with ABCA1 (12, 15), which suggests a possible receptor-ligand type interaction between ABCA1 and apoA-I. Several other exchangeable-type apolipoproteins have also been shown, however, to efflux lipid from ABCA1-transfected cells (14). Although the exchangeable-type apolipoproteins do not share a similar primary amino acid sequence, they all contain amphipathic helices, a structural motif known to facilitate the interaction of proteins with lipids (16, 17). An alternative to the receptor-ligand-type interaction model of ABCA1 (12, 15) is that ABCA1, by acting as a lipid floppase, may modify the adjacent plasma membrane and promote lipid efflux by facilitating the binding of apolipoproteins to cell membrane lipids (13, 14, 18).

Short synthetic peptide mimics of apolipoproteins have long been used as a model for understanding the physical properties of apolipoproteins (19). Recently, treatment of mice with such peptides has been shown to protect against the development of atherosclerosis (20), and they are currently being explored as possible therapeutic agents. Although these peptides may have several different benefi-
cial effects in preventing atherosclerosis (20), they have also been shown to promote lipid efflux from cells (21, 22). For example, the 37pA peptide (23), which contains two identical class A amphipathic helices linked by a proline, stimulates cholesterol and phospholipid efflux from cells (21, 22). The mechanism whereby the 37pA peptide and other similar peptides can promote lipid efflux has not been fully established, but it has been shown to also depend upon the presence of amphipathic helices and on their ability to bind and solubilize lipids (24, 25). Based on these results and the near-simultaneous efflux of both phospholipid and cholesterol from cells, it has been proposed that amphipathic helical peptides, as well as apoA-I, may promote lipid efflux by causing the microsolvulbilation of the plasma membrane of cells (24–25). The role, however, of ABCA1 in this microsolvulbilization-type process and whether synthetic amphipathic helical peptides can also efflux lipid by the ABCA1 transporter has not been directly determined. Previous studies of lipid efflux from cells by synthetic peptides were done prior to the discovery of ABCA1 and were done under various cellular conditions in which ABCA1 may or may not have been expressed. Furthermore, inducing endogenous ABCA1 expression in cell lines by various differentiation agents and or cholesterol loading results in numerous changes in gene expression (26), as well as changes in membrane lipid composition (27, 28), which could also modulate lipid efflux by the peptides.

In order to gain further insight into the protein structural requirements of synthetic peptides for promoting lipid efflux, the 37pA peptide (23) and several structurally-related peptides were tested using a well-defined experimental system, namely ABCA1-transfected HeLa cells and wild-type HeLa cells that do not express significant amounts of ABCA1 (13, 14). Results from this study reveal that, unlike apoA-I, synthetic amphipathic helical peptides can, in fact, efflux lipid from cells by both an ABCA1-dependent and an ABCA1-independent pathway. Furthermore, no difference in lipid efflux from ABCA1-transfected cells was observed between the L and D stereoisomer forms of the 37pA peptide, which suggests that there may not be a typical receptor-ligand-type interaction between the peptides and ABCA1. Overall, the results from this study are consistent with a model that reconciles the previous proposal for lipid efflux by a microsolvulbilization-type mechanism (24, 25) with a role for ABCA1 in this process. Lipid efflux from both ABCA1-expressing and nonexpressing cells appears to depend upon the detergent-like action of the amphipathic helices on synthetic peptides, but for apolipoproteins and synthetic peptides with only moderate lipid affinity, it is necessary for this process to be facilitated by ABCA1.

MATERIALS AND METHODS

Peptide studies

The 37pA peptide (DWLKAFYDKVAEKLKEAFP DWLKAFYDKVAEKLKEAF) and the γ crystalline peptide (RMKRERDDDF-RGQMSEITDDCPdLQDRHIHSLRLVLEG) were synthesized by a solid-phase procedure using a Fmoc/DIC/HOBt protocol on a Biospec 9600 peptide synthesizer.

The L2D-37pA and L3D-37pA peptides have the same amino acid sequence as the 37pA peptide and were synthesized with l amino acids, except that α amino acids were used for valine and tyrosine for L2D-37pA, and δ amino acids were used for alanine, lysine, and aspartic acid for L3D-37pA. All peptides were purified to greater than 98% homogeneity by reverse-phase HPLC on an Aquaprep RP-300 column.

The solubilization of multilamellar dimyristoyl phosphatidyl cholines (DMPC) vesicles (2 mg/ml) by the peptides (0.4 mg/ml) was performed in the presence of 8.5% NaBr, and the absorbance at 350 nm was measured after a 2 h incubation at room temperature, as previously described (29).

Cell culture studies

HeLa cells stably transfected with human ABCA1 cDNA (ABCA1 cells) and HeLa cells transfected with only a hygromycin-resistant control plasmid (control cells) were produced and grown in α-modified Eagle’s medium (αMEM) media plus 10% fetal calf serum, as previously described (14). Cholesterol and phospholipid efflux was performed for 18 h on noncholesterol-loaded cells radiolabeled with either cholesterol or cholines (11). Percentage efflux was calculated after subtracting the radioactive counts in the blank media (αMEM plus 1 mg/ml of BSA), and expressed as the percent of total radioactive counts removed from the cells during the efflux period.

Cell fixation was performed by a 10 min treatment with 3% paraformaldehyde in PBS, followed by three washes with blank media. Lactate dehydrogenase (LDH) release from cells into the media was measured enzymatically (Roche Diagnostics, Indianapolis, IN) and expressed, after subtraction of LDH released into blank media, as the percentage of total cell LDH. Total cell LDH was determined after cell solubilization with 1% Triton X-100.

Binding assay

The L-37pA peptide was labeled with 125I using iodine monochloride. Confluent cells grown on 12-well plates were incubated for 3 h at 4°C with the indicated concentration of the unlabeled competitor peptide in αMEM media plus 10 mg/ml of BSA. The cells were then washed three times and incubated for 1 h at 4°C with 1 μg/ml of the radiolabeled L-37pA peptide dissolved in αMEM media plus 10 mg/ml of BSA. Cells were washed three times, and cell bound counts were determined after solubilization with 0.1 N NaOH.

RESULTS

HeLa cells (control cells), which express undetectable levels of ABCA1 as determined by immunoblot analysis (13, 14), and HeLa cells stably transfected (14) with human ABCA1 cDNA (ABCA1 cells) were used to assess the ability of apoA-1 and synthetic peptides to efflux lipid from cells (Fig. 1). As previously described (13, 14), control HeLa cells do not efflux significant amounts of cholesterol and phospholipid to apoA-I, but do so after transfection with ABCA1 (Fig. 1A, B). The L-37pA peptide, which was synthesized with all l amino acids and only has two amphipathic helices instead of the 10 present in apoA-I, also effluxed approximately 2- to 4-fold more cholesterol and phospholipid from ABCA1 cells than from
control cells (Fig. 1C, D). Both the L-37pA peptide and apoA-I began to show saturation for lipid efflux at approximately the same protein concentration of 10 μg/ml, but because the L-37pA peptide is significantly smaller in molecular weight than apoA-I, this corresponds to a molar concentration of 2 μM for L-37pA and 0.36 μM for apoA-I. The 37pA peptide synthesized with all d amino acids, D-37pA, was also effective in promoting cholesterol and phospholipid efflux from ABCA1 cells (Fig. 1E, F). D-37pA had a similar dose-response curve as L-37pA, which suggests that there is not a need for a stereoselective interaction between the 37-pA peptide and the ABCA1 transporter for lipid efflux. Unexpectedly, both L-37pA and D-37pA also consistently removed more cholesterol (5% at 40 μg/ml) and phospholipids (8% at 40 μg/ml) from control cells (Fig. 1C–F) than did apoA-I (Fig. 1A, B).

In Fig. 2, the time course for cholesterol efflux to apoA-I, L-37pA, and blank media (αMEM plus 1 mg/ml BSA) was examined for ABCA1 cells and control cells. Cholesterol efflux from ABCA1 cells to apoA-I was first detectable after 2 h and continued to increase throughout the 30 h efflux period (Fig. 2A). In contrast, there was no significant increase above background in cholesterol efflux from control cells (Fig. 2B). Overall, the kinetics for cholesterol efflux to L-37pA from ABCA1 cells was similar to that of apoA-I, except that cholesterol efflux was first detectable after 30 min (Fig. 2A). Similar to what was observed in (Fig. 1), L-37pA peptide, unlike apoA-I, also promoted cholesterol efflux from control cells but at approximately half the rate (Fig. 2B). A small amount of cholesterol efflux to L-37pA from control cells was first detectable at 30 min, and then it slowly continued to increase throughout the efflux period similar to what was observed for L-37pA with ABCA1 cells.

The introduction of d amino acids into a peptide that otherwise contains l amino acids is known to interfere with the ability of a peptide to form an α helix (30). In order to test the importance of amphipathic α helices in peptide lipid affinity and in the ability of peptides to promote lipid efflux from cells, the following two peptides with the same sequence as 37pA were made with a mixture of l and d amino acids: 1) L2D-37pA, all amino acids except for V and Y; and 2) L3D-37pA, all l amino acids except for A, K, and d. As predicted, the peptides made with a mixture of l and d amino acids had lower lipid affinity, as assessed by monitoring their ability to act as detergents in the solubilization of DMPC vesicles (29). After a 2 h incubation, the L-37pA and D-37pA peptides nearly com-
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Completely solubilized the DMPC vesicles, whereas the L3D-37pA peptide caused only a minimal decrease in turbidity (Fig. 3). The L2D-37pA peptide and apoA-I caused an intermediate level of DMPC vesicle solubilization compared with the L-37pA and L3D-37pA peptides.

When the L2D-37pA peptide was tested for lipid efflux (Fig. 4), the substitution of d amino acids for V and Y residues caused a greater than 75% reduction in cholesterol and phospholipid efflux from ABCA1 cells compared with the L-37pA peptide, which contains all l amino acids (Fig. 1C, D). Interestingly, even though lipid efflux was reduced from ABCA1 cells to the L2D-37pA peptide, the peptide still retained some ability to efflux lipid from ABCA1 cells, but it was unable, like apoA-I (Fig. 1A, B), to promote any lipid efflux from control cells. In contrast, L3D-37pA, which caused only minimal DMPC vesicle solubilization (Fig. 3), was also unable to promote detectable amounts of lipid efflux from either ABCA1 cells or control cells (Fig. 4). A peptide based on the /H9253 crystalline protein (31), which contains two nonamphipathic /H9251 helices of approximately the same length as the helices on 37pA (see Materials and Methods), was tested and also found to be completely ineffective in promoting cholesterol and phospholipid efflux from either cell line (data not shown). Overall, these results are consistent with previous studies (24, 25) that demonstrated the importance of the amphipathic /H9251 helix in promoting lipid efflux. Based on the relative level of lipid efflux from the two cell lines (Figs. 1, 4), it appears, however, that amphipathic helical peptides can promote lipid efflux with and without ABCA1, although the expression of ABCA1 is necessary for those apolipoproteins and peptides, such as apoA-I and L2D-37pA, with only moderate lipid affinity, as assessed by DMPC vesicle solubilization (Fig. 3).

In order to confirm that the residual lipid efflux from the control HeLa cells to L-37pA and D-37pA observed in Fig. 1 was not due to a low level of endogenous ABCA1, a Tangier disease fibroblast cell line with a truncated non-functional ABCA1 transporter (7) was evaluated for lipid efflux (Fig. 5). ApoA-I, L-37pA, and D-37pA all effluxed

Fig. 2. Time course for lipid efflux by ABCA1 cells and control cells to apoA-I and L-37pA. Cholesterol efflux from either ABCA1 cells (A) or control cells (B) to (10 μg/ml) apoA-I (square), (10 μg/ml) L-37pA peptide (triangle), and blank media (circle) (αMEM plus 1 mg/ml of BSA) was determined at the time points indicated on the x axis. Results represent the mean of triplicates ± 1 SD.

Fig. 3. Solubilization of dimyristoyl phosphatidyl choline (DMPC) vesicles by synthetic peptides. The indicated peptides [L-37pA (L), D-37pA (D), L2D-37pA (L2D), L3D-37pA (L3D), and apoA-I (A)] at a final concentration of 0.4 mg/ml were incubated with DMPC vesicles (2 mg/ml) for 2 h and the decrease in turbidity was monitored at an absorbance of 350 nm. Results represent the mean of triplicates ± 1 SD.

Fig. 4. Lipid efflux by ABCA1 cells and control cells to mixed l and d amino acid 37pA peptides. ABCA1 cells (closed symbols) and control cells (open symbols) were examined for their ability to efflux cholesterol (A) and phospholipid (B) over a 18 h period to (10 μg/ml) L2D-37pA (closed circle, open circle) and (10 μg/ml) L3D-37pA (closed square, open square). Results represent the mean of triplicates ± 1 SD.
cholesterol from normal fibroblasts, but as has been previously described (10, 11), apoA-I did not efflux significant amounts of cholesterol from Tangier disease fibroblasts. In contrast, both L-37pA and D-37pA were still able to efflux cholesterol from Tangier disease fibroblasts, albeit at a reduced level, thus confirming the ability of these peptides to efflux lipid from cells in the absence of ABCA1 activity.

The ABCA1-independent pathway for lipid efflux was further evaluated by examining the effect of cell fixation with paraformaldehyde on cholesterol efflux to apoA-I (A), L-37pA (L), and D-37pA (D) (Fig. 6). In addition, 0.02% of taurodeoxycholate (T) was also tested for lipid efflux after 1 h, in order to determine if a sublytic concentration of a simple detergent would also promote more lipid efflux from ABCA1 cells than from control cells. As expected, based on the ATP requirement of the ABCA1 transporter (1, 32), fixation of ABCA1 cells with paraformaldehyde completely blocked the ability of apoA-I to efflux cholesterol (Fig. 6A). In contrast, cell fixation of ABCA1 cells only partially reduced cholesterol efflux to the L-37pA and D-37pA peptides; ~30% of the baseline cholesterol efflux was still retained after cell fixation. When cholesterol efflux was tested on nonfixed control cells (Fig. 6B), the level of cholesterol efflux to L-37pA and D-37pA was similar to the level obtained with fixed ABCA1 cells (Fig. 6A). Furthermore, unlike ABCA1 cells, fixation of control cells did not further reduce cholesterol efflux to the two peptides (Fig. 6B). These results indicate that lipid efflux by the peptides from ABCA1 cells occurs by both an ABCA1-dependent and an ABCA1-independent pathway, whereas lipid efflux from control cells only occurs by the ABCA1-independent pathway, which is a passive, energy-independent process that does not require viable cells.

The addition of a relatively low concentration (0.02%) of taurodeoxycholate to the cell culture efflux media for 1 h did not alter the morphology of the cells, as assessed by light microscopy, but did result in a small amount of cholesterol efflux from ABCA1 cells (Fig. 6A), which slightly increased after fixation. Approximately the same amount of cholesterol efflux also occurred from control cells after the taurodeoxycholate treatment (Fig. 6B). Nearly identical results were also obtained with several other detergents (TX-100, NP-40, and CHAPS) when tested at sublytic concentrations (data not shown). This indicates that ABCA1 promotes lipid efflux to amphipathic helical proteins, but does not increase the overall propensity of cells to efflux lipids to simple detergents.

The inability to completely block peptide-mediated lipid efflux by cell fixation (Fig. 6) and the correlation between DMPC vesicle solubilization by the peptides with lipid efflux (Fig. 1, 3) suggests that lipid efflux from control cells occurs as the result of the microsolubilization of the cell membrane lipids by the detergent-like action of the amphipathic helices on the peptides. The microsolubilization of the plasma membrane of cells could, therefore, be potentially cytotoxic, but no morphologic effect was observed on the cells after incubation with the peptides or apoA-I during the efflux experiments (data not shown). Incubation of the cells with L-37pA and D-37pA at the maximum concentration and time used for the efflux studies (40 μg/ml for 18 h) did, however, consistently result in the release of a small amount of LDH from both cell lines [control cells: L-37pA (6.1% ± 0.2), D-37pA (6.6% ± 0.1); ABCA1 cells: L-37pA (4.3% ± 0.04), D-37pA (5.7% ± 0.1)]. In contrast, L2D-37pA, L3D-37pA, and apoA-1, which did not cause lipid efflux from control cells.
cells (Fig. 2, 3) and therefore appear to be incapable of effluxing lipid by the ABCA1-independent pathway, also did not cause any significant release of cell LDH above baseline (<0.5%) from either cell line.

The competition of the peptides and apoA-I for the binding of radiolabeled L-37pA to ABCA1 cells and control cells is shown in Fig. 7. A two-step sequential competitive binding assay was performed (21) in order to prevent any potentially interfering interaction of the radiolabeled peptide with the competitor proteins. The cells were first incubated with the competitor proteins for 3 h, washed, and then the cell binding of the radiolabeled L-37 peptide was measured. At 8 μM, which is equivalent to the maximum peptide protein concentration of 40 μg/ml used in the lipid efflux studies (Fig. 1), the unlabeled L-37pA peptide blocked the binding of ~40% of the labeled L-37pA peptide (Fig. 7A). D-37pA was similarly effective in competing for the binding of L-37pA, indicating a lack of stereoselectivity in the binding of the peptides to ABCA1 cells. L3D-37pA, in contrast, was completely ineffective in competing for the binding of radiolabeled L-37 peptide. At the maximum concentration tested, both peptides blocked less than 5% of the radiolabeled L-37pA from binding to control cells, which is, in fact, similar to the result obtained with the inactive L3D-37pA peptide. Overall, these results indicate that there is a lack of stereoselectivity in the binding of the 37pA peptide to either ABCA1 cells or control cells and that the cell binding of the peptides appears to be partly dependent upon their lipid affinity.

![Fig. 7](image-url)

**Fig. 7.** Competitive binding of L-37pA peptide to ABCA1 cells and control cells. ABCA1 cells (A) and control cells (B) were incubated for 3 h at 4°C with the indicated concentration of the competitor proteins [L-37pA (triangle), D-37pA (open square), apoA-I (closed circle), L2D-37pA (star), and L3D-37pA (open circle)], and were then washed and incubated for 1 h at 4°C with 1 μg/ml of radiolabeled L-37pA peptide. Results are expressed as the mean of triplicates ± 1 SD.

**DISCUSSION**

The observation that synthetic amphipathic helical peptides can efflux lipid with and without the ABCA1 transporter is potentially important in understanding the mechanism for lipid efflux. The L-37pA and D-37pA peptides were found to promote more lipid efflux from HeLa cells after transfection with ABCA1 (Fig. 1), unlike apoA-I, they were still able to promote a significant level of lipid efflux even from untransfected control cells (Fig. 1) and/or from Tangier disease fibroblasts (Fig. 5), which do not express a functional ABCA1 transporter. Furthermore, the results from the cell fixation experiments (Fig. 6) indicate that the synthetic peptides can, in fact, efflux lipids by a passive, energy-independent process that must not involve ABCA1. Like other ABC transporters (1), ABCA1 activity is dependent upon ATP (1, 32); therefore, the ability of synthetic peptides to remove lipid from nonviable, fixed cells indicates that this process must be occurring without the ABCA1 transporter. The most likely mechanism for the ABCA1-independent pathway for lipid efflux is that the amphipathic helical domains on the peptides, by a detergent-like action, lead to the partial solubilization of the lipids on the plasma membrane of cells, as has been previously proposed (22, 24, 25). The observation that the substitution of t amino acids into the L-37pA peptide decreases the ability of the mixed l and D amino acid peptides to both solubilize DMPC vesicles (Fig. 3) and to efflux lipid from control cells as well as ABCA1-transfected cells (Fig. 4) suggests that this type of mechanism may also partly account for lipid efflux when it occurs by the ABCA1 transporter. Interestingly, the L2D-37pA peptide, like apoA-I, retained some ability to efflux lipid from ABCA1 cells but not from control cells (Fig. 4). A possible explanation for this result is that lipid efflux from control cells by the ABCA1-independent pathway requires that a peptide have a higher lipid affinity than what is necessary for when the peptide promotes lipid efflux via the ABCA1 transporter. ABCA1, by modifying the lipid composition of the adjacent plasma membrane (13, 18), may lower the threshold required for the lipid affinity of
the peptides (24), thus enabling the L2D-37pA peptide to efflux lipid from ABCA1 cells but not from control cells. Even for when lipid efflux to the peptides occurs via the ABCA1 transporter, there appears to be a necessary minimum threshold for lipid affinity of the peptide based on the inability of the L3D-37pA peptide to efflux lipid from ABCA1 cells (Fig. 4). This is supported by the recent observation that apoA-I mutants with reduced lipid affinity have a decreased ability to efflux lipid from ABCA1 expressing cells (35). Interestingly, no difference was observed in the ability of simple detergents, like taurodeoxycholate, to efflux lipid from ABCA1 cells versus control cells (Fig. 6), which may occur because the higher lipid affinity of simple detergents enables them to interact with the cell membrane lipids completely independent of the ABCA1 transporter.

ApoA-I, unlike L-37pA and D-37pA, may be unable to directly efflux lipid from cells without ABCA1 (Figs. 1, 2), because only two of its 10 amphipathic helices have a high affinity for lipids and can promote lipid efflux from cells when synthesized as individual peptides (24). The surface exclusion pressure of L-37pA for the binding to lipid monolayers was found, in fact, to be greater than that of any of the individual amphipathic helices on apoA-I, and even more than the intact apoA-I protein (24, 36, 37). The higher lipid affinity of L-37pA may enable it, unlike apoA-I, to bind to and microsolubilize the lipids on the plasma membrane of cells in the absence of ABCA1 activity. The amphipathic helices on apoA-I may have possibly evolved to only have moderate lipid affinity in order to limit their ability to efflux lipid by only the ABCA1 transporter, and thus possibly avoid the type of cytotoxicity that was observed with the synthetic peptides. For example, the L-37pA and D-37pA peptides, but not apoA-I, were found, based on cell LDH release, to be partially cytotoxic. Interestingly, although several-fold more lipid efflux occurred from ABCA1 cells than from control cells with L-37pA and D-37pA (Fig. 1), there was not a substantially greater release of LDH from ABCA1 cells than from control cells with the synthetic peptides. This indicates that the integrity of the plasma membrane is somehow maintained when the ABCA1 transporter mediates lipid efflux to apoA-I and or to the peptides.

The nature of the interaction between apolipoproteins and ABCA1 is not fully understood, but the results from this study suggest that there is not a stereoselective requirement for lipid efflux in the interaction of the synthetic peptides with the ABCA1 transporter. The L and D stereoisomer forms of the 37pA peptide were found to be equally effective in cell binding (Fig. 7) and in mediating lipid efflux from ABCA1 cells (Fig. 1C–F), which suggests that the formation of a typical receptor-ligand type complex between ABCA1 and the peptides may not be necessary for lipid efflux. This is supported by fluorescent photobleaching experiments that have revealed that apoA-I primarily interacts with lipids on the plasma membrane and not with the ABCA1 transporter (18). Mutations in the domain responsible for the ATPase activity of ABCA1 have also been shown to decrease apoA-I cell binding (18), which further suggests that ABCA1 may not be acting as a simple receptor and that the binding of apoA-I may instead depend on the transport of a ligand by the transporter. Trypsin treatment of cell-surface proteins has also recently been shown (33) not to interfere with the initial rate of lipid efflux from ABCA1 expressing cells to apoA-I. Finally, ABCA1 is also known to enhance lipid efflux to a wide variety of amphipathic helical containing apolipoproteins (14). This finding indicates that there also may not be primary amino acid sequence specificity in the interaction of apolipoproteins with ABCA1, although this is apparently also true for many of the protein ligands that bind SR-B1 (34).

One factor that appears to be important in determining the overall level of cell binding of the peptides is their lipid affinity. The L3D-37pA peptide, which was ineffective in solubilizing DMPC vesicles (Fig. 3) and in promoting lipid efflux (Fig. 1) also did not act as an effective competitor for radiolabeled L-37pA in the binding to ABCA1 cells (Fig. 7). L-37pA and D-37pA, which were the best in solubilizing DMPC vesicles (Fig. 3), also showed the greatest cell binding to ABCA1 cells. There was also a considerable amount of binding of L-37pA and D-37pA to control cells, although it was reduced compared with ABCA1 cells (Fig. 7). This result indicates that peptides with high lipid affinity can bind to cells by both an ABCA1-dependent and an ABCA1-independent mechanism. L2D-37pA and apoA-I showed an intermediate level of competition for the binding of radiolabeled L-37pA to ABCA1 cells, but were not as effective as competitors in control cells that do not express ABCA1 (Fig. 7). Based on this result, L2D-37pA and apoA-I appear to be more dependent upon the expression of ABCA1 for both cell binding and for lipid efflux (Fig. 1). This is consistent with a previous study that showed that L-37pA can bind to the same site on cells as apoA-I, and also to additional sites not shared by apoA-I (21).

The main evidence in support of ABCA1 acting as a receptor for apolipoproteins is cross-linking experiments that have shown that at least a fraction of apoA-I forms a close complex with ABCA1 (12, 15). As previously suggested (13, 14, 18), one possible explanation that is compatible with all of the results is that the ABCA1 may create a modified-lipid plasma membrane domain by acting as a lipid floppase, which may then promote the binding of apoA-I to the membrane lipids adjacent to the ABCA1 transporter, thus accounting for the cross-linking between apoA-I and ABCA1. A multi-step binding process has also recently been proposed for ABCA1-mediated lipid efflux (35). A binding domain on apoA-I may facilitate the initial interaction of apoA-I with ABCA1, and the amphipathic helices on apoA-I may then interact with a modified lipid-binding domain adjacent to the transporter, which ultimately leads to the microsolubilization of lipids and the dissociation of the newly formed HDL. A protein-binding domain that promotes a direct interaction with ABCA1 may not be discernible with the relatively short and structurally simple synthetic peptides used in this study and, therefore, the existence of such a ligand-binding domain
on apolipoproteins cannot be completely excluded based on this study. The lipid efflux results with the peptides (Fig. 1) do indicate, however, that a classic receptor-ligand type interaction between ABCA1 and the peptides is not necessary for ABCA1-mediated lipid efflux. Future studies aimed at mapping and identifying the exact amino acid residues on apolipoproteins and ABCA1 that interact will likely be necessary to better understand the role of apolipoprotein binding to the ABCA1 transporter in lipid efflux.

In summary, synthetic peptide mimics of apolipoproteins were shown, in a nonstereoselective manner, to be capable of mediating lipid efflux by an ABCA1-dependent and an ABCA1-independent pathway. The results are consistent with a model wherein the ABCA1-independent pathway occurs by the microsolubilization of the cell membrane mediated by the amphipathic helices on synthetic peptides, whereas the ABCA1 pathway occurs by a facilitated microsolubilization-type process for those proteins and peptides, such as apoA-I and L2D-37pA, that only have moderate lipid affinity. Because apoA-I is constitutively expressed, its dependence upon ABCA1, which is highly regulated by the cholesterol content of cells (2), may provide a mechanism for physiologically controlling the overall level of lipid efflux from cells. Based on the lower lipid affinity of the natural amphipathic helices on apoA-I compared with the 37pA peptide (36, 37), the ABCA1-independent pathway of lipid efflux may not occur in vivo, but the identification of this alternative pathway has provided insight into the mechanism of action of the ABCA1 transporter. In addition, the results of this study suggest that lipid efflux assays from ABCA1-expressing and nonexpressing cells, as well as cytotoxicity type assays, may be useful for further examining the mechanism for the antiatherogenic effect of synthetic amphipathic helical peptides (20), and for potentially optimizing their structure and therapeutic index.

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