Molecular Basis of the Interaction between Plasma Platelet-activating Factor Acetylhydrolase and Low Density Lipoprotein*

(Received for publication, October 19, 1998, and in revised form, December 8, 1998)

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The platelet-activating factor acetylhydrolases are enzymes that were initially characterized by their ability to hydrolyze platelet-activating factor (PAF). In human plasma, PAF acetylhydrolase (EC 3.1.1.47) circulates in a complex with low density lipoproteins (LDL) and high density lipoproteins (HDL). This association defines the physical state of PAF acetylhydrolase, confers a long half-life, and is a major determinant of its catalytic efficiency in vivo. The lipoprotein-associated enzyme accounts for all of the PAF hydrolysis in plasma but only two-thirds of the protein mass. To characterize the enzyme–lipoprotein interaction, we employed site-directed mutagenesis techniques. Two domains within the primary sequence of human PAF acetylhydrolase, tyrosine 205 and residues 115 and 116, were important for its binding to LDL. Mutation or deletion of those sequences prevented the association of the enzyme with lipoproteins. When residues 115 and 116 from human PAF acetylhydrolase were introduced into mouse PAF acetylhydrolase (which normally does not associate with LDL), the mutant mouse PAF acetylhydrolase associated with lipoproteins. To analyze the role of apoprotein (apo) B100 in the formation of the PAF acetylhydrolase–LDL complex, we tested the ability of PAF acetylhydrolase to bind to lipoproteins containing truncated forms of apoB. These studies indicated that the carboxyl terminus of apoB plays a key role in the association of PAF acetylhydrolase with LDL. These data on the molecular basis of the PAF acetylhydrolase–LDL association provide a new level of understanding regarding the pathway for the catalysis of PAF in human blood.

Platelet-activating factor (PAF)1 is a phospholipid messenger synthesized by a variety of cells involved in host defense, such as endothelial cells, neutrophils, and monocytes (1). PAF functions both in normal physiological events and in pathological responses, particularly inflammation and allergy (1). High levels of PAF are associated with a variety of human diseases such as asthma, necrotizing enterocolitis, and sepsis, as judged by direct measurement of PAF levels (2–5), by the effects of PAF receptor antagonists (6–9), and by the effects of an enzyme that inactivates PAF (10, 11). PAF is inactivated by hydrolysis of the sn-2 acetyl group, a reaction catalyzed by PAF acetylhydrolases (12, 13). The secreted form of PAF acetylhydrolase circulates in human plasma as a hydrophobic protein complexed with low density lipoproteins (LDL) and high density lipoproteins (HDL) (14, 15).

In addition to defining the physical state of PAF acetylhydrolase in the plasma compartment, the association of this enzyme with lipoproteins has important implications for catalysis. The association of the enzyme with LDL is a major determinant of its catalytic efficiency in vivo; when the substrate concentration is limiting, the LDL-associated activity accounts for virtually all of the PAF hydrolysis in plasma (16). Thus, the failure of PAF acetylhydrolase to bind to LDL would be predicted to block the physiological function of the enzyme, even if its intrinsic catalytic activity remained intact. In addition, the association of PAF acetylhydrolase with lipoproteins allows this relatively hydrophobic enzyme to circulate in blood and gain unfettered access to sites of inflammation and cellular activation. Finally, it is possible that the lipoprotein lipids might facilitate access of hydrophobic lipid substrates to the active site of the enzyme, which may account for the efficiency of substrate hydrolysis when the enzyme is bound to lipoproteins. Therefore, identifying the factors that define PAF acetylhydrolase’s association with lipoproteins is an important issue with clear cut physiologic consequences.

In this study, we characterized the molecular basis for the interaction between PAF acetylhydrolase and LDL. Using site-directed mutagenesis, we identified two domains of PAF acetylhydrolase that are important for its interaction with LDL. In addition, we tested the hypothesis that the principal protein component of LDL, apolipoprotein (apo) B100, is important for the PAF acetylhydrolase–LDL interaction by analyzing the binding of PAF acetylhydrolase to lipoproteins containing truncated forms of human apoB. Our data indicate that the carboxyl terminus of human apoB100 plays a key role in the association of PAF acetylhydrolase with LDL.

EXPERIMENTAL PROCEDURES

Materials—PAF(1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine) was purchased from Avanti Polar Lipids (Birmingham, AL). A polyclonal antibody against PAF acetylhydrolase was prepared by immunizing New Zealand White rabbits with the purified recombinant protein (Quality Controlled Biochemicals, Hopkinton, MA). [acetyl-3H]PAF was from NEN Life Science Products.

Site-directed Mutagenesis and Deletions—Oligonucleotide-directed

* This work was supported by National Institutes of Health Grants HL35828 and HL41633 and by the Huntsman Cancer Institute. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1754 solely to indicate this fact.

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¶ The abbreviations used are: PAF, platelet-activating factor; LDL, low density lipoprotein(s); HDL, high density lipoprotein(s); DFP, diisopropylfluorophosphate; apo, apolipoprotein; CHAPS, 3-(3-cholamidopropyl)dimethylammonio)-1-propanesulfonic acid.

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mutagenesis of various sites within PAF acetylhydrolase was performed as described (11). The presence of the desired mutations or deletions was confirmed by automated analysis of DNA sequences. All mutants were expressed in a pUC vector to which the tryptophan promoter was added to allow expression of PAF acetylhydrolase in *Escherichia coli* (11). The promoter was derepressed by depletion of tryptophan during overnight incubation at 37 °C. The cultures were harvested by centrifugation, and the cells were resuspended in buffer A (100 mM succinate, 100 mM NaCl, 1 mM EDTA, 20 mM CHAPS, pH 6.0), lysed by sonication, and allowed to incubate on ice for 60 min. The soluble fraction was recovered by centrifugation, and the pellet was discarded. Supernatant fluids were assayed for PAF acetylhydrolase activity as described below and subjected to immunoblot analysis (11). Recombinant PAF acetylhydrolase was detected with a polyclonal anti-PAF acetylhydrolase antiserum (diluted 1:2000) as the primary antibody and a horseradish peroxidase–labeled goat anti-rabbit IgG antibody (diluted 1:5000) from Kirkegaard and Perry Laboratories (Gaithersburg, MD). The blots were developed with the enhanced chemiluminescence (ECL) system (Amersham Pharmacia Biotech).

Assessment of PAF Acetylhydrolase Binding to LDL—Human LDL were isolated from the plasma of healthy subjects by a single spin ultracentrifugation technique (15). The endogenous PAF acetylhydrolase activity in the LDL fraction was irreversibly inactivated by treatment with 5.0 mM diisopropylfluorophosphate (DFP, Sigma) for 60 min at 37 °C and dialysis against phosphate-buffered saline. The ability of mutant and wild-type PAF acetylhydrolase proteins to associate with LDL was assessed by incubating DFP-treated LDL (DFP-LDL) with 5 μg of solubilized extract from *E. coli* for 2 h at 37 °C. In control experiments, the amount of CHAPS carried over to the assay had no effect on the association of endogenous PAF acetylhydrolase activity with LDL or the ability of exogenously added PAF acetylhydrolase to bind to DFP-LDL. After the incubation, the mixtures of DFP-LDL and *E. coli* extracts were subjected to ultracentrifugation, as above. The LDL fraction and the lipoprotein-free “bottom fraction” were collected and assayed for PAF acetylhydrolase activity by incubation with [acetyl-³H]PAF and separation of the reaction products by reverse phase liquid chromatography, as described by Stafforini et al. (17). A unit was defined as a μmol of substrate hydrolyzed per h at 37 °C. LDL binding was assessed by calculating the fractional area in the LDL peak with Matlab software.

The generation and characterization of transgenic mice expressing the full-length human apoB100 have been reported (18). Transgenic mice expressing truncated versions of apoB (apoB80 (apo B amino acids 1–3619; Ref. 19), and apoB90 (apoB amino acids 1–4084; ref. 20)) have been isolated and stored at 4 °C. Endogenous PAF acetylhydrolase in the plasma was inactivated with DFP (5.0 mM, 60 min at 37 °C). The plasma from these mice was harvested by centrifugation, and the cells were resuspended in buffer A (100 mM succinate, 100 mM NaCl, 1 mM EDTA, 20 mM CHAPS, pH 6.0), lysed by sonication, and allowed to incubate on ice for 60 min. The soluble fraction was recovered by centrifugation, and the pellet was discarded. Supernatant fluids were assayed for PAF acetylhydrolase activity by incubation with [¹⁴C]PAF and density gradient ultracentrifugation. Fractions were collected and assayed for PAF acetylhydrolase activity, as described by Stafforini et al. (17). The results are representative of two experiments performed under identical conditions.

**RESULTS**

**The Amino and Carboxyl Termini of PAF Acetylhydrolase Are Not Necessary for Association with LDL—**Our initial studies were aimed at establishing the minimal segment of the PAF acetylhydrolase molecule required for specific binding to LDL. We tested several deletion constructs for their ability to associate with LDL in human plasma or with purified LDL fractions that had been pretreated with DFP to inhibit endogenous PAF acetylhydrolase activity. Deletion of up to 60 amino acids from the amino terminus and 21 amino acids from the carboxyl terminus of PAF acetylhydrolase had no effect on the ability of these constructs to bind to LDL (>80% of the total activity was associated with LDL in both cases). Further deletions abolished enzymatic activity (22). Therefore, we did not use the deletion approach to test other domains in the PAF acetylhydrolase molecule.

**Tyrosine 205 Is Required for Binding of PAF Acetylhydrolase to LDL—**Next, we systematically mutated all tyrosine residues in the PAF acetylhydrolase molecule to phenylalanines. Tyrosines were analyzed because they are important in the interaction of proteins with macromolecules in other systems (23). Tyrosine 440 was not analyzed because it lies within the carboxyl-terminal 20 amino acids of the protein, which has no effect on LDL binding. Mutation of tyrosine residues 20, 26, 63, 84, 85, 103, 144, 160, 188, 189, 307, 321, 324, and 335 had no effect on enzymatic activity or immunoreactivity (data not shown). All but one of the tyrosine mutants bound to purified, DFP-inactivated human LDL particles and subjected to density gradient ultracentrifugation. Fractions were collected and assayed for PAF acetylhydrolase activity, as described by Stafforini et al. (17). The results are representative of two experiments performed under identical conditions.

**Fig. 1. Tyrosine 205 is involved in the interaction between PAF acetylhydrolase (AH) and LDL.** Three PAF acetylhydrolase constructs (wild-type (WT) PAF acetylhydrolase (A); Y205F (B); and Y205A (C)) starting from the initiating methionine were cloned into a pUC 19 vector under the control of a tryptophan promoter and expressed in *E. coli*, as described (22). After harvest, the cells were resuspended in CHAPS-containing solubilization buffer, and the insoluble material was removed by centrifugation. The extracts were incubated with DFP-inactivated human LDL at pH 2.8 and subjected to density gradient ultracentrifugation. Fractions were collected and assayed for PAF acetylhydrolase activity, as described by Stafforini et al. (17). The results are representative of two experiments performed under identical conditions.

Tyrosine 440 was not analyzed because it lies within the carboxyl-terminal 20 amino acids of the protein, which has no effect on LDL binding. Mutation of tyrosine residues 20, 26, 63, 84, 85, 103, 144, 160, 188, 189, 307, 321, 324, and 335 had no effect on enzymatic activity or immunoreactivity (data not shown). All but one of the tyrosine mutants bound to purified, DFP-treated human LDL in a manner similar to the wild-type protein (>75%). Mutant Y205F had decreased binding (59%, Fig. 1), suggesting that residue 205 might be important for the interaction. A mutant with alanine at this position (Y205A) did not bind to LDL (Fig. 1). To determine if other residues in the region were also important for the interaction, we generated alanine mutants of the six adjacent amino acid residues. Changing residues 202–204 and 206–208 to alanines had no effect on enzymatic activity or on the association with LDL (not shown). Therefore, a conformational change resulting from the replacement of tyrosine at position 205 with alanine is unlikely to account for altered binding to LDL. Tyrosine 205 is present in all species examined to date, including human, bovine, dog, chicken, and mouse (22). When
we tested the ability of the dog and mouse recombinant PAF acetylhydrolases to bind human LDL, the dog protein associated with LDL in a normal fashion, but the mouse protein did not (Fig. 2). These observations suggest that tyrosine 205 is necessary but not sufficient for binding to LDL and that an additional region or regions are involved in the interaction of the enzyme with LDL. In LDL binding assays, all of the histidine mutants had normal binding except for H114A, which had altered binding to LDL (Fig. 3). Replacement of histidine 114 with glutamate or glutamine also abolished binding to LDL (Fig. 3), suggesting that the effect was independent of charge. Further analysis indicated that a residue or residues important for binding to LDL exist near histidine 114, and several findings suggest that histidine 114 is not directly involved. First, a deletion mutant lacking histidine 114 was enzymatically active and bound normally to LDL (Fig. 3). Second, the dog homolog, which has a proline residue at position 114, also bound normally to LDL (Fig. 2). Third, the mouse PAF acetylhydrolase protein, which has a proline residue at position 113 (equivalent to histidine 114 in the human molecule), did not associate with human LDL (Fig. 2B); however, mutation of proline 113 in the mouse sequence to histidine did not result in binding (Fig. 3). Thus, while histidine 114 in human PAF acetylhydrolase is near a region that is important for binding, it is not required for the association with LDL.

To identify the residue or residues near histidine 114 that are key for binding of PAF acetylhydrolase to LDL, we mutated the neighboring residues to alanines and expressed the mutant constructs in E. coli. The recombinant proteins were expressed at levels comparable with those of the wild-type construct (Fig. 4A). Mutation of residues on the amino-terminal side of histidine 114 had little effect on binding (Fig. 4). In contrast, replacement of tryptophan 115, leucine 116, and methionine 117 with alanine altered the association with LDL (Fig. 4B). Mutation of tryptophan 115 had the greatest effect, but changing leucine 116 and methionine 117 also altered binding. We next generated additional constructs in which tryptophan 115, leucine 116, and methionine 117 were individually deleted (Fig. 4C). We found that these deletion mutants retained expression of enzymatic activity, but binding to LDL was severely reduced (Fig. 4C). Thus, in contrast to histidine 114 (Fig. 3), the deletion of tryptophan 115, leucine 116, and, to a lesser extent, methionine 117 resulted in molecules that lacked the ability to associate with LDL, indicating that these residues are necessary for binding to the lipoprotein.

The role played by tryptophan 115 and leucine 116 in the binding of PAF acetylhydrolase to LDL was confirmed in the following experiment. We made use of the fact that the primary sequence of the mouse PAF acetylhydrolase in the region of residues 114–117 is quite different from the human sequence (Fig. 5). We systematically mutated the residues in the mouse PAF acetylhydrolase to mimic the domain present in the human molecule. As the homology in this region was increased to match that of the human PAF acetylhydrolase, binding to LDL was restored (Fig. 5). Changing proline 113 to histidine did not significantly affect binding (1% for the wild-type versus 7.5% for the P113H mutant). Replacing proline 113 and serine 114 to histidine and tryptophan, respectively, increased binding to LDL to 40.9%. When proline 113, serine 114, and isoleucine 115 of the mouse enzyme were converted to histidine, tryptophan, and leucine, respectively, binding to LDL increased to 64.4%. Notably, the dog PAF acetylhydrolase (which binds to human LDL) is identical to human PAF acetylhydrolase in this region. Thus, site-directed mutagenesis and deletion analyses showed that residues 115 and 116 are critical for binding of PAF acetylhydrolase to LDL. Moreover, the introduction of these residues into the mouse sequence yielded an enzyme that was

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Fig. 2. Association of dog and mouse PAF acetylhydrolase (AH) with human LDL. The cDNAs encoding dog (A) and mouse (B) PAF acetylhydrolase homologs (22) were cloned into the pUC 19 and expressed from the initiating methionine, as described in the legend to Fig. 1. Binding to human LDL was assessed after incubation with the lipoprotein, ultracentrifugation, fractionation, and enzymatic activity determinations, as described under "Experimental Procedures." The results are representative of two experiments performed under identical conditions.

An amino-terminal region in PAF acetylhydrolase is necessary for binding to LDL—Next, we tested the possibility that histidine residues in PAF acetylhydrolase are important for binding to LDL. We previously observed that the association of this enzyme with LDL is dependent on pH and that its pH profile is consistent with the notion that a histidine residue plays a role in the process (15). All histidines (residues 7, 32, 74, 114, 152, 170, 179, 216, 241, 272, 367, 395, 399, and 428) were changed to alanines (except histidine 351, which is part of the catalytic triad in PAF acetylhydrolase (22)), and the resulting mutants were tested for binding to LDL. In general, mutation of histidines to alanines had no effect on the enzymatic activity of the recombinant products. However, changing histidine 272 to alanine abolished enzymatic activity. Histidine 272 is next to serine 273, which is part of a catalytic triad that is characteristic of lipases (22). It is not surprising that the H272A mutant expressed no enzymatic activity, because altering the conformation of the protein in this region would be likely to disrupt the three-dimensional structure near the active site. Western blot analysis of the histidine mutants showed expression of protein at levels corresponding to the activity in the samples (not shown).

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The role of histidine 114 was examined by comparing the binding of wild-type PAF acetylhydrolase to LDL with that of three mutants in which the residue was mutated (H114A, H114Q, and H114E) or deleted (H114ø). The mouse PAF acetylhydrolase homolog was used to generate a mutant in which the proline present at position 113 (equivalent to position 114 in the human cDNA) was replaced with a histidine residue. The results are representative of two experiments performed under identical conditions.

Cysteine Residues Are Not Involved in the PAF Acetylhydrolase-LDL Interaction—The full-length PAF acetylhydrolase cDNA encodes eight cysteines (22), but the mature form of the enzyme is predicted to contain only five cysteines (22). Therefore, the mature protein is likely to contain at least one unpaired sulfhydryl group, which might be involved in binding to LDL. In addition, PAF acetylhydrolase has been reported to have lower affinity for LDL than for lipoprotein(a) (24), which differs from LDL by the covalent attachment of apo(a), a glycoprotein that is highly homologous to plasminogen (25). Apo(a) associates with apoB by a disulfide bond (26, 27). To examine the possibility that PAF acetylhydrolase and LDL interact through a disulfide bond, we tested the effect of reducing agents on the distribution of PAF acetylhydrolase in lipoproteins. Relatively high concentrations of dithiothreitol (1, 5, and 10 mM) had no effect on enzymatic activity or association with LDL (not shown). The failure of dithiothreitol to dissociate PAF acetylhydrolase and LDL makes it very unlikely that their interaction depends on a disulfide bond. However, to further explore this issue, we generated five mutants in which the cysteine residues were converted to serines. Cysteine residues 11, 13, and 15 were not examined, because they lie within a region of the amino terminus shown by the deletion studies to be unimportant for binding to LDL. We tested the ability of C67S, C229S, C291S, C334S, and C407S to bind LDL. The mutants retained enzymatic activity and associated with LDL in a manner identical to that of the wild-type construct (data not shown).

