Interaction of Apolipoprotein AII with the Putative High-Density Lipoprotein Receptor†

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ABSTRACT: There is strong evidence to indicate that binding of HDL by cells is due to recognition of apoproteins residing on the surface of the lipoprotein by the putative HDL receptor(s). Although both of the major HDL apoproteins, AI and AII, are recognized by the putative receptor, the nature of the binding interaction and the domains of the apoproteins involved are largely unknown. Previous data from this laboratory led to the proposal of a model to explain how HDL particles containing AII interacted with the HDL receptor in a different manner as compared to HDL particles which contain apoAI but not apoAII [Vadiveloo, P. K., & Fidge, N. H. (1992) *Biochem. J. 284*, 145–151]. The model predicted that each chain of the apoAII homodimer contained a binding domain capable of interacting with the HDL receptor. This model was tested in the current study by preparing apoAII monomers, complexing them with phospholipid, and determining the ability of these complexes to bind to putative HDL receptors in rat liver plasma membranes (RLPM) and bovine aortic endothelial cell membranes (BAECM) by ligand blotting. The data showed that these complexes were bound by HB1 and HB2 from RLPM, and to the 110-kDa HDL binding protein from BAECM, providing critical evidence to support the model. Further investigation into the binding interaction revealed that apoAII complexed with phospholipid (apoAII–PC) bound more than delipidated apoAII, which bound more than delipidated apoAII monomers. Thus, optimum binding required the presence of lipid. Since the amount of binding correlated with the amount of α-helical content of the molecules (i.e., apoAII–PC > delipidated apoAII > delipidated apoAII monomers), these findings are consistent with the hypothesis that α-helical regions are involved in recognition of apoAII by the putative HDL receptor. ApoAII has a single methionine residue at position 26. Investigations into whether the oxidation state of the methionine residue affected receptor interaction were undertaken. The results of these experiments showed that apoAII with methionine residues in either the reduced or the oxidized state was able to bind to the putative HDL receptors.

The findings that plasma levels of HDL are negatively correlated with the risk of developing heart disease [see Hopkins and Williams (1981) for a review] have prompted numerous investigations into possible mechanisms of regulation of plasma HDL concentration. A number of laboratories have identified specific cell membrane proteins capable of binding HDL (Tozuka & Fidge, 1989; Graham & Oram, 1987; Keso et al., 1987; Eder et al., 1989), and the ability to regulate expression of these proteins strongly suggests that they may be HDL receptors (Matthai et al., 1990; Graham & Oram, 1987).

While many studies have concluded that both of the major proteins present on the surface of HDL (i.e., apoAI and apoAII)[1] can mediate the binding of HDL to cells and putative HDL receptors (Fidge & Nestel, 1985; Tozuka & Fidge, 1989; Vadiveloo & Fidge, 1990, 1992; Graham & Oram, 1987; Hwang & Menon, 1985; Fong et al., 1985; Hoeg et al., 1985), there is little understanding of the mechanisms involved. It is quite likely that interaction of apoAI with the putative receptor may differ from that of apoAII since each apoprotein appears to be bound with different affinity and capacity (Vadiveloo & Fidge, 1992; Hwang & Menon, 1985; Barbaras et al., 1986; Steinmetz et al., 1990). It is important to gain a full understanding of the apoprotein binding mechanisms since they may explain the fact that HDL particles are metabolized differently depending upon the presence of apoAI or apoAII (Atmeh et al., 1983; Barbaras et al., 1987a; Fong et al., 1987; Schreiber et al., 1985). Of particular interest are the findings that particles containing apoAII are less effective at promoting both cholesterol efflux from the cell (Barbaras et al., 1987a) and cholesterol influx to the cell (Fong et al., 1987; Schreiber et al., 1985). These observations have led to the suggestion that HDL containing apoAII may antagonize the "reverse cholesterol transport" pathway (Vadiveloo & Fidge, 1992). Such a conclusion is consistent with the findings that (a) high levels of Lp(AI without AII) may offer protection against the development of coronary heart disease (Puchois et al., 1987) and (b) levels of HDL_2, which are comprised mainly of AI without AII particles, more strongly correlate with a decreased risk of heart disease than levels of HDL_3, which contain mostly AI with AII particles (Miller et al., 1981; Ballantyne et al., 1982).

