Efflux of Cellular Cholesterol and Phospholipid to Apolipoprotein A-I Mutants*

(Received for publication, March 27, 1996, and in revised form, October 8, 1996)

Dmitri Sviridov, Louise E. Pyle, and Noel Fidge

From the Baker Medical Research Institute, Commercial Road, Prahran, Victoria 3181, Australia

Human plasma apolipoprotein A-I (apoA-I) and recombinant full-length proapoA-I (apoA-I(–6–243)) as well as four truncated forms of proapoA-I were used as acceptors to study cholesterol and phospholipid efflux from HepG2 cells. Efflux of both cholesterol and phospholipid to the lipid-free plasma apoA-I was twice that of apoA-I(–6–243). When apoA-I was incorporated into reconstituted high density lipoprotein, cholesterol efflux increased, phospholipid efflux decreased and the difference between plasma apoA-I and apoA-I(–6–243) disappeared. Truncation of recombinant apoA-I to residues 222 (apoA-I(–6–222)) and 210 (apoA-I(–6–210)) resulted in a 70–95% decrease in their ability to promote the efflux of both intracellular and plasma membrane cholesterol. Further truncation to residues 150 (apoA-I(–6–150)) and 135 (apoA-I(–6–135)) fully restored the ability of apoA-I to promote cholesterol efflux. Phospholipid efflux closely paralleled the efflux of cholesterol. Interaction of 125I-labeled apoA-I with the cells was similar for apoA-I(–6–243), apoA-I(–6–222), and apoA-I(–6–210), but slightly higher for apoA-I(–6–150) and apoA-I(–6–135). When complexed with phospholipid, all forms except apoA-I(–6–210) formed discoidal reconstituted high density lipoprotein particles. When the same amounts of free or lipid-associated apoA-I were compared, association of apoA-I with phospholipid increased cholesterol efflux and decreased phospholipid efflux, and the difference in the ability of different mutants to promote cholesterol and phospholipid efflux disappeared. We conclude that the capacity of lipid-free apoA-I to promote cholesterol efflux is related to its ability to mobilize cellular phospholipid, which apparently involves a region around residues 222–243. A second lipid-binding region is exposed when the carboxy-terminal half of apoA-I is absent.

It is currently accepted that apolipoprotein A-I (apoA-I)1-containing lipoproteins play a key role in the efflux of cellular cholesterol, the first step in the reverse cholesterol transport pathway (1–3). Thus, apoA-I-containing lipoproteins are the most efficient initial acceptors of cellular cholesterol (1, 2), and removal of these lipoproteins from the plasma greatly reduces the release of cholesterol from cells (4). The mechanism of how apoA-I executes this function remains, however, unresolved. It has been demonstrated that short peptides mimicking the secondary structure of apoA-I are able, to a certain extent, to imitate its ability to facilitate removal of cellular cholesterol (5–7), suggesting that a rather nonspecific mechanism, which relies mainly on the ability of apoA-I to bind lipids, appears to be involved. On the other hand, new evidence suggests that specific sequences of apoA-I are responsible for cholesterol efflux (8–10), pointing to the involvement of a more specific and possibly receptor-dependent mechanism.

In our previous work using monoclonal antibodies against specific epitopes of apoA-I, we identified a sequence, apoA-I(140–150), that was associated with the ability of apoA-I to promote efflux of intracellular cholesterol (10). In order to further investigate the involvement of various domains of apoA-I in cholesterol efflux, we used recombinant proapoA-I, either intact or truncated, in the lipid-free form or incorporated into reconstituted high density lipoprotein (rHDL), to study the effect of the carboxyl-terminal domain truncations on the efflux of cholesterol and phospholipid from cultured cells. While the proform of apoA-I is a minor component of circulating apoA-I, its known properties do not deviate strongly from mature apoA-I (11–14) and the biological principles underlying structure-function relationships between cholesterol efflux and proteins should be equally relevant for mature apoA-I and proapoA-I.

MATERIALS AND METHODS

Cells—Human hepatoma cells HepG2 were grown in a CO2 incubator (5% CO2, 95% air) in 75-cm2 flasks or 6- or 12-well cell culture clusters (Costar, Cambridge, MA) coated with collagen (15). Cultures were maintained in Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum, 20 mM HEPES, 1% nonessential amino acids, 2 mM l-glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin, and 3.7 mg/ml sodium bicarbonate (all reagents from ICN (Seven Hills, NSW, Australia)).

Expression of Recombinant ApoA-I—Recombinant human proapoA-I was expressed as a fusion protein in an Escherichia coli/pGEX vector expression system (16). The apoA-I fusion protein was purified by glutathione-agarose affinity chromatography, cleaved with thrombin, and repurified to remove the glutathione S-transferase carrier. The final product was analyzed by electrophoresis on 12% sodium dodecyl sulfate (SDS)-polyacrylamide gels and by NH2-terminal sequencing and was confirmed to be highly purified proapoA-I (apoA-I(–6–243)). The following truncated forms of apoA-I were also generated using this system: apoA-I(–6–222), apoA-I(–6–210), apoA-I(–6–150), and apoA-I(–6–135). Use of the prosegment of apoA-I to allow for an effective thrombin cleavage site made it possible to produce useful amounts and a variety of apoA-I mutants, as compared with the low levels of mature apoA-I shown to be produced when the fusion protein is cleaved with Factor Xa (17). Details of the expression system and physicochemical properties of the different truncated forms of recombinant apoA-I are described elsewhere (18).

Human plasma apoA-I was isolated and purified as described previously (19).

Preparations of Reconstituted High Density Lipoprotein—rHDL was prepared by the sodium cholate dialysis method according to Jonas et
Cholesterol in HepG2 cells was labeled by incubation with $^{[14]}$C]acetate for 3 h at 15°C, and phospholipid was labeled by incubation with $^{[3]}$H]choline for 24 h at 37°C (see "Materials and Methods"). After labeling cells were washed and incubated for 3 h at 37°C with either lipid-free apoA-I (final concentration 1 μM) or apoA-I incorporated into rHDL (final concentration 66 μM POPC). Cholesterol and phospholipid were isolated as described under "Materials and Methods."


table I

| Acceptor | Efflux | APOA-I | APOA-I/POPC |
|----------|--------|--------|-------------|
| A. Cholesterol efflux | ApoA-I (1–243) | 7.41 ± 0.13 | 9.61 ± 0.38^b |
| ProapoA-I (1–6–243) | 3.84 ± 0.21^b | 8.32 ± 0.22^b |
| B. Phospholipid efflux | ApoA-I (1–243) | 1.9 ± 0.07 | 0.23 ± 0.02^b |
| ProapoA-I (1–6–243) | 0.74 ± 0.06^b | 0.18 ± 0.03^b |

*p < 0.001 (versus apoA-I).

^p < 0.01 (versus lipid-free apoA-I).

RESULTS

Cholesterol and Phospholipid Efflux in the Presence of Recombinant and Human Plasma ApoA-I—We compared release of intracellular cholesterol and cellular phospholipid from HepG2 cells to the medium containing recombinant proapoA-I and human plasma apoA-I both in lipid-free form or reconstituted into rHDL. In the presence of lipid-free apoA-I, cholesterol efflux to human apoA-I was twice as much as that to the recombinant proapoA-I (Table I). When either of the proteins were incorporated into discoidal rHDL (see below), their ability to promote cholesterol efflux was significantly increased and the difference between human apoA-I and recombinant proapoA-I virtually disappeared (Table I). Efflux of phospholipid to lipid-free apoA-I was analogous to the efflux of cholesterol; human plasma apoA-I was more than twice as active compared with recombinant proapoA-I (Table I). In contrast to cholesterol, phospholipid efflux to rHDL was only 12–24% of that observed with similar amounts of the corresponding lipid-free human plasma or recombinant apoA-I. There was no statistically significant difference in efflux between rHDL containing either human plasma or recombinant apoA-I (Table I).

Cholesterol and Phospholipid Efflux to Lipid-free ApoA-I Mutants—Labeled cells were incubated for 3 h at 37°C with equimolar concentrations (1 μM) of the different forms of the recombinant apoA-I. Efflux of intracellular cholesterol in the presence of full-length apoA-I compared with carboxyl-terminally truncated forms is shown in Fig. 1A. Truncation of the last 21 amino acids (apoA-I (6–22)) decreased the ability of apoA-I to promote cholesterol efflux by 80%; the decrease was not significantly changed by further truncation to residue 210...

