Phosphorylation and Stabilization of ATP Binding Cassette Transporter A1 by Synthetic Amphiphilic Helical Peptides

Received for publication, December 19, 2003 Published, JBC Papers in Press, December 29, 2003, DOI 10.1074/jbc.C300553200

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To investigate structural requirement of helical apolipoprotein to phosphorylate and stabilize ATP-binding cassette transporter A1 (ABCA1), synthetic peptides (Remaley, A. T., Thomas, F., Stonik, J. A., Demosky, S. J., Bark, S. E., Neufeld, E. B., Bocharov, A. V., Vishnyakova, T. G., Patterson, A. F., Eggerman, T. L., Santamarina-Fojo, S., and Brewer, H. B. (2003) J. Lipid Res. 44, 828–836) were examined for these activities. L37pA, an L amino acid peptide that contains two class-A amphiphilic helices, and D37pA, the same peptide with all D amino acids, both removed cholesterol and phospholipid from differentiated THP-1 cells more than apolipoproteins (apo) A-I, A-II, and apoE and by all the peptides tested except apoA-I and apoA-II (8, 9), which is observed not only with apoA-I but also with other apolipoproteins capable of lipid removal such as apoA-II or apoE (8, 9), which is consistent with the hypothesis that the removal of lipid triggers the signaling for ABCA1 stabilization rather than some other type apolipoprotein interaction with the cells.

Synthetic peptides that mimic amphiphilic helical segments of apolipoproteins were demonstrated to reproduce the lipid removal reaction by apolipoproteins, indicating that structural requirement for apolipoproteins to carry out this reaction is not highly specific (11, 12). Furthermore, both L and D stereoisomer forms of the peptides were equally efficient in promoting lipid release (13). This is similar to the structural requirements described for reactions of lecithin:cholesterol acyltransferase (14) and cholesteryl ester transfer protein (15).

In this study, we therefore examined the phosphorylation and stabilization of ABCA1 with series of synthetic peptides that mimic amphiphilic helical proteins (13). In addition, L or D stereoisomers, as well as peptides with a mixture of L and D amino acids were tested (13). The results are consistent with a model whereby peptides with an amphipathic helical structure that are competent in promoting lipid efflux from cells also promote the phosphorylation and stabilization of ABCA1.

EXPERIMENTAL PROCEDURES

Materials—ApoA-I and apoA-II were isolated from HDL fraction prepared from fresh human plasma as described elsewhere (16). Recombinant human apoE3 was a generous gift from Mitsubishi Pharma Corp. (Yokohama, Japan). ApoC-III was prepared from human plasma as described previously (17). The L37pA peptide (DWLKAFYDKVAEKLKEAF) was synthesized by a solid phase procedure as previously described with all L amino acids (13). The D37pA peptide was synthesized with the same sequence but with D amino acids (13). Valine and tyrosine were replaced with D amino acids for the L37pA peptide, and alanine, lysine, and asparatic acid were replaced with D amino acids for the L37pA peptide (13).
**Stabilization of ABCA1 by Synthetic Peptides**

**Results and Discussion**

Lipid release was examined from differentiated THP-1 cells by apoA-I, apoA-II, apoE, and apoC-III and for the peptides L37pA, D37pA, L2D37pA, and L3D37pA (Fig. 1). ApoA-I, apoA-II, and apoE showed similar lipid release activity on a per weight basis, with approximately a 1:1 mass ratio of cholesterol to choline-phospholipid. ApoC-III, however, exhibited only slight activity for lipid release. The peptide L37pA resulted in approximately a 4-fold more choline-phospholipid release and a 2-fold more cholesterol release compared with the apolipoproteins when used at the same protein mass concentration. Similarly, the D37pA peptide also promoted more cholesterol and phospholipid release than the apolipoproteins, although it was slightly less effective than the L37pA peptide. L2D37pA removed more lipid than apoC-III, but it was much less effective than the other apolipoproteins and the L37pA and D37pA. L3D37pA was completely ineffective in promoting lipid removal from cells, as described previously (13).

Similar lipid release experiments were carried out with WI-38 cells (Fig. 2). This cell line releases relatively high amount of choline-phospholipid at base line without any apolipoproteins or any other “lipid acceptor” in the media (18). We have shown previously that this is mostly due to lysophosphatidylcholine release (18). Interestingly, unlike THP-1 cells, L37pA and D37pA showed similar lipid releasing activity to that by apoA-I. Furthermore, in contrast to THP-1 cells, L2D37pA also demonstrated equivalent capability of lipid removal compared with apoA-I, apoE, L37pA, and D37pA. L3D37pA, however, was incapable of removing lipid from either WI-38 cells (Fig. 2) or THP-1 cells (Fig. 1).

ABCA1 in THP-1 cells was analyzed by immunoblotting for its protein level, by protein pulse labeling for the rate of decay, and by using 32P-labeling for monitoring its state of phosphorylation (Fig. 3).