Recently we proposed a model to explain the mechanism of HDL_3 binding to cells (Vadiveloo & Fidge, 1992). This model was based upon the observation that HDL_3 containing only apoAII (i.e., AII–HDL_3) bound to cell membranes with a higher affinity and lower capacity than HDL_3 containing only apoAI [HDL_3(AI without AII)]. A major feature of

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[1] Abbreviations: BAEC, bovine aortic endothelial cells; RLP, rat liver plasma; apoAI, apolipoprotein AI; apoAII, apolipoprotein AII; rc-apoAII, reduced and carboxymethylated apoAII; rc-apoAII–PC, reduced and carboxymethylated apoAII complexed with phosphatidylcholine; rc-apoAIImet, reduced and carboxylated apoAII with methionine in the reduced state; rc-apoAIImetO, reduced and carboxymethylated apoAII with methionine in the oxidized state.

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this model predicted that each subunit of an apoAII dimer was capable of binding to the putative HDL receptor. One aim of the current study was to test this hypothesis by preparing apoAII monomers (reduced and carboxymethylated apoAII, i.e., rc-apoAII) and assessing their ability to bind to putative HDL receptors present in either bovine aortic endothelial cell (BAEC) membranes or rat liver plasma membranes (RLPM).

When complexed with lipid, apoAII contains about 70% α-helical structure. A second aim of these investigations into the binding of apoAII to the HDL receptor was to determine whether the α-helical content of apoAII was important for receptor recognition. The final part of this investigation examined whether the oxidation state of the methionine residue at position 26 of apoAII influenced binding to the receptor, since it has been shown that oxidation of methionine residues leads to a loss of biological properties of a number of proteins [see Brot and Weissbach (1983) for a review].

MATERIALS AND METHODS

Preparation of ApoAII. ApoAII was prepared by the method of Cheung and Albers (1977). Briefly, human HDL (d = 1.063–1.21 g/mL) was incubated for 3 h at 37 °C with 6 M guanidine hydrochloride to dissociate apoAI. Following extensive dialysis against buffer A (150 mM NaCl, 10 mM Tris, and 1 mM EDTA, pH 7.4; d = 1.006 g/mL), the solution was adjusted to d = 1.21 g/mL with KBr and centrifuged at 70 000 rpm in a Beckman 70.1 Ti rotor for 16 h at 4 °C. The top fraction (containing apoAII-enriched lipoprotein) was collected, dialyzed against 5 mM NH_4HCO_3, lyophilized, and delipidated as previously described (Vadiveloo & Fidge, 1990). The proteins were resuspended in urea buffer (6 M urea/20 mM Tris, pH 8) and applied to a DEAE-Sepharose column (Pharmacia, Sweden) equilibrated in urea buffer. The proteins were eluted with a linear gradient of 0–125 mM NaCl, and fractions containing pure apoAII (as assessed by 15% PAGE with SDS) were collected, dialyzed against buffer A, and stored under N_2(g) at –20 °C.

Preparation of ApoAII Monomers. ApoAII was incubated with 10 mM dithiothreitol (Calbiochem, La Jolla, CA) under N_2 for 30 min at 4 °C to reduce the disulfide bonds. Carboxymethylation of the sulfhydryl groups was achieved by addition of iodoacetic acid (Calbiochem) to a final concentration of 22 mM, followed by incubation as described above, in the dark, after which unreacted dithiothreitol and iodoacetic acid were removed by extensive dialysis against buffer A. Samples were analyzed by 15% PAGE in the presence of 0.1% SDS to confirm complete conversion of apoAII to the monomeric form, hereafter referred to as rc-apoAII (i.e., reduced, carboxymethylated apoAII).

Purification of Met and MetO Forms of Rc-apoAII by HPLC. The Met and MetO forms of rc-apoAII were purified by reversed-phase HPLC on an Aquapore C8 column (10 × 100 mm) equilibrated in 0.1% TFA. Up to 300 μg of protein was loaded, and elution was by a linear 60-min gradient from 36 to 48% CH_3CN at a flow rate of 0.8 mL/min. Eluted fractions, monitored at 215 nm, were collected, and those estimated to contain either pure Met or MetO forms were pooled separately, concentrated in a Speed Vac, and resuspended in buffer A, and an aliquot was taken and rechromatographed to check for purity. For this analysis, the same conditions were used except the gradient was established over 30 min. Amino acid analysis showed that the oxidized form of the protein eluted earlier, as previously reported by Anatharamiah et al. (1988) (data not shown). The proteins were used within 48 h of preparation, and chromatographic analysis showed that there was no change in the profiles after this time, indicating that no changes in the oxidation states had occurred.

Preparation of ApoAII–Egg Yolk Phosphatidylcholine (PC) Complexes. ApoAII–egg yolk PC complexes were prepared essentially as described by Blanche et al. (1988). Briefly, L-α-phosphatidylcholine prepared from egg yolk (type XI-E, Sigma) and supplied in a chloroform solution was dried under N_2 and resuspended in buffer A to a concentration between 1 and 2 mg/mL. This solution was placed on ice and sonicated (Branson sonifier) on setting 3 for 30–40 min, under a stream of N_2. Following sonication, the solution was centrifuged in a Beckman 50.3 rotor at 20 000 rpm for 1 h at 25 °C to pellet the titanium particles loosened from the sonicating probe. Protein was added at a molar ratio of 16:1 (PC:protein ratio); the mixture was vortexed and incubated at 4 °C for 24 h. The solution was adjusted to d = 1.085 g/mL using KBr and centrifuged in a TL 100.2 rotor (Beckman) at 100 000 rpm, 4 °C, for 16 h. The bottom fraction was collected, adjusted to d = 1.21 g/mL, and centrifuged under the same conditions as described above. The top fraction from this second ultracentrifugation was collected, dialyzed against buffer A, and analyzed by 4–30% native PAGE which confirmed that liposomes had been formed (data not shown). Liposomes were radioiodinated with ^{125}I (Amersham) as previously described (Fidge et al., 1984) and showed specific activities of approximately 600 cpm/ng.

Preparation of Bovine Aortic Endothelial Cell (BAEC) Membranes. Cultured BAE cells were prepared and maintained as described elsewhere (Vadiveloo & Fidge, 1990), and cell membranes were prepared essentially according to Basu et al. (1978). Briefly, confluent cells grown on 90-mm dishes were scraped with a rubber policeman and stored in buffer B (buffer A plus 1 mM $CaCl_2$ and 1 mM PMSF) at –80 °C until required. Upon thawing, cells were immediately disrupted on ice in buffer B using a Polytron homogenizer for 20 s on setting 4. The homogenate was centrifuged at 1000g for 5 min at 4 °C. The supernatant was collected and examined by light microscopy which showed the presence of membrane structures, but not whole cells. The pellet was homogenized and centrifuged again, and the supernatants of the 1000g centrifugations were pooled. These supernatants were further centrifuged for 60 min at 100000g at 4 °C to pellet the membranes, which were then resuspended in buffer A by passing through 18- and then through 20- and 25-gauge needles consecutively. Aliquots of the membranes were stored at –20 °C.

Preparation of HDL Binding Proteins from Rat Liver Plasma Membranes. Rat liver plasma membranes (RLPM) were prepared as previously described (Tozuka & Fidge, 1989). A membrane protein fraction rich in HDL binding proteins (HB1 and HB2) was prepared by DEAE chromatography as previously described (Tozuka & Fidge, 1989).

Ligand Blotting Assay. BAEC membranes (250 μg) or RLPM fractions rich in HB1 and HB2 (50 μg) were dissolved in buffer A containing 3% SDS, and proteins were separated on either 8% or 10% polyacrylamide gels containing 0.1% SDS. Proteins were electrophoretically transferred to nitrocellulose paper (100 mA, 18 h, 4 °C), which was then incubated for at least 1 h at room temperature in a blocking buffer (50 mM Tris, 90 mM NaCl, and 3% skim milk powder, pH 8.0). For the assay using radiolabeled ligands, the nitrocellulose sheet was incubated with 10 μg/mL radiolabeled ligand (in blocking buffer) for 3 h at 25 °C and then washed in PBS (5 × 5 min) to remove nonbound ligand. The nitrocellulose was

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          10        20        30        40        50
QAKEPCVESL VSQYFQTVTD YGKDLMEKVK SPELQAEAKS YFEKSKEQLT

          60        70
PLIKKAGTEL VNFLSYFVEL GTQPATQ
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FIGURE 1: Amino acid sequence of the reduced (or monomeric) form of human plasma apoAII. In the native state, the protein is a dimer linked by a disulfide bond at residue 6. The three predicted α-helical regions are underlined. A = Ala, C = Cys, D = Asp, E = Glu, F = Phe, G = Gly, H = His, I = Ile, K = Lys, L = Leu, M = Met, N = Asn, P = Pro, Q = Gln, R = Arg, S = Ser, T = Thr, V = Val, W = Trp, and Y = Tyr.

exposed to Hyperfilm-MP (Amersham) for 24–48 h at –70 °C in cassettes containing Cronex Lightning Plus intensifying screens (Du Pont).

In some assays, ligand binding was detected using specific antibodies for the ligand, rather than by autoradiography following the use of labeled ligands. In these instances, after transfer of the membrane proteins, the nitrocellulose filter was incubated with the appropriate ligand (in blocking buffer) for at least 2 h at 25 °C and then washed in PBS (3 × 10 min) to remove nonbound ligand. The nitrocellulose filters were then incubated with 4 μg/mL anti-apoAII IgG [prepared as described in Vadiveloo and Fidge (1992)] for 1 h, after which they were washed as described above. After being washed, they were incubated with 1:500 dilution of goat anti-rabbit IgG–HRPO conjugate (BioRad) for 30 min at 25 °C. The filters were washed again, and bands were detected using a color reagent comprising 100 mL of buffer A, 60 μL of hydrogen peroxide, and 10 mL of dianisidine solution (3 mg of dianisidine/mL of methanol).

Other Methods. Protein was assayed by the method of Lowry et al. (1951) using bovine serum albumin as a standard.

RESULTS

The first aim of these studies was to determine whether apoAII monomers were able to recognize putative HDL receptors, thereby providing further evidence to support our previously proposed model describing the nature of HDL binding to its receptor (Vadiveloo & Fidge, 1992). Monomers of apoAII were prepared by cleavage of the disulfide bond at position 6 (see Figure 1) followed by carboxymethylation of the resultant free thiol groups to prevent reannealing (see Materials and Methods). Analysis of samples using 15% PAGE containing SDS indicated that conversion from dimer (M_r 17 000) to monomer (M_r 8500) was complete (see Figure 2). Both native apoAII and reduced, carboxymethylated apoAII (rc-apoAII) were then complexed with egg PC, as described under Materials and Methods, and tested for their ability to recognize the putative HDL receptors, HB1 and HB2, present in rat liver plasma membranes (Tozuka & Fidge, 1989). The ligand blotting data in Figure 3 show that HDL_3 (lane 1), apoAII complexed with PC (apoAII–PC) (lane 2), and rc-apoAII complexed with PC (rc-apoAII–PC) (lane 3) were all bound by putative HDL receptors (although weak in this experiment, rc-apoAII–PC did bind to the 120-kDa HB1, as shown in Figure 6). Similarly, the results presented in Figure 4 indicate that both apoAII–PC and rc-apoAII–PC bound to the 110-kDa HDL binding protein identified in BAEC membranes (Graham & Oram, 1987).

The second aim of these studies was to assess whether α-helical structures of apoAII play a role in its recognition by putative HDL receptors. When complexed with lipid, apoAII has about 70% α-helical structure, while in the absence

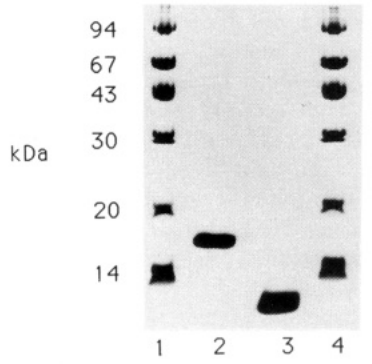

FIGURE 2: Analysis of reduced and carboxymethylated apoAII (rc-apoAII). ApoAII (17 kDa) was reduced and carboxymethylated as described under Materials and Methods. Conversion of native apoAII to its monomer form (8.5 kDa) was assessed by 15% SDS–PAGE. Lanes 1 and 4; Pharmacia low molecular mass standards; lane 2, apoAII; lane 3, reduced and carboxymethylated apoAII. The gel was stained with Coomassie blue.