*Table II*

| Acceptor | Efflux | APOA-I | APOA-I/POPC |
|----------|--------|--------|-------------|
| A. Cholesterol efflux | ApoA-I (1–243) | 7.41 ± 0.13 | 9.61 ± 0.38^b |
| ProapoA-I (1–6–243) | 3.84 ± 0.21^b | 8.32 ± 0.22^b |
| B. Phospholipid efflux | ApoA-I (1–243) | 1.9 ± 0.07 | 0.23 ± 0.02^b |
| ProapoA-I (1–6–243) | 0.74 ± 0.06^b | 0.18 ± 0.03^b |

*p < 0.001 (versus apoA-I).

^p < 0.01 (versus lipid-free apoA-I).
Lipid Efflux to ApoA-I Mutants

[Image 97x438 to 249x729]

Fig. 1. Efflux of intracellular (A) and plasma membrane (B) cholesterol onto lipid-free apoA-I mutants. Cholesterol in HepG2 cells was labeled by incubation with [14]Cacetate for 3 h at 15 °C (A) or with [14]Ccholesterol/PC liposomes for 3 h at 37 °C (B) (see “Materials and Methods”). After labeling, cells were washed and incubated for 3 h at 37 °C with the different forms of lipid-free apoA-I (final concentration 1 μM). [14]CCholesterol from the medium was isolated and quantitated as described under “Materials and Methods.” *, p < 0.05; **, p < 0.001 (versus apoA-I(−6–243)).

(a) (apoA-I(−6–210)). Unexpectedly, truncation to residue 150 (apoA-I(−6–150)) completely restored the ability of apoA-I to promote cholesterol efflux, and in the presence of the mutant truncated to the residue 135 (apoA-I(−6–135)) efflux was slightly less than that of full-length proapoA-I or apoA-I(−6–150).

The difference in ability of the truncated forms of apoA-I to promote intracellular cholesterol efflux may be due to the removal or unmasking of specific sequences that signal translocation of intracellular cholesterol to PM, as was the case with the inhibition of cholesterol efflux by monoclonal antibodies (10), or due to the changes in their ability to accept cholesterol released from the PM. To distinguish between these two possibilities, we compared the efflux of cholesterol from the intracellular compartment (ER) and the plasma membrane. When PM cholesterol was labeled, the amount of cholesterol released and the relationship between the abilities of different mutated forms to facilitate the efflux remained the same as for ER cholesterol (r = 0.98, p < 0.005) (Fig. 1B). It appears, therefore, that truncations affect the lipid acceptor properties of apoA-I.

To determine if the rates of cholesterol transfer are affected by structural changes of the acceptor, kinetics of cholesterol efflux were studied. The relative rates of cholesterol efflux onto differently truncated forms of apoA-I are shown in Fig. 2A. Initial velocities of cholesterol efflux (V0) in the presence of apoA-I(−6–222) (V0 = 0.008 ± 0.01 nmol/h) and apoA-I(−6–210) (V0 = 0.26 ± 0.01 nmol/h) were significantly lower compared with the full-length apoA-I(−6–243) (V0 = 1.96 ± 0.1 nmol/h) (p < 0.001). In contrast, initial velocities of cholesterol efflux in the presence of apoA-I(−6–150) (V0 = 1.43 ± 0.03 nmol/h) and apoA-I(−6–135) (V0 = 1.14 ± 0.03 nmol/h) were just marginally lower than that with apoA-I(−6–243) (p < 0.03) and significantly higher than that for apoA-I(−6–222) and apoA-I(−6–210) (p < 0.001). At low concentrations of an acceptor, differences in the initial velocity reflect the differences in the apparent rate constants for cholesterol efflux (28). This must be due to a difference in the fraction of “effective collisions,” in other words, an ability of the mutants to bind cholesterol released from the plasma membrane. Cholesterol efflux to the full-length apoA-I, however, showed signs of saturation earlier (3 h) compared with other mutants (6 h). After 21 h of incubation with apoA-I(−6–222), the amount of [14]Ccholesterol found in the medium was only 5% of the control value.

A possible explanation for the differences in efflux may also result from changes in the ability of apoA-I mutants to interact with the cell surface. We have previously demonstrated, using monoclonal antibody, that the carboxyl-terminal end of apoA-I may be involved in the interaction of HDL with cells (29). However, we were unable to confirm this finding in the current study using recombinant apoA-I; when [125]I-labeled apoA-I mutants were incubated with the cells at similar concentrations to those used in efflux experiments, we observed no difference in the interaction with cells between full-length apoA-I(−6–243) and two truncated forms, apoA-I(−6–222) and apoA-I(−6–210) (Fig. 3). The interaction of the two other truncated forms, apoA-I(−6–150) and apoA-I(−6–135), was 30–50% greater compared with the full-length form (Fig. 3). It should be noted...
that nonspecific binding, which amounted to about 30% of the total, was determined in the presence of an excess of HDL rather than the corresponding form of lipid-free apoA-I. It cannot be excluded, therefore, that the increased binding of apoA-I(--6–150) and apoA-I(--6–135) reflects a nonspecific interaction. There was no correlation between the interaction of different forms with the cell surface and the mutants’ ability to promote cholesterol efflux. The differences in cholesterol efflux therefore cannot be explained by the differences in the interaction of apoA-I mutants with the cells.

It was proposed by Forte et al. (30) and Yancey et al. (7) that lipid-free apoA-I is unable to bind cholesterol before it forms a complex with phospholipid. Formation of phospholipid-protein complexes was suggested to be a prerequisite to cholesterol binding and thus essential to the process of cholesterol efflux (30, 31). The observed differences in efflux of cholesterol with the differently truncated forms of apoA-I may therefore result from their unequal ability to promote cholesterol efflux. The differences in cholesterol efflux therefore cannot be explained by the differences in the interaction of apoA-I mutants with the cells.

Generation of rHDL Containing ApoA-I Mutants—In order to study cholesterol efflux independently of the efflux of phospholipid, different apoA-I mutants were complexed with phospholipid prior to efflux experiments. The resulting discoidal rHDL particles were analyzed by gradient gel electrophoresis (Fig. 4), and densitometric analysis of the gels is presented in Fig. 5. In order to determine the amount of lipid-free protein still present after particle formation, complexes were subject to native gradient gel electrophoresis under conditions shown to retain free apoA-I on the gel. Corresponding forms of lipid-free apoA-I were run in adjacent lanes. None of the lipid-free apoA-I self-associated oligomers reached the size of the rHDL particles. The results of densitometric quantitation of these gels are presented in Table III. The composition of the particles and results of cross-linking experiments are also presented in Table III.

Human plasma apoA-I and recombinant apoA-I(--6–243) formed almost homogeneous populations of particles; 90% of particles were represented by rHDL with diameters of 10.0 and 9.8 nm and predominantly 3 molecules of apoA-I per particle, while incorporation of apoA-I into rHDL was complete (Fig. 4, lanes A and B; Fig. 5, A and B; Table III). The size and degree of homogeneity of the particles was similar to that reported by other laboratories (21, 28, 32) for rHDL formed from POPC and apoA-I under similar conditions, except that these studies identified predominantly two molecules of apoA-I per particle. Despite its low efficiency in binding cellular phospholipid in short term experiments, when assembled using the sodium-cholate method, apoA-I(--6–222) formed almost homogeneous complexes with POPC with a diameter of 9.0 nm. The particles contained 3 molecules of apoA-I/particle, and the amount of unincorporated apoA-I was negligible (Fig. 4, lane C; Fig. 5C; Table III). Mutant apoA-I(--6–210), however, formed a very heterogeneous population of particles, ranging from 7.5 to 17 nm in diameter.

**Table II**

**Efflux of phospholipid onto truncated forms of lipid-free apoA-I and apoA-I incorporated into rHDL**

Phospholipid in HepG2 cells was labeled by incubation with [14C]choline for 24 h at 37 °C (see “Materials and Methods”). After labeling cells were washed and incubated for 3 h at 37 °C with different forms of either lipid-free apoA-I (final concentration 1 μM) or apoA-I incorporated into rHDL (final concentration 68 μM POPC). Phospholipid was isolated as described under “Materials and Methods.”

| Truncation          | Efflux apoA-I | Efflux apoA-I/POPC |
|---------------------|---------------|--------------------|
| apoA-I(--6–243)     | 1.2 ± 0.05    | 0.53 ± 0.12        |
| apoA-I(--6–222)     | 0.96 ± 0.98   | 0.50 ± 0.08        |
| apoA-I(--6–210)     | 0.4 ± 0.22    | ND                 |
| apoA-I(--6–150)     | 2.5 ± 0.22    | 0.75 ± 0.04        |
| apoA-I(--6–135)     | 1.2 ± 0.3     | 0.69 ± 0.04        |

a p < 0.001 (versus apoA-I(--6–243)).

b p < 0.02 (versus lipid-free apoA-I).

c Not determined.
Lipid Efflux to ApoA-I Mutants

STOKES DIAMETER (nm)

Fig. 5. Densitometric scans of non-denaturating gel electrophoresis patterns (stained for protein) during the preparation of rHDL particles. The rHDL particles were prepared from a molar ratio POPC:apoA-I:sodium cholate (80:1:80) as described under “Materials and Methods.” The forms of apoA-I used for rHDL preparation are: A, human plasma apoA-I(1–243); B, apoA-I(−6–210); C, apoA-I(−6–222); D, apoA-I(−6–210); E, apoA-I(−6–150); F, apoA-I(−6–135).

Table III

Composition and size of rHDL

Preparation of rHDL and gradient gel electrophoresis are described under “Materials and Methods” and in the legend to Fig. 3.

| Truncation | Composition PC/apoA-I | No. of apoA-I/particle | Diameter (nm) | Amount of free apoA-I |
|------------|------------------------|------------------------|--------------|----------------------|
|            | mol/mol nm %           |                        |              |                      |
| ApoA-I(1–243) | 84:1 3 10.0 | 0                     |              |                      |
| ApoA-I(−6–243) | 66:1 3 9.8 | 2.1                   |              |                      |
| ApoA-I(−6–222) | 66:1 3 9.0 | 4.9                   |              |                      |
| ApoA-I(−6–210) | 143:1 1 9.0 | 13.6 | 44.1          |                      |
| ApoA-I(−6–150) | 71:1 5–6 10.0 | 12.4 | 0           |                      |
| ApoA-I(−6–135) | 66:1 6 9.0 | 10.4 | 0           |                      |

a Protein and phospholipid assays are described under “Materials and Methods.”

b Determined by cross-linking as described under “Materials and Methods.”

c As determined by the gradient gel electrophoresis.

Fig. 6. Efflux of intracellular (A and B) and plasma membrane (C and D) cholesterol onto apoA-I mutants incorporated into rHDL. Cholesterol in HepG2 cells was labeled by incubation with [14C]acetate for 3 h at 15 °C (A and B) or with [3H]cholesterol/PC liposomes for 3 h at 37 °C (C and D) (see “Materials and Methods”). After labeling cells were washed and incubated for 3 h at 37 °C with the different forms of apoA-I incorporated into rHDL (final POPC concentration 66 μmol). [3H]Cholesterol from the medium was isolated and quantitated as described under “Materials and Methods.” Amount of [3H]cholesterol released from the cells was calculated relative to the POPC content (per 33 nmol of POPC) (A and C) or relative to the number of particles (per 3 × 10^14 (0.5 nmol) particles) (B and D). * p < 0.05; ** p < 0.001 (versus apoA-I(−6–243)).

Cholesterol and Phospholipid Efflux to rHDL Containing ApoA-I Mutants—To study intracellular cholesterol efflux, cells were labeled with [14C]acetate and incubated for 3 h at 37 °C with rHDL containing at equimolar concentrations of POPC (66 μmol). Since the POPC/apoA-I ratio in the particles was always close to 66:1, the concentration of added apoA-I was close to 1 μmol, i.e., the same as that used in the experiments described above with the lipid-free apoA-I. When the particles were compared on the basis of their phospholipid content for their ability to promote intracellular cholesterol efflux, no differences were observed between rHDLs containing full-length or truncated forms of recombinant apoA-I (Fig. 6A). The particles formed from the shortest forms, apoA-I(−6–150) and apoA-I(−6–135), contained more apoA-I molecules per particle than the other two forms, so the data were recalculated on a per particle basis. This determination showed that cholesterol efflux to rHDL containing apoA-I(−6–150) and apoA-I(−6–135) was almost twice that of the apoA-I(−6–243) and apoA-I(−6–222) (Fig. 6B). When plasma membrane cholesterol was labeled with [14C]cholesterol/PC liposomes, a modest but statistically

2 K.-A. Rye, L. Pyle, D. Sviridov, and N. Fidge, manuscript in preparation.
significant difference in cholesterol efflux to apoA-I-(6–243) and apoA-I-(6–222) versus apoA-I-(6–150) and apoA-I-(6–135) was observed (Fig. 6C). Comparison of efflux from either PM or ER revealed that this difference was due to an increase in $^{13}$C]cholesterol released to the longer forms when cholesterol in the PM was labeled, while efflux to the shorter forms was similar for PM and ER (Fig. 6, A and C). This points to the possibility that rHDL containing the longer forms may be more efficient acceptors of cholesterol when the amount of cholesterol is not limited by transfer from ER to PM. However, after recalculating cholesterol efflux from PM on a per particle basis, the relationship was similar to that observed for efflux of intracellular cholesterol (Fig. 6, B and D).