The Carboxyl Terminal of ApoB100 Participates in the Association between PAF Acetylhydrolase and LDL—There is some evidence that apoB100 is the protein component that mediates the binding of PAF acetylhydrolase to LDL. First, our early studies on the purification of PAF acetylhydrolase showed that a carboxyl-terminal fragment of apoB100 (including residues 4119–4536) co-purified with PAF acetylhydrolase through numerous purification steps, strongly suggesting that the carboxyl-terminal portion of apoB100 and PAF acetylhydrolase interact with each other (28). Second, apoB100 is the major protein component of LDL. Third, in human subjects who are deficient in apoB100, PAF acetylhydrolase associates entirely with HDL (16). To determine which domains in apoB100 are involved in the interaction of LDL with PAF acetylhydrolase, we analyzed plasma from transgenic mice expressing human apoB100, apoB90, or apoB80. The endogenous mouse PAF acetylhydrolase was also observed with plasma samples from all of the human apoB transgenic mice (which have high levels of LDL), indicating that the endogenous PAF acetylhydrolase does not bind to LDL particles containing either full-length human apoB100 or the truncated human apoB proteins. This result is consistent with our in vitro studies on the association of mouse PAF acetylhydrolase with human LDL (Fig. 2).

To evaluate binding of human PAF acetylhydrolase to LDL produced by transgenic mice, we inactivated the endogenous, LDL-associated PAF acetylhydrolase activity in the plasma samples by pretreatment with DFP. The pretreated plasma samples were then combined with purified recombinant human PAF acetylhydrolase or with solubilized extracts from bacteria overexpressing the human enzyme. After a 2-h incubation at 37 °C, the lipoproteins were fractionated by ultracentrifugation, and each fraction was assayed for enzymatic activity. Human plasma pretreated with DFP served as the control source of acceptor lipid particles. When plasma derived from transgenic mice overexpressing human apoB100 was used as the acceptor, PAF acetylhydrolase associated with LDL and HDL (Fig. 6B) in a manner virtually identical to that observed with human plasma (Fig. 6A). In contrast, when samples from control mice (not shown) or mice expressing human apoB90 or human apoB80 were used as the source of lipoprotein acceptor particles, PAF acetylhydrolase associated exclusively with HDL (Fig. 6, C and D, respectively). Identical results were obtained when the plasma lipoproteins were separated by chromatography on Superose 6 columns (not shown). These results indicate that the carboxyl-terminal portion of apoB100 is required for binding of PAF acetylhydrolase to LDL.

DISCUSSION

This study demonstrates that the interaction between human PAF acetylhydrolase and human LDL is likely to involve protein–protein interactions between specific residues in the plasma PAF acetylhydrolase molecule and residues in apoB100, the principal apolipoprotein of LDL. Mutation of tyrosine 205, which is completely conserved among species (22),
abolished binding to LDL. None of the other tyrosine residues seem to be required for binding, because replacing them with alanines did not affect the ability of the recombinant proteins to associate with LDL. The fact that mutating residues near tyrosine 205 did not affect binding to LDL suggests that the effect observed is specific to tyrosine 205 and not simply a consequence of conformational changes in that region of the PAF acetylhydrolase molecule. It is also possible that tyrosine 205 interacts with a distal site within PAF acetylhydrolase, inducing or stabilizing a conformation necessary for binding to LDL. The inability of mouse PAF acetylhydrolase to bind human LDL despite the presence of tyrosine 205 is supportive of this possibility.

Several findings suggest that a region centered on tryptophan 115 and leucine 116 is also involved in the interaction between PAF acetylhydrolase and LDL. First, replacement of these residues with alanines abolished binding to LDL. Second, deletion of these residues also prevented association with the lipoprotein. Third, introduction of these residues into the wild-type mouse PAF acetylhydrolase (which normally does not associate with LDL) resulted in a mutant mouse enzyme that bound to LDL almost as efficiently as the wild-type human enzyme.

The observation that tyrosine 205 as well as tryptophan 115 and leucine 116 are necessary for binding to LDL suggests that these two domains might have adjacent locations within the three-dimensional structure of the plasma form of PAF acetylhydrolase. However, an x-ray crystallographic analysis of secreted PAF acetylhydrolase is not yet available, and there is no homology of these domains to other PAF acetylhydrolases or lipases whose three-dimensional structure is known (30, 31).

Our findings also suggest that the carboxyl terminus of human apoB100 is involved in binding to PAF acetylhydrolase. ApoB90 and apoB80 lacked the ability to bind to PAF acetylhydrolase, consistent with participation of the carboxyl terminus of apoB100 in the interaction with the enzyme. Our results, however, do not completely exclude the possibility that the structural features for the interaction with PAF acetylhydrolase are present within apoB80 and apoB90 but are not in the proper conformation for the interaction. For example, altered composition of apoB90-containing lipoproteins might change the conformation on the surface of the lipoprotein, affecting its ability to interact with PAF acetylhydrolase. However, additional evidence suggests that the carboxyl terminus of apoB100 and PAF acetylhydrolase interact directly (i.e. without the involvement of lipid components). In our early studies on the purification of PAF acetylhydrolase, a carboxyl-terminal fragment of apoB100 (including apoB100 residues 4119–4536) co-purified with PAF acetylhydrolase, suggesting that a tight interaction exists between the enzyme and this domain of apoB100 (28).

Interestingly, the mutant LDL containing the human–mouse hybrid apoB bound to PAF acetylhydrolase normally, sug-
suggesting that apoB100 residues 4279–4536 may not be critical for the interaction between PAF acetylhydrolase and human apoB100.2 These results, taken together with our earlier studies (28) and the current studies on human apoB90 and human apoB80, suggest the possibility that human apoB100 amino acids 4119–4279 could be important for binding to PAF acetylhydrolase. That possibility will be tested in future studies.

It is interesting that mice, which have very low plasma concentrations of LDL, also synthesize a PAF acetylhydrolase that is intrinsically defective in its ability to bind to LDL. It is tempting to speculate that these two features (low LDL levels and a “nonbinding” PAF acetylhydrolase) may have arisen together during mammalian evolution; it seems possible that the extremely low levels of LDL in mouse plasma may have obviated any selective pressure to have a PAF acetylhydrolase that binds to apoB100. In any case, the nonbinding mouse PAF acetylhydrolase has provided us with a useful experimental tool, the ability to analyze the binding of human PAF acetylhydrolase to human apoB proteins without having to worry about interference from the endogenous mouse protein. In humans, LDL-associated PAF acetylhydrolase plays a key role in the clearance of PAF and PAF analogs from the plasma, while the HDL-associated enzyme is less important (15). In mice, the absence of LDL-associated PAF acetylhydrolase appears to be compensated for by the HDL-associated enzyme; this could explain why the total serum PAF acetylhydrolase activity is approximately 5 times higher in mice than in humans (32).

Acknowledgments—We are indebted to Dr. Guy A. Zimmerman for insightful comments and critical review of this manuscript. We thank Drs. T. L. Innerarity and J. Boren for providing human apoB80 transgenic mice. We are grateful to Dr. Massimiliano Zaniboni for performing area calculations, Diana Lim for expert assistance on figure preparation, and Connie Zlot for technical assistance. We also thank the DNA Sequencing and Peptide/Oligo DNA Synthesis Core Facilities at the University of Utah, which are supported by National Institutes of Health Grant CA42014.

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