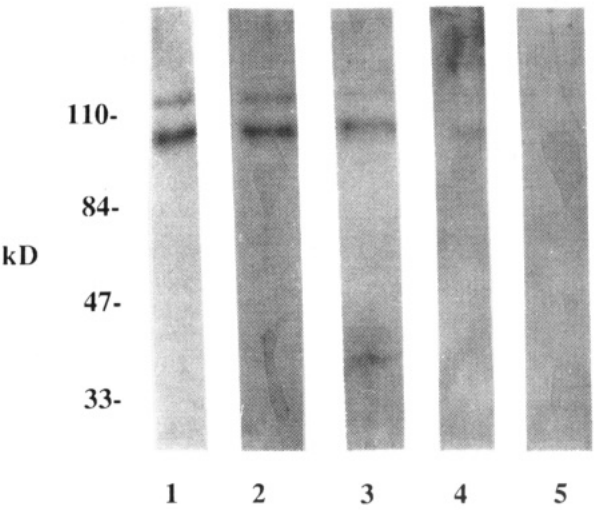

FIGURE 3: Ligand blot showing binding of HDL_3, apoAII–PC, rc-apoAII–PC, apoAII, and rc-apoAII to partially purified HDL binding proteins present in rat liver plasma membranes. Membrane proteins were separated by 8% SDS–PAGE and then electroblotted onto nitrocellulose filters. Strips were then incubated with either 10 μg/mL HDL_3 (lane 1), 20 μg/mL apoAII–PC (lane 2), 20 μg/mL rc-apoAII–PC (lane 3), 20 μg/mL apoAII (lane 4), or 20 μg/mL rc-apoAII (lane 5) for 2 h at 25 °C. Following washing, the strips were incubated with anti-apoAII IgG, followed by a second antibody conjugated to horseradish peroxidase. Bands were detected using dianisidine color developer (see Materials and Methods for details).

of lipid it contains only about 35% α-helical structure (Assman & Brewer, 1974; Massey et al., 1981). The data in Figure 3 show that when not complexed with PC, apoAII bound only very weakly (lane 4) and rc-apoAII did not bind at all (lane 5).

The next aim of these studies was to determine whether the oxidation state of apoAII methionine residue had any influence upon binding. Previous investigators have shown that approximately 50% of the methionine residues present in purified apoAII are in the reduced (Met) form and the other 50% in the oxidized (MetO) form (Anantharamiah et al., 1988). To investigate whether this difference in oxidative state affected the binding, the two forms were purified by HPLC of rc-apoAII (see Materials and Methods for details). Panel A of Figure 5 shows the elution profile following the loading of 200 μg rc-apoAII. The rc-apoAIImetO form eluted earlier (56 min) than the rc-apoAIImet form (64 min). The large peak at 12 min represents the solvent peak as the gradient was increased from 0% CH_3CN at loading to 36% CH_3CN to

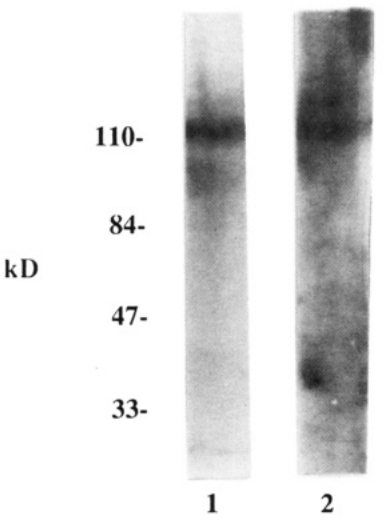

FIGURE 4: Ligand blot showing binding of apoAII–PC and rc-apoAII–PC to the HDL binding protein present in bovine aortic endothelial cell membranes. Membrane proteins were separated using 10% SDS–PAGE and then electroblotted onto nitrocellulose filters. Strips were then incubated with 10 μg/mL either ^{125}I-apoAII–PC (lane 1) or ^{125}I-rc-apoAII–PC (lane 2) for 3 h at 25 °C. Following washing, the strips were then exposed to autoradiograph film (Materials and Methods for details).

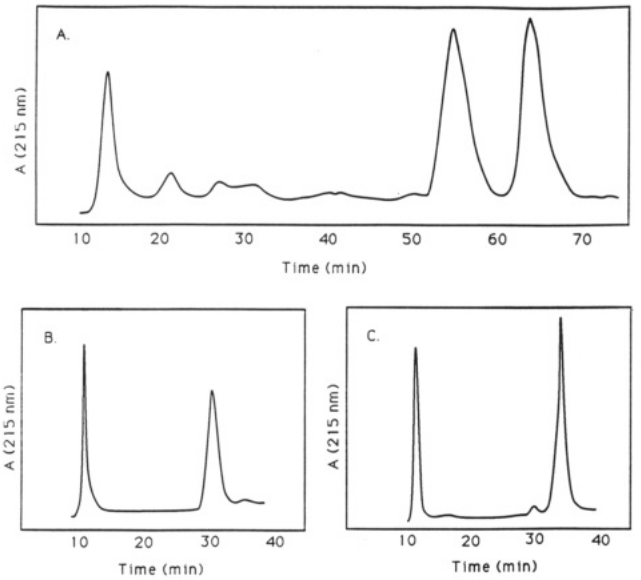

FIGURE 5: HPLC chromatograms showing separation of apoAIImet from apoAIImetO. Panel A shows the absorbance profile following the application of 200 μg of apoAII to an Aquapore C8 column (10 × 100 mm) equilibrated in 0.1% TFA (AUFS = 1.28). Protein was eluted by a linear 60-min gradient from 36 to 48% CH_3CN at a flow rate of 0.8 mL/min. Peaks were collected separately, and fractions containing rc-apoAIImetO (panel B, AUFS = 0.64) and rc-apoAIImet (panel C, AUFS = 0.64) pools were rechromatographed to check for purity (for this analysis, the same conditions were used to except the gradient was over 30 min) (see Materials and Methods for details).

start the elution. Fractions predicted to contain either pure Met or MetO forms were processed as described under Materials and Methods, and an aliquot was taken and checked for purity. Panel B depicts the elution profile of the rc-apoAIImetO fraction, showing only trace amounts of the Met form. Panel C shows the elution profile of the rc-apoAIImet fraction, again showing only very minor amounts of the MetO form.

Both rc-apoAIImetO and rc-apoAIImet were complexed with egg PC as described under Materials and Methods, and

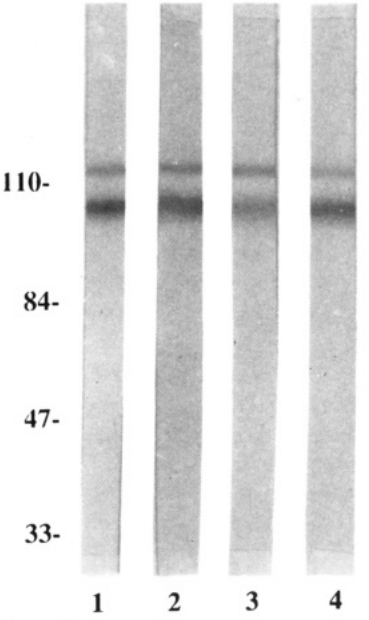

FIGURE 6: Ligand blot showing binding of apoAII–PC (lane 1), rc-apoAII–PC (lane 2), rc-apoAIImet–PC (lane 3), and rc-apoAI-ImetO–PC (lane 4) to partially purified HDL binding proteins present in rat liver plasma membranes. The procedure was the same as outlined in the Figure 3 legend.

used in the ligand blotting assay in order to determine whether the oxidation state of the Met residue affected the ability of the complexes to bind to HB1 and HB2. The data in Figure 6 show that both forms were able to bind to the putative HDL receptors present on rat liver plasma membranes.

DISCUSSION

ApoAI and apoAII are the major proteins present on the surface of HDL. While both proteins have been shown to be capable of facilitating HDL binding to cells (Fidge & Nestel, 1985), membranes (Vadiveloo & Fidge, 1992; Fong et al. 1985; Hoeg et al., 1985), and isolated HDL binding proteins (Tozuka & Fidge, 1989; Graham & Oram, 1987), little is known about the mechanism of binding. The current experiments were designed to investigate the interaction of apoAII with putative HDL receptors isolated from both BAEC and RLP membranes.

The results from the present studies showed that apoAII monomers when complexed with lipid were able to bind to the putative HDL receptors. This indicates that each chain of the native apoAII molecule contains a binding domain capable of interacting with the HDL binding proteins and that binding of apoAII is not the result of a conformation imposed by the disulfide bond. These data suggest that each apoAII molecule is capable of occupying (at least) two putative receptors, which supports a model recently proposed to explain the observation that HDL_3 particles which contain apoAII bound to BAEC membranes with higher affinity and lower capacity compared to particles which contained only apoAI (Vadiveloo & Fidge, 1992). Such observations may have major physiological consequences with respect to the reverse cholesterol transport pathway since it appears that HDL particles containing apoAII inhibit the cholesterol flux between cells and particles containing apoAI (Barbaras et al., 1987; Fong et al., 1987; Schreiber et al., 1985).

It has been shown in monkeys that the apoAII present on the HDL particle is in a monomeric form due to a substitution of the Cys residue at position 6 (Edelstein et al., 1976). The current finding that monomeric apoAII can bind to the putative

HDL receptor suggests that in monkeys apoAII could be recognized by an HDL receptor in this species.

The data in the present studies indicated that lipid is required to maintain the optimum conformation of apoAII for its interaction with the receptor. That apoAII complexed with lipid bound to a greater extent than delipidated apoAII is unlikely to be explained by an interaction between the putative receptors and the lipid since LDL does not compete for HDL binding to HB1 and HB2 (Tozuka & Fidge, 1989) and binding to the 110-kDa protein from BAEC membranes appears to be specifically via the apoprotein moiety of HDL (Oram et al., 1991). Another explanation for why delipidated apoAII monomers cannot bind could be that the proteins self-associated, thereby masking the receptor binding domains. However, this is unlikely to be the case in these studies since it has been shown that self-association of either native apoAII or apoAII monomers requries concentrations of protein above 200 μg/mL (Osborne et al., 1975; Teng et al., 1978; Donovan et al., 1987).

Given that the addition of lipid to apoproteins results in an increase in α-helical structure, and that the strength of binding of the three ligands tested correlated with the amount of α-helical structure present in each, i.e., apoAII-PC has more α-helix than apoAII, which has more than rc-apoAII (Assman & Brewer, 1974; Massey et al., 1981; Scanu, 1970), the data are consistent with the proposition that α-helical structure is involved in the recognition of apoAII with the putative HDL receptor. Herzyk et al. (1988) concluded that the α-helical structures in the apoproteins may play a role in HDL binding since tetranitromethane-treated HDL, which has a marked decrease in α-helical structure, was unable to compete for native HDL binding to HepG2 cells. During the preparation of this paper, Leblond and Marcel (1991) reported that antibodies directed against the α-helical regions of apoAI were effective in inhibiting HDL binding to HepG2 cells, further supporting the earlier observation by Herzyk et al. (1988). The involvement of α-helical structure in HDL binding is supported by other data from this laboratory which indicated that the carboxy-terminal region of apoAI, which contains up to five α-helical domains (Segrest et al., 1992), was the region recognized by the putative HDL receptor (Morrison et al., 1991).

Since a loss of biological activity accompanies oxidation of the Met residues in a number of proteins and peptides [see Brot and Weissbach (1983) for a review], studies were undertaken here to investigate whether the oxidation state of the Met at residue 26 influenced the ability of apoAII to interact with the putative HDL receptor. These experiments showed that both rc-apoAIImet–PC and rc-apoAIImetO–PC were able to bind, indicating that the oxidation state of the methionine residue at position 26 had no influence on the ability of apoAII to bind the putative HDL receptor.

There are three predicted α-helical regions in apoAII (between residues 9–29, 37–50, and 52–70; see Figure 1), and the Met residue lies within the N-terminal helix (Mao et al., 1981; Massey et al., 1984; Massey & Pownall, 1989). Anantharamiah et al. (1988) reported that the oxidation of a Met residue within an amphipathic α-helix disrupts the structure. Given that α-helical structures appears to be an important factor in the putative receptor/ligand interaction, it may be postulated that the binding domain incorporates one or both of the predicted α-helical regions found further toward the carboxy-terminal end of the molecule. Further support for this proposition comes from the observation that there is strong homology between the receptor binding domain in the carboxy-terminal region of apoAI and the carboxy-terminal region of apoAII (Allan et al., 1992).

The experiments described here investigated the binding of apoAII to the putative HDL receptor. The finding that apoAII monomers were able to bind provides additional evidence to support a previously postulated model describing a mechanism in which HDL interacts with its putative receptor on cells (Vadiveloo & Fidge, 1992). In terms of the structure/function relationships, the current data indicate that optimal binding of apoAII requires the presence of lipid, which may reflect the involvement of α-helical domains in the binding interaction. However, it appears that the oxidation state of the Met residue does not influence binding of apoAII to the putative receptor.

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