In the presence of 32P for 1 h, and ABCA1 was analyzed by electrophoresis and autoradiography. To study phosphorylation of ABCA1, the cells were incubated with the apolipoproteins or the synthetic peptides in the presence of 32P for 1 h, and ABCA1 was analyzed by electrophoresis and autoradiography after immunoprecipitation.
amino acids. Thus, stabilization of ABCA1 by helical apolipoproteins was reproduced with the use of synthetic amphiplic helical peptides that are also capable of removing cellular lipid. Phosphorylation of ABCA1 was enhanced by stimulating the cells with apoA-I, apoA-II, apoE, L37pA, and D37pA. ABCA1 in WI-38 was also analyzed in a similar manner (Fig. 4). Increase of ABCA1 was demonstrated with apoA-I, apoE, L37pA, D37pA, and L2D37pA, and also with apoC-III but to less extent. Phosphorylation of ABCA1 was also shown with these apolipoproteins and peptides. Neither protein increase nor phosphorylation of ABCA1 was observed with L3D37pA. Fig. 5 shows the extent of ABCA1 phosphorylation standardized by protein level for THP-1 and WI-38 cells.

Relationships among the various measured parameters was analyzed in Figs. 6–8. Fig. 6 shows the extent of ABCA1 phosphorylation as a function of phospholipid release by various apolipoproteins and their model peptides. It appears that there is a threshold for the effect of phospholipid release on ABCA1 phosphorylation. Phosphorylation of ABCA1 reaches a maximum at a phospholipid release of ~2 μg/mg cell protein and increases no further, which is consistent with a model whereby removal of phospholipid is a trigger for signal transduction to phosphorylate ABCA1 (10). Phosphorylation of ABCA1 in WI-38 cells (Fig. 6) also increased with phospholipids release, and a similar threshold effect was demonstrated. It should be noted that apoC-III removed a significant amount of phospholipid from WI-38 cells and induced a maximum phosphorylation of ABCA1, while it was inefficient in both reactions in THP-1 cells. Fig. 7 shows the relationship between phospho-
lipids release and ABCA1 protein levels. A direct positive correlation was observed between ABCA1 phosphorylation and ABCA1 protein level (Fig. 7). Finally, phospholipid release increased along with ABCA1 protein level, shown in Fig. 8.

We thus conclude that structural requirement of apolipoprotein for removal of lipid to generate HDL is the presence of a stable type A amphiphilic helical segment (13) and that this structural motif is also strongly associated with the potential of a protein to promote the phosphorylation and stabilization of ABCA1.

There was, however, some interesting differences in the ability of the various peptides to promote lipid efflux from THP-1 cells and WI-38 cells (Figs. 1 and 2). The L37pA and D37pA peptides were more effective in lipid release in THP1 cells than apolipoproteins and the L2D37pA peptide. ApoA-I and L2D27pA peptide were previously shown to be completely dependent upon ABCA1 for lipid release, whereas L37pA and D37pA, because of their higher lipid affinity, are also able to promote lipid release without ABCA1 (13). It is, therefore, possible a lower level of ABCA1 activity in THP1 cells compared with WI-38 cells could account for this difference. Alternatively, a difference in the lipid composition between THP-1 cells and WI-38 cells could also account for a greater amount of ABCA1-independent lipid efflux from the THP1 cells for the L37pA and D37pA peptides. Previously, it was shown in HeLa cells transfected with ABCA1 (13) that there was not a stereoselective interaction with synthetic amphiphilic helical peptides in the lipid release process, which is consistent with the equal amounts of lipid release observed for the l and d stereoisomer forms of the 37pA peptide observed in WI-38 cells. It was consistently observed in this study, however, that the D37pA peptide was not as effective as the L37pA peptide for lipid release in THP1 cells. This suggests that there must be some sort of chiral interaction of the peptides with the THP1 cells, which could be with some other auxiliary protein besides ABCA1 to promote the cell interaction with the L37pA peptide and/or in some other way to promote lipid release. In regard to the effect of the peptides on ABCA1 phosphorylation and ABCA1 protein stabilization, the two cell lines yielded similar results. Those peptides and apolipoproteins that stimulated phospholipid efflux also promoted the phosphorylation of ABCA1 and its stabilization against degradation (Figs. 3 and 4). Because the 37pA peptides do not share any primary amino acid homology with any apolipoprotein (13), but do have type A amphiphilic helices mimicking apolipoproteins, ABCA1 phosphorylation and stabilization is unlikely to be the result of a specific interaction of the peptides or apolipoproteins with the ABCA1 transporter. This view is further supported by the observation that the l and d stereoisomer forms of the peptides were equally effective in promoting the phosphorylation and stabilization of ABCA1 (Figs. 3 and 4). A positive correlation was observed between phospholipid release and ABCA1 phosphorylation and stabilization (Figs. 6–8), which as proposed previously (10) suggests that the release of phospholipid from cells provides the signal for ABCA1 phosphorylation and stabilization. Such a mechanism would provide an alternative means besides gene regulation for regulating the amount of functional ABCA1 transporter present in cells and for coupling the amount of ABCA1 in cells with the availability of HDL-apolipoproteins in extracellular fluid.

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J. Biol. Chem. 2004, 279:6217-6220.
doi: 10.1074/jbc.C300553200 originally published online December 29, 2003

Access the most updated version of this article at doi: 10.1074/jbc.C300553200

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