To study phospholipid efflux, cellular phospholipid was labeled with $^{14}$C]cholate and cells were then incubated with rHDLs at conditions identical to those used to study cholesterol efflux. The ability of the full-length recombinant apoA-I to promote phospholipid efflux was decreased by 56% when it was incorporated into rHDL (Table II), but full-length and all truncated forms of the recombinant apoA-I were similarly as effective in promoting phospholipid efflux when complexed with POPC to form rHDL (Table II).

**DISCUSSION**

The present study continues our investigations into the identification of putative structural features of apolipoprotein A-I that determine its ability to promote efflux of cholesterol and phospholipid from cultured cells. In our previous work we demonstrated that monoclonal antibodies reacting with epitope within the region apoA-I-(140–150) specifically inhibited the ability of apoA-I-containing lipoproteins to promote efflux of intracellular but not plasma membrane cholesterol (10). For this study we have produced several versions of recombinant proapoA-I selectively truncated at loci suspected of involvement in cellular cholesterol efflux. The ability of these mutants, either in the lipid-free form or incorporated into rHDL, to mobilize cellular cholesterol and phospholipid were compared.

At least two stages have been identified in the process of cholesterol efflux: transfer of cholesterol from intracellular compartments to the plasma membrane and transfer from plasma membrane to the extracellular acceptor (1). While the first stage may be receptor-dependent (35), the second stage mainly depends on the secondary structure of the apolipoproteins (36). In the present study, we found that varying degrees of truncation produced marked differences in the ability of lipid-free apoA-I to promote efflux of cellular cholesterol. In contrast to the selective inhibition of the efflux of intracellular cholesterol by monoclonal antibodies (10), truncations of apoA-I exerted a similar effect on the efflux of both intracellular and plasma membrane cholesterol. The changes in cholesterol efflux resulting from truncation of apoA-I did not correlate with binding of apoA-I to cells. Taken together, these observations suggest that the removal of the varying proportion of the carboxyl terminus of apoA-I does not affect intracellular stage of cholesterol efflux, a stage that may depend on a specific interaction between apoA-I and cells. Instead, it appears to reflect changes in the ability of truncated forms of apoA-I to interact and form complexes with cholesterol and phospholipid, processes that are associated with the second stage of the efflux.

It was suggested by Forte et al. (30, 31) that association with phospholipid is a prerequisite for the ability of lipid-free apoA-I to facilitate cholesterol efflux. Several findings in this work support that hypothesis. First, the effect of truncations on cholesterol efflux paralleled their effect on the efflux of phospholipid. Second, differences in the ability of the various forms of apoA-I to promote cholesterol efflux disappeared when apoA-I was complexed with phospholipid prior to incubation with cells. The cholesterol/phospholipid ratio in the medium rose quickly during the first 1–3 h of incubation and remained constant thereafter. Taken together, these data suggest that any differences observed in the promotion of cholesterol efflux from HepG2 cells most likely reflect a difference in capacity to associate with cellular phospholipid and subsequently form the apoA-I/phospholipid complexes required for efflux of cholesterol.

The data obtained with truncations that involved the last 21 amino acids suggest that this domain of apoA-I plays a crucial role in interaction with cellular lipids. This region includes a class Y amphipathic helix, which is suggested to bind phospholipid strongly and may play an important role in assembling apoA-I into lipid-containing complexes (37). It was also demonstrated that truncation of apoA-I to residue 189 (38), 192 (39), or 212 (33) significantly diminished its ability to react with phospholipid. Truncation to residues 226, 217, and 201, which decreased the ability of apoA-I to associate with HDL in vivo, also produced a marked increase in the clearance of apoA-I (17). The important lipid binding properties of this region has been also demonstrated with other techniques; peptide consisting of residues apoA-I-(220–241), together with peptide apoA-I-(44–65), were the only fragments of apoA-I apparently able to bind phospholipid (40). In this work we have demonstrated that truncation to residue 222 caused a significant reduction in the ability of apoA-I to promote efflux of cellular lipids suggesting that the 223–243 region is most likely involved in the formation of apoA-I-phospholipid complex.

Unexpectedly, truncation of apoA-I to residue 150 completely restored and even enhanced promotion of cholesterol and phospholipid efflux. At least three explanations can be offered for this finding. First, another cryptic lipid-binding domain exists in the center of the apoA-I, which is unmasked by truncation. The most likely candidates are a “hinge” region apoA-I-(87–142), which contains the only two remaining class Y amphipathic helices of apoA-I and which has a strong ability to bind lipids (37), or a region apoA-I-(44–65), which also shows a strong lipid-binding ability (40). Second, this other domain is a primary lipid binding domain in full-length apoA-I, but is structurally compromised when the last 21 amino acids are truncated. Third, the lipid-binding properties of apoA-I do not depend on a specific sequence or a domain, but rather on the overall secondary structure of the whole protein which is affected when some parts of the protein are truncated. Evidence from the work with synthetic peptides suggests the existence of two lipid-binding domains in apoA-I and therefore favors the first possibility (40). ApoA-I with point mutations are currently being produced in order to distinguish between these possibilities.

The recombinant proform of apoA-I was used in most experiments. While the properties of proapoA-I and mature apoA-I were almost identical when complexed to lipids (11–14, 18), the ability of lipid-free mature apoA-I to promote lipid efflux was different from that of proapoA-I. We evaluated the contribution of the propeptide moiety to the facilitation of lipid efflux and reasoned that this contribution would influence equally all the carboxyl-terminally truncated forms. It cannot be totally excluded, however, that some of the effects observed with lipid-free apoA-I may be due to changes in the interaction of the propeptide with the rest of the molecule. For example, the propeptide may interact with a site at the carboxyl terminus, which could explain how it decreases the lipid-binding ability of apoA-I. Truncation to residue 150 may have eliminated this site and therefore negated the effect of the propeptide. While this possibility cannot be excluded, it is, nevertheless, unlikely; the truncation restored the activity of the mutant to the level of full-length proapoA-I, not to the level of mature apoA-I as one...
would expect if the mutation negated the effect of the propetide. Alternatively, the difference in the folding of the recombinant protein, compared with the native apoA-I rather than the presence of propetide, may have contributed to the differences observed. When complexed with phospholipid, recombinant prosaposin and mature plasma apoA-I were equally able to facilitate cholesterol and phospholipid efflux, a phenomenon also noticed by Westman et al. (13).

When various forms of apoA-I were incorporated into rHDL, all of them, except apoA-I(6–210), formed rHDL particles. Full-length apoA-I and apoA-I(6–222) formed mostly single-size populations, while apoA-I(6–150) and apoA-I(6–135) produced rather heterogeneous populations. All rHDL particles were of pre-

REFERENCES

1. Fielding, C. J., and Fielding, P. E. (1995) J. Lipid Res. 36, 211–228
2. Forte, T. M., and McCall, M. R. (1993) J. Biol. Chem. 268, 534–364
3. Barter, P. (1993) Curr. Opin. Lipidol. 4, 210–217
4. Fielding, C. J., and Moser, K. (1982) J. Biol. Chem. 257, 10955–10960
5. Davidson, W. S., Lund-Katz, S., Johnson, W. J., Anantharamaiah, G. M., Palghamachari, M. N., Segrest, J. P., Rothblat, G. H., and Phillips, M. C. (1994) J. Biol. Chem. 269, 22975–22982
6. Mendez, A. J., Anantharamaiah, G. M., Segrest, J. P., and Oram, J. F. (1994) J. Clin. Invest. 94, 1688–1705
7. Yancey, P. G., Bielicki, J. K., Johnson, W. J., Lund-Katz, S., Palghamachari, M. N., Anantharamaiah, G. M., Segrest, J. P., Phillips, M. C., and Rothblat, G. H. (1995) Biochemistry 34, 7955–7965
8. Bari, C. L., Black, A. S., and Curtiss, L. K. (1994) J. Biol. Chem. 269, 10288–10297
9. Fielding, P. E., Kawano, M., Catapano, A. L., Zoppo, A., Marcovina, S., and Fielding, C. J. (1994) Biochemistry 33, 6981–6985
10. Segrest, J. P., Pyle, L. L., and Fidge, N. (1996) Biochemistry 35, 189–196
11. Sorci-Thomas, M. G., Parks, J. S., Kearns, M. W., Pate, G. N., Zhang, C., and Thomas, M. J. (1996) J. Lipid Res. 37, 673–683
12. Roobol, C., Heymans, C., Carlson, L. A., and Wulfert, E. (1993) Scand. J. Clin. Lab. Invest. 53, 773–782
13. McGuire, K. A., Davidson, W. S., and Jonas, A. (1996) J. Lipid Res. 37, 1519–1528
14. Dixon, J. L., Furukawa, S., and Ginsberg, H. N. (1991) J. Biol. Chem. 266, 5080–5086
15. Hakes, D. J., and Dixon, J. E. (1992) Anal. Biochem. 202, 293–298
16. Schmidt, H. H.-J., Remaley, A. T., Stuck, J. A., Ronan, R., Wellman, A., Thomas, F., Zech, L. A., Brewer, H. B., and Hoeg, J. M. (1995) J. Biol. Chem. 270, 5469–5475
17. Pyle, L. E., Sawyer, W. H., Fujiwara, Y., Mitchell, A., and Fidge, N. H. (1996) J. Biol. Chem. 271, 12046–12052
18. Morrisson, J. R., Fidge, N. H., and Grego, B. (1990) Anal. Biochem. 186, 145–152
19. Jonas, K. Z., and Jonas, A. (1982) J. Biol. Chem. 257, 4535–4540
20. Yancey, P. G., Bielicki, J. K., Johnson, W. J., Lund-Katz, S., Palghamachari, M. N., Anantharamaiah, G. M., Segrest, J. P., Phillips, M. C., and Rothblat, G. H. (1995) J. Biol. Chem. 270, 25037–25046
21. Davidson, W. S., Gillotte, K. L., Lund-Katz, S., Johnson, W. J., Rothblat, G. H., and Phillips, M. C. (1995) J. Biol. Chem. 270, 5882–5890
22. Allan, C. M., Fidge, N. H., Morrison, J. R., and Kanellos, J. (1993) Biochem. J. 290, 449–455
23. Forte, T. M., Goth-Goldstein, R., Nordhausen, R. W., and McCall, M. R. (1993) J. Lipid Res. 34, 317–324
24. Forte, T. M., Bielicki, J. K., Goth-Goldstein, R., Selmke, J., and McCall, M. R. (1995) J. Lipid Res. 36, 148–157
25. Rye, K.-A., Hime, N. J., and Barter, P. J. (1996) J. Biol. Chem. 271, 4243–4250
26. Minnich, A., Collet, X., Boghanie, A., Colfarras, C., Hamilton, R. L., Fielding, C. J., and Zannis, V. I. (1994) J. Biol. Chem. 269, 16553–16560
27. Davidson, W. S., Rodriguez-Grasa, W. V., Lund-Katz, S., Johnson, W. J., Rothblat, G. H., and Phillips, M. C. (1995) J. Biol. Chem. 270, 17106–17113
28. Mendez, A. J., Oram, J. F., and Bierman, E. L. (1995) J. Biol. Chem. 270, 16104–16111
29. Segrest, J. P., Jones, M. K., DeLoof, H., Broulllette, C. G., Venkatacharalapati, Y. V., and Anantharamaiah, G. M. (1992) J. Lipid Res. 33, 141–166
30. Broulllette, C. G., and Anantharamaiah, G. M. (1995) Biochem. Biophys. Acta 1250, 103–129
31. Holvoet, P., Zhao, Z., Vanloob, B., Ves, R., Deridder, E., Dheest, A., Taveraine, J., Brouwers, P., Demars, E., Engelferghs, Y., Rosseneu, M., Colleens, D., and Brasseur, R. (1995) Biochemistry 34, 13334–13342
32. Ji, Y., and Jonas, A. (1995) J. Biol. Chem. 270, 11290–11297
33. Palghamachari, M. N., Mishra, V. K., Lund-Katz, S., Phillips, M. C., Adeeye, S. O., Alluri, S., Anantharamaiah, G. M., and Segrest, J. P. (1996) Arterioscler. Thromb. Vasc. Biol. 16, 328–333

Acknowledgment—We are grateful to B. Sherrard-Cooper for performing enzymatic fluorometric assays.