Carboxyl-Terminal Truncation of Apolipoprotein B Results in Gradual Loss of the Ability To Form Buoyant Lipoproteins in Cultured Human and Rat Liver Cell Lines

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ABSTRACT: Apolipoprotein B has an obligatory role in the production of chylomicrons, VLDL, and LDL. Familial hypobetalipoproteinemia is a codominant disorder characterized by reduced levels of apo B containing lipoproteins in plasma. We have previously described mutations of the apo B gene in persons with hypobetalipoproteinemia that predict truncated forms of apo B designated apo B29 (1305 amino acid residues) and apo B39 (1799 residues). Apo B39 was present in the VLDL and LDL fractions of plasma, but apo B29 was not detected in the lipoprotein or infranatant fractions of plasma. Here we have investigated the regions of apo B necessary for apo B containing lipoprotein secretion by expression of constructs designed to express truncated forms of apo B. Apo B13 (583 residues), apo B17 (784 residues), apo B23 (1084 residues), apo B29 (1306 residues), and apo B41 (1880 residues) were transiently expressed in HepG2 cells, and apo B23 and apo B41 were stably expressed in McArdle 7777 cells. Lipoprotein ($d < 1.25$ g/mL) and infranatant ($d > 1.25$ g/mL) fractions of conditioned medium were analyzed by immunoprecipitation and SDS-PAGE. The distribution between lipoprotein and infranatant fractions varied: apo B41 was found solely in the lipoprotein fraction; apo B29, apo B23, and apo B17 were present in both fractions, but with stepwise truncation, progressively more apo B was recovered in the infranatant; apo B13 was only in the infranatant. These results demonstrate that deletion from the carboxyl terminal of apo B41 results in a gradual loss of the ability of the truncated proteins to form buoyant lipoprotein particles.

Human apolipoprotein B100 is synthesized in the liver and is a major protein component of VLDL and of LDL. Apo B100 is uniquely equipped structurally for its role in hepatic lipoprotein assembly and secretion, and for the transport of cholesterol and triglyceride in the circulation. Lipid binding structures alternate with hydrophilic sequences throughout the 4536 amino acid length of apo B100 (Knott et al., 1986; Yang et al., 1986, 1989; Olofsson et al., 1987). These structures consist of short stretches of amphipathic β-strand and α-helix, much larger amphipathic α-helical domains and proline-rich amphipathic β-sheets, and short stretches of hydrophobic amino acids (Figure 1). In addition, apo B100 is covalently associated with fatty acid, and these linkages may also be associated with lipid binding (Hoeg et al., 1988; Huang et al., 1988). The lipid binding structures in apo B100 account for its insolubility in aqueous media, and inability to exchange between lipoprotein fractions in the circulation. Thus, after secretion from the liver as VLDL, apo B100 remains firmly associated with its core lipid, until it is cleared from the circulation, and unlike the smaller apolipoproteins does not exchange between lipoprotein particles. Apo B48 is synthesized in the small intestine and has an essential role in dietary lipid absorption and transport. Apo B48 is collinear with the amino-terminal 2152 amino acids residues of apo B100 and is produced from the same single-copy gene by editing of the apo B100 RNA transcript to create a translation stop at codon 2153 (Powell et al., 1987).

Familial hypobetalipoproteinemia is a rare autosomal codominant disorder caused by mutation of the apo B gene (Young et al., 1987a,b, 1988, 1989, 1990; Collins et al., 1988; Leppert et al., 1988; Ross et al., 1988; Huang et al., 1989; Krul et al., 1989). Homozygous individuals have very low levels of apo B containing lipoproteins in the circulation and may also have profound malabsorption of fat and fat-soluble vitamins. Fat-soluble vitamin deficiency can lead to spinocerebellar and retinal degeneration, and acanthocytosis. In the heterozygous state, affected persons are frequently asymptomatic, but have LDL cholesterol levels less than half of normal. In persons with hypobetalipoproteinemia, truncated variants of apo B designated apo B31 (1425 amino acid residues) (Young et al., 1990), apo B37 (1728 residues) (Young et al., 1987a,b, 1988), apo B39 (1799 residues) (Collins et al., 1988), apo B40 (~1814 residues) (Krul et al., 1989), apo B46 (2057 residues) (Young et al., 1989), and apo B90 (4082 residues) (Krul et al., 1989) on the centile system have been found in plasma lipoproteins at reduced levels compared to apo B100. Apo B31 can also be detected in the infranatant fraction of plasma (Young et al., 1990). Additional persons with hypobetalipoproteinemia have been described, one with a point mutation in the apo B gene, which predicts apo B29[1] (1305 residue) (Collins et al., 1988), and another with a 694 bp deletion, which predicts apo B25 (1085 residues) (Huang et al., 1989). Neither apo B29 nor apo B25 was detected in the lipoprotein or nonlipoprotein fraction of plasma (Collins et al., 1988; Huang et al., 1989). These truncated variants are caused by frame shift or point mutations in the apo B gene.

The mechanism by which truncated variants of apo B cause hypobetalipoproteinemia remains unclear. It is possible that their rate of secretion is reduced relative to apo B100 or that

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[1] Apo B29 was previously described as apo B26 (Collins et al., 1988). This predicted protein is just larger than the amino-terminal thrombin cleavage product of apo B100 at amino acid residue 1297, which has 26% of the size of apo B100 on SDS-PAGE.

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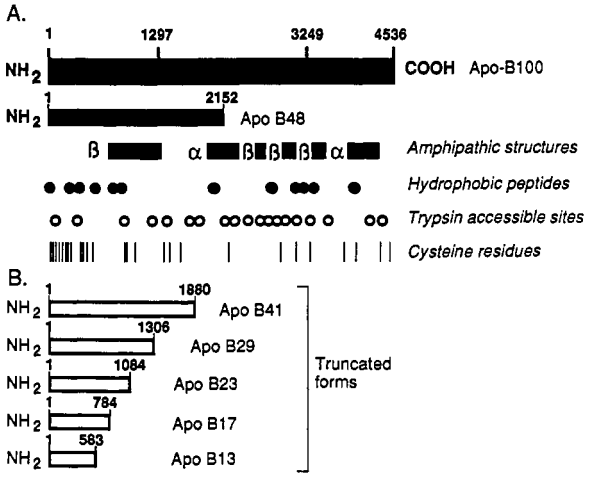

FIGURE 1: Apo B100 and apo B48 and carboxyl-terminally truncated apo B constructs. (A) The principal lipid binding structures in apo B are shown. Fragments of apo B cDNA were subcloned into the eukaryotic expression vector PSV7d. (B) Predicted secreted product sizes are shown at the carboxyl terminal of each construct. Apo B 29 was previously described as apo B26 (Collins et al., 1988). This protein is just larger than the product of thrombin cleavage of apo B at residue 1297, which has 26% of the size of apo B100 on SDS–PAGE.

truncated apo B variants may be inadequate for the assembly of normal triglyceride-rich lipoproteins. Alternatively, short forms of apo B may be rapidly cleared from the circulation. To investigate the regions of apo B which are necessary for lipoprotein assembly and secretion, we have prