Structural Domains of Human Apolipoprotein B-100

Differential Accessibility to Limited Proteolysis of B-100 in Low Density and Very Low Density Lipoproteins

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Human apolipoprotein B-100 is one of the largest polypeptides known, consisting of 4536 amino acid residues as deduced from cDNA clones (1-3). Moreover, B-100 is the sole protein component of low density lipoproteins (LDL)

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* The abbreviations used are: LDL, low density lipoproteins; VLDL, very low density lipoproteins; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.
and the conformational changes of B-100 that accompany the conversion of VLDL to LDL, we first investigated whether exposed flexible peptide regions that are highly accessible to limited proteolysis (16) by various endoproteases exist in B-100 of LDL and then determined whether such peptide regions are also accessible to limited proteolysis in the conformation adopted by B-100 in VLDL.

EXPERIMENTAL PROCEDURES

Isolation of VLDL and LDL—Human VLDL (ρ = 1.006 g/ml) and LDL (ρ = 1.025-1.055 g/ml) were isolated from the plasma of normolipemic fasting donors by sequential ultracentrifugation in a 40.3 rotor at 12 °C for 18 h at 36,000 rpm (17). Solvent densities were adjusted with KBr. To prevent degradation of lipoproteins, benzamidine (0.3 mg/ml), phenylmethylsulfonyl fluoride (0.17 mg/ml), N-ethylmaleimide (1.3 mg/ml), and α-macroglobulin (2.5 mg/ml) were added to the plasma. In addition, NaN₃ (0.05%), EDTA (0.04%, pH 7.5), and gentamicin (0.05 mg/ml) were also added to the plasma and to all centrifugal solutions. The isolated LDL and VLDL were dialyzed extensively at 4 °C against 0.15 M NaCl containing 0.05% NaN₃ and 0.04% EDTA (pH 7.5). The concentration of total protein was determined by the method of Lowry et al. (18). ApoB was determined by its insolubility in 4.2 M urea, and apolipoprotein concentrations were estimated from the approximate molecular weight determined by its relative mobilities in 4-15% SDS-PAGE (20). Prior to electrophoresis, the proteolytic digests of LDL or VLDL were combined with SDS, glycerol, discontinuous buffer system (20). Prior to electrophoresis, the proteolytic digests of LDL or VLDL were combined with SDS, glycerol, discontinuous buffer system (20). The enzyme reaction was stopped, and samples (15 μg) were treated with 2-mercaptoethanol (2%) prior to SDS-PAGE (4-7%), as described elsewhere (22).

Peptide Mapping by Analytical SDS-PAGE—Analytical SDS-PAGE was performed on 4-7% linear gradient slab gels and then determined by the method of Lowry et al. (18). ApoB, determined by its insolubility in 4.2 M urea, and apolipoprotein concentrations were estimated from the approximate molecular weight determined by its relative mobilities in 4-15% SDS-PAGE (20). Prior to electrophoresis, the proteolytic digests of LDL or VLDL were combined with SDS, glycerol, bromophenol blue, with or without 2-mercaptoethanol and thioglycolic acid to final concentrations of 2.5, 10, 0.003, 2, and 0.4%, respectively; and the mixtures were then boiled for 1 min. Gels were stained with Coomassie Brilliant Blue R-250.

Isolation and Sequence Analysis of Proteolytic Fragments of B-100—To isolate B-100 fragments for NH₂-terminal sequence analysis, proteolytic digests of LDL and VLDL were separated by SDS-PAGE on linear gradient slab gels and then electroblotted onto polyvinylidene difluoride membranes (Millipore Corp.) (21). Polypeptides transferred onto polyvinylidene difluoride membranes were incubated with Coomassie Brilliant Blue R-250, and the bands were cut out. Sequence analysis of the isolated proteolytic fragments on polyvinylidene difluoride membranes was performed on an Applied Biosystems Model 470A protein microsequencer (22).

Immunoblotting—Antisera against sequence-specific synthetic peptides of B-100 (residues 259-280, 890-908, 2404-2425, 3492-3511, and 3958-3971) raised in rabbits (23, 24) were used to identify the original positions of proteolytic fragments in the B-100 molecule by immunoblotting. Polypeptides were transferred to nitrocellulose filters following SDS-PAGE, as described previously (25). The antisera were the gift of Dr. A. A. Protter (California Biotechnology, Inc.) and Drs. S. G. Young and T. L. Innerarity (Gladstone Foundation Laboratories, San Francisco).

RESULTS

Limited Proteolysis of B-100 in LDL and Identification of Proteolytic Fragments—To investigate whether exposed peptide regions that are highly susceptible to proteolytic cleavage exist in B-100 of LDL, limited proteolysis was performed on LDL with 12 enzymes of various specificities. Under the conditions used, only a limited number of characteristic fragments were generated from B-100 by each of the enzymes, as shown by the SDS-PAGE patterns (Fig. 1). Note that the presence of 10 mM CaCl₂ slightly retarded the electrophoretic mobilities of the fragments (lanes 7-12). Digestion with pepsin appeared to result in more extensive degradation of B-100 (lane 13). More limited degradation was observed at a lower ratio of enzyme to substrate. The major bands generated by digestion of B-100 by various enzymes appeared to have relative mobilities similar to the known B-100 fragments, namely B-74 (K1), T1, T3, T2, and B-26 (K2 or T4) (top to bottom).

In order to locate the sites of cleavage in B-100 by these enzymes, the next step was to isolate and characterize as many fragments as possible. Two approaches were used to identify the proteolytic fragments. First, fragments were isolated, and their NH₂-terminal sequences were determined. In addition, the COOH-terminal ends of some of these fragments were estimated from the approximate molecular weight determined by mobility on SDS-PAGE gel and the enzyme specificity. Alternatively, fragments were identified by means of immunoblotting using antisera raised against sequence-spe-
cific synthetic peptides of B-100. The results of these analyses are summarized in Fig. 2.

Fifteen fragments, were identified by NH$_2$-terminal sequence analysis. Three fragments of approximate molecular mass similar to B-26 (145 kDa) (9, 10), generated by clostridin, dispase, and S. aureus V8 protease, respectively, and one ~98-kDa fragment, generated by pepsin, were determined to contain the NH$_2$ terminus of B-100. Four fragments of approximate molecular mass similar to T3 (238 kDa), generated by S. aureus V8 protease, clostridin, endoprotease Lys-C, and pepsin, respectively, were determined to have residues 1288, 1291, 1298, and 1319, respectively, as their NH$_2$ termini. Another fragment of ~140 kDa, generated by pepsin, was determined to have residue 1308 as its NH$_2$ terminus. Six fragments of approximate molecular mass similar to T2 (170 kDa), generated by clostridin, S. aureus V8 protease, thermolysin, trypsin, endoprotease Lys-C, and pepsin, respectively, were determined to have residues 3186, 3199, 3201 (for two fragments), 3206, and 3278, respectively, as their NH$_2$ termini. These results clearly demonstrate that B-100 in LDL contains two regions that are highly susceptible to cleavage by a variety of endoproteases. These two regions appear to connect three segments that are relatively resistant to limited proteolysis. One of these susceptible regions encompasses residues 1280–1320 (designated the NH$_2$-terminal region), and the other region encompasses residues 3180–3280 (designated the COOH-terminal region). Note that the two cleavage sites (located after Lys-1297 and Lys-3249) reported for both kallikrein and thrombin are also contained within these regions of high susceptibility to proteolysis.

The remaining B-100 fragments generated by other enzymes appeared to have molecular masses comparable to the identified fragments. Results of immunoblotting with sequence-specific peptide antisera indicated their relative locations in the B-100 molecule (data not shown), suggesting that the two highly susceptible peptide regions also contained their cleavage sites. Additional evidence for the positioning of the fragments in the B-100 molecule was obtained from studies of disulfide linkages (see below, Fig. 6).

The time course of digestion of B-100 in LDL revealed a difference in the accessibility to limited proteolysis by various enzymes between the two highly susceptible peptide regions (Figs. 3 and 4). The NH$_2$-terminal region appeared to be the preferential cleavage site for submaxillaris protease (Fig. 3B) and kallikrein (data not shown), each yielding initially two fragments with approximate molecular masses of 145 and 410 kDa.

**FIG. 2.** Cleavage sites resulting from limited proteolysis of B-100 by various endoproteases and location of sequence-specific peptide antisera and some cysteine residues. The long horizontal line represents the B-100 molecule. Above the horizontal line, the roman numbers designate the NH$_2$-terminal residues of five synthetic peptides used to raise the antisera, and the italic numbers represent the positions of 8 cysteine residues. After limited proteolysis of B-100 in LDL or VLDL, the cleavage products were first separated by SDS-PAGE and then electroblotted onto polyvinylidene difluoride membranes and analyzed by NH$_2$-terminal sequencing, as described under "Experimental Procedures." The numbers in parentheses designate the NH$_2$-terminal residues of the proteolytic fragments generated by the individual enzymes listed in front of it: CL, clostridin; DS, dispase; LC, endoprotease Lys-C; PE, pepsin; SP, S. aureus V8 protease; TH, thermolysin; TR, trypsin. The cleavage sites for kallikrein (KL) and thrombin (TB) digestions of B-100 in LDL (1) and their cleavage products (B-26, T3, and T2) are also shown.

**FIG. 3.** Time course of digestion of B-100 in LDL by various enzymes. LDL (0.5 mg/ml of B-100) in 0.15 M NaCl, 0.125 M Tris-HCl, 0.04% EDTA, and 0.05% NaN$_3$ (pH 8.4) were incubated with the indicated enzymes at 37 °C. After the addition of enzymes, aliquots were removed at timed intervals, the reaction was stopped, and samples (15 μg) were treated with 2-mercaptoethanol (2%) and thioglycolic acid (0.4%) prior to SDS-PAGE, as described under "Experimental Procedures." In A–F, 0 indicates no enzyme addition, and the other numbers indicate time points of incubation in minutes. A, dispase (ES = 1:190); B, submaxillaris protease (ES = 1:40); C, endoprotease Lys-C (ES = 1:370); D, trypsin (ES = 1:1500); E, chymotrypsin (ES = 1:1500); F, thermolysin (ES = 1:1500). The mobilities of known B-100 fragments and their apparent molecular masses (in kilodaltons) are indicated. The arrow in D indicates the additional tryptic fragment.

**FIG. 4.** Effect of reduction of disulfide bonds on time course of digestion of B-100 in LDL. LDL (0.45 mg/ml of B-100) were incubated with S. aureus V8 protease (ES = 1:375) (A) and clostridin (ES = 1:375) (B) and analyzed by SDS-PAGE, as described in the legend to Fig. 3. In A and B, lane 0 indicates no enzyme addition, lanes 1–5 represent native (unreduced) LDL, and lanes 6–10 represent reduced LDL (pretreated with 15 mM dithiothreitol at room temperature for 2 h). In each time course, aliquots of sample were removed at 15, 30, 60, 120, and 180 min (lanes 1–5 and 6–10, respectively). The arrows indicate additional bands released from the reduced LDL samples. The mobilities of known B-100 fragments and their apparent molecular masses (in kilodaltons) are indicated in the left margins, and two molecular mass standards are indicated in the right margin of A.
kDa, whereas the COOH-terminal region appeared to be the preferential cleavage site for endoprotease Lys-C, chymotrypsin (Fig. 3, C and E, respectively), and thrombin (data not shown), each yielding initially two fragments with approximate molecular masses of 170 and 385 kDa. In addition, both NH2-terminal and COOH-terminal cleavage sites appeared to be readily accessible to digestion by dispase, trypsin, thermolysin, and S. aureus V8 protease, and clostripain (Fig. 4, A and B, lanes 1–5, respectively); but the COOH-terminal products (~170 kDa) appeared to be more abundant than the NH2-terminal products (~145 kDa) for trypsin and S. aureus V8 protease. Upon prolonged digestion, further degradation at either of the two cleavage sites yielded the middle-portion B-100 fragments with approximate molecular masses similar to T3 (238 kDa) for all enzymes used.

In addition to the major fragments mentioned so far, trypsin digestion (Fig. 3D) also produced a very prominent, relatively diffuse large band with an apparent molecular mass of ~310 kDa, as indicated by the arrow. A few minor smaller fragments (~90–120 kDa) were also produced by chymotrypsin (Fig. 3E) and S. aureus V8 protease (Fig. 4A), indicating the presence of additional cleavage sites for these enzymes.

There are 25 cysteine residues in B-100 (1,2). To investigate the effect of disulfide linkages on the accessibility of cleavage sites, LDL samples were reduced with dithiothreitol before the addition of enzymes. Clostripain and S. aureus V8 protease, which proved to be functional in the presence of the reducing agent, were used for these studies. Pretreatment with the reducing agent resulted in some notable changes in the cleavage patterns of B-100 in LDL generated by these enzymes (Fig. 4). Within 30 min of digestion, three major bands of B-100 fragments were apparently released from native LDL (lanes 1 and 2), whereas five major and one minor band of B-100 fragments were released from reduced LDL (lanes 6 and 7), suggesting that more cleavage sites became exposed after the disulfide bonds were reduced. The three additional bands, as indicated by arrows, appeared to have approximate molecular masses of 105, 275, and 450 kDa, respectively. In the case of native (unreduced) LDL incubated with S. aureus V8 protease, all the cleavage products of B-100 became progressively more intense with time (Fig. 4A, lanes 1–5). In the case of reduced LDL, by contrast, the NH2-terminal product (~145 kDa) was only a minor fine band at every time point; the middle-portion product (~238 kDa) appeared less intense than that from native LDL; and the three additional bands became the major cleavage products (lanes 6–10). The effect of pretreatment with the reducing agent was less pronounced on the production of the NH2-terminal fragment (~145 kDa) by clostripain, which became slightly more prominent with time (Fig. 4B, lanes 6–10). Moreover, the rate of B-100 degradation increased in reduced LDL. For example, at the 180-min time point, a large amount of intact B-100 still remained in the digestion of native LDL (lane 5), but substantially less intact B-100 remained in the case of reduced LDL (lane 10). Nevertheless, the cleavage patterns still showed a limited number of characteristic B-100 fragments generated from reduced LDL samples by the enzymes used.

The NH2-terminal residues of the apparent 105-kDa fragments generated by S. aureus V8 protease and clostripain were determined to be Asn-7 and Glu-1, respectively. Furthermore, immunoblotting showed that sequence-specific peptide antisera 259–280 and 890–908 reacted strongly with both fragments (Fig. 5). These results indicated that both of the 105-kDa fragments arose from the NH2-terminal portion of B-100. Based on the enzyme specificities and assuming a mean residue M, of 112, the COOH termini of these two 105-kDa fragments were predicted to lie at Gln-924 or Glu-931 (S. aureus V8 protease) and Lys-922 or Arg-933 (clostripain), respectively. The two other additional bands produced from reduced LDL (~275 and ~450 kDa) each reacted with peptide antisera 2404–2425 (Fig. 5). The 450-kDa band also reacted with antisera 3492–3511 (see below, Fig. 8) and 3958–3971 (data not shown). These results indicated that the 450-kDa band corresponded to the COOH-terminal fragment of B-100 and that the 275-kDa band corresponded to the middle portion.

Comparison of Cleavage Patterns of B-100 Fragments Generated by Limited Proteolysis of LDL and VLDL—To ascertain whether the two highly susceptible peptide regions identified in B-100 of LDL were also accessible to proteolytic cleavages in the conformation adopted by B-100 in VLDL, limited proteolysis was performed on both native (unreduced) LDL and VLDL with 12 enzymes of various specificities. As shown in Fig. 6, the cleavage patterns of the B-100 fragments revealed significant differences in susceptibility to limited proteolysis between LDL B-100 and VLDL B-100. Note that the presence of 10 mM CaCl2 (lanes 5–8) slightly retarded the electrophoretic mobilities of the fragments. Under the digestion conditions used, B-100 in VLDL, like B-100 in LDL, was cleaved into only a limited number of characteristic fragments by each of the enzymes, as shown by SDS-PAGE performed in the presence of reducing agent (4% 2-mercaptoethanol) (Fig. 6A). However, B-100 in VLDL appeared to be less degraded than B-100 in LDL by all enzymes used. One noteworthy difference between the cleavage patterns of LDL B-100 and VLDL B-100 was the absence or very faint appearance of two major B-100 fragments in the digests of VLDL (Fig. 6A, denoted by V, lanes 1, 3, and 5–7), namely the ~145- and ~238-kDa fragments observed in the digests of LDL, which had been identified as the NH2-terminal portion and the middle portion of the B-100 molecule, respectively, as described above. Thus, the prominent band of approximate molecular mass similar to T2 (170 kDa), generated from VLDL B-100 by all enzymes, might be the COOH-terminal portion of B-100. Accordingly, NH2-terminal sequencing and immunoblotting with sequence-specific peptide antisera were performed to identify them.

Two such fragments (~170 kDa) of VLDL B-100, generated
For example, in the digests of VLDL, two prominent B-100 fragments with approximate molecular masses similar to T1 (385 kDa) and T2 (170 kDa), generated by various enzymes, appeared on SDS-PAGE performed under reducing conditions (Fig. 6A, denoted by V, lanes 1–3 and 5–7). However, when SDS-PAGE was performed in the absence of reducing agent, these two fragments were no longer visible (Fig. 6B), indicating that they were cross-linked by a disulfide bond. Similarly, in the digests of LDL, the two fragments representing the COOH-terminal (~170 kDa) and central (~238 kDa) fragments of B-100 were absent in the unreduced SDS-PAGE patterns. In addition, the intensity of staining of the largest B-100 fragments (~385–410 kDa) decreased markedly. On the other hand, two major fragments representing the NH₂-terminal fragment (~145 kDa) and its complementary fragment still remained in the unreduced SDS-PAGE patterns of LDL digests (Fig. 6B, denoted by L, lanes 1, 3, and 5–8), in contrast to the very faint bands present in the unreduced SDS-PAGE patterns of VLDL digests. The appearance of the faint bands in some VLDL digests (Fig. 6B, denoted by V, lanes 3, 5, and 6) may indicate more sites for degradation of B-100 in VLDL than in LDL. One exception is thrombin-treated LDL B-100 (Fig. 6, lane 2) in that both B-100 fragments (T1 and T2) disappeared because they are products from the COOH-terminal cleavage site and hence are cross-linked. Thus, of the five possible cleavage products of LDL B-100 generated at both the NH₂- and COOH-terminal cleavage sites, three could be disulfide-linked, namely the COOH-terminal fragment cross-linking with either the rest of the B-100 molecule or its degraded middle-portion fragment. Disulfide bonds linking various B-100 fragments were also apparent in pepsin-treated LDL B-100 and VLDL B-100 (Fig. 6, A and B, lane 4), although the patterns appeared more complicated. On the other hand, the 105-kDa fragment, generated by S. aureus V8 protease (lane 3), from native (unreduced) LDL, as indicated by the arrow, and the other tryptic fragments (lane 7) produced from both LDL and VLDL were not linked by disulfide bonds.

Taken together, these results provided further evidence that cleavage sites in both the NH₂- and COOH-terminal susceptible peptide regions were readily accessible to limited proteolysis in LDL B-100, but that only those in the COOH-terminal region were readily accessible in VLDL B-100. Upon prolonged digestion of VLDL B-100, a cleavage product (~238 kDa) was also released from the NH₂-terminal cleavage site, as shown in a representative time course of digestion of B-100 in VLDL by S. aureus V8 protease (Fig. 7A, lanes 5 and 6); but the rate of digestion appeared slower for VLDL B-100

![Fig. 6. Comparison of SDS-PAGE patterns of B-100 fragments generated from native (unreduced) LDL and VLDL by various endopeptidases.](image)

![Fig. 7. Effect of reduction of disulfide bonds on time course of digestion of B-100 in VLDL as compared with that in LDL by S. aureus V8 protease.](image)
As described above (Fig. 4), the cleavage patterns of LDL B-100 generated by S. aureus V8 protease and clostripain were greatly influenced by pretreatment with a disulfide reducing agent. To ascertain whether VLDL B-100 would also be affected, the same experiment was performed on VLDL. As shown in Fig. 7, the effect of reduction with dithiothreitol on VLDL B-100 (Fig. 7C) was very similar to that on LDL B-100 (Fig. 7D), resulting in the release of three additional bands with approximate molecular masses of 105, 275, and 450 kDa, respectively, as indicated by arrows. Nevertheless, the rate of digestion was much slower for reduced VLDL B-100 than for reduced LDL B-100. Results of immunoblotting with peptide antisera (residues 259–280) (Fig. 257, 258) showed that the 105-kDa fragment generated from reduced VLDL B-100 was identical to that from LDL B-100, corresponding to the NH2-terminal portion of B-100. Thus, reduction of disulfide bonds exposed NH2-terminal cleavage sites of VLDL B-100. Immunoblotting with antisera 2404–2425 indicated that similar additional cleavage fragments were generated by S. aureus V8 protease from both reduced LDL B-100 and reduced VLDL B-100. In addition, immunoblotting with antisera 3492–3511 revealed increased production of COOH-terminal fragments of ~170 kDa, indicating that reduction of disulfide bonds also promoted cleavage in the COOH-terminal susceptible region.

**DISCUSSION**

In this study, two peptide regions that are highly susceptible to proteolytic cleavage, an NH2-terminal region (spanning residues 1280–1320) and a COOH-terminal region (spanning residues 3180–3280), were identified in B-100 of LDL by characterizing the cleavage products generated by limited proteolysis with 12 endoproteases of widely different specificities. Although numerous potential cleavage sites could be expected from the amino acid sequence of B-100, these two peptide regions appear to contain all of the readily accessible cleavage sites for the enzymes used, strongly suggesting that these two regions must be relatively exposed on the surface of LDL. These regions are located within two of the four proline-rich clusters which may participate in β-turn formation (1). Unlike LDL, only one of the two peptide regions, namely the COOH-terminal region, appears to be readily accessible to limited proteolysis by the same battery of enzymes in B-100 of VLDL. This suggests that conformational or environmental differences affect the spatial arrangement of the NH2-terminal peptide region of B-100 in VLDL, rendering the potential cleavage sites inaccessible to limited proteolysis. This seems to explain the observation that the complementary B-26 and B-74 fragments of B-100 are present in the LDL of some individuals, but are not found in VLDL (9). These results indicate the presence of three principal structural domains in B-100 of LDL that are relatively resistant to proteolytic cleavage. These three domains are connected by the two susceptible peptide regions. The results also demonstrate differential accessibility of cleavage sites in B-100 of LDL and VLDL to limited proteolysis. Although proteolytic enzymes can unequivocally identify peptide regions in B-100 that are accessible to proteolytic cleavage, the approach we used cannot differentiate between potential causes of inaccessibility. For example, peptide bonds may be inaccessible to cleavage because they are buried within the folded structures of the protein itself or because they are embedded in lipids. In addition, lipid-protein interactions may induce conformational changes that render the peptide bonds inaccessible.

The two identified NH2- and COOH-terminal susceptible peptide regions in B-100 of LDL exhibited differential accessibility to various enzymes, indicating effects of both chemical environment (sequences neighboring the cleavable bond) and the folding of the surrounding residues (16). For example, within the NH2-terminal susceptible region (about 40 residues in length), three enzymes (endoprotease Lys-C, kallikrein, and thrombin) cleaved at a single site (Lys-1297). Within the COOH-terminal susceptible region (about 100 residues in length), kallikrein and thrombin also cleaved at a single site (Lys-3249), whereas endoprotease Lys-C cleaved at a site 44 residues away (Lys-3205) despite the presence of a cluster of other potentially cleavable sites containing lysine residues in the sequence. These results indicate that specific conformations are required to interact with the active site of each enzyme. Although the COOH-terminal susceptible region comprises a peptide segment more than twice the length of the NH2-terminal susceptible region, its folded structure could actually be as compact as the NH2-terminal region due to the presence of a disulfide linkage between the COOH-terminal and middle-portion fragments. The cysteine residues at positions 3167 and 3297 have been reported to be cross-linked,2 which would span the COOH-terminal susceptible region. Thus, it is likely that they form the disulfide bond linking the B-100 fragments generated from the COOH-terminal cleavage sites by various enzymes. One noteworthy feature of the COOH-terminal susceptible region is the presence of an N-linked glycosylated asparagine at position 3197 (2) which is contained in the COOH-terminal fragment generated by clostripain (cleavage site at Arg-3186). The presence of carbohydrate in this fragment presumably accounts for a greater mass, estimated from SDS-PAGE patterns, than would be predicted from the peptide mass alone.

The observation that reduction of disulfide bonds before proteolysis by S. aureus V8 protease and clostripain not only exposed additional cleavage sites but also increased the rate of B-100 degradation suggests that the breaking of disulfide bonds may result in the unfolding of the structural domains. For example, the rapid release of the prominent 105-kDa fragments upon reduction appears to result from unfolding in the region of residues 920–950. Support for this possibility is that the cysteine residues at positions 939 and 949 are cross-linked, as reported by Yang et al. (26) at the completion of this work. Our data in Fig. 6 indicate that the 105-kDa fragments, generated from cleavage sites in this susceptible region (residues 920–935), as well as the larger fragments (~145 and ~410 kDa), generated from cleavage sites in the NH2-terminal susceptible region (residues 1280–1320), were not linked by disulfide bond. However, the presence of 6 cysteine residues in the vicinity of this sequence (as indicated by italic numbers in Fig. 2) suggests that these cysteine

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2 C. H. Spilman et al., unpublished data in Ref. 1.
residues probably exert important conformational constraints on the NH2-terminal susceptible regions in B-100 of both LDL and VLDL. The observation that the rate of digestion for reduced VLDL B-100 was slower than for reduced LDL B-100 suggests that other environmental factors also affect accessibility for limited proteolysis. This peptide segment of about 400 amino acids (residues 920-1320), defined by these proteolytically susceptible regions, appears to be consistent with the previously reported B-100 domain in LDL between residues 1001 and 1360 which consists of alternating trypsin-accessible and -inaccessible peptides (2).

The differential accessibility of cleavage sites in B-100 of LDL and VLDL suggests that there are substantial changes in the conformation of B-100 as VLDL are transformed into LDL. This is consistent with observations of different reactivity of polyclonal and monoclonal antibodies against B-100 with lipoprotein particles at different levels of the VLDL catabolic cascade. Despite our observation that the most striking differences are seen in the NH2-terminal susceptible peptide region of B-100 between LDL and VLDL, subtle differences detectable in the COOH-terminal susceptible region with a battery of endoproteases suggest that the conformation of B-100 also changes in the COOH-terminal peptide region, which is in the vicinity of the putative LDL receptor-binding domains (1, 2). This subtle change in conformation may correlate with the differential expression of LDL receptor-binding domains of B-100 in LDL and VLDL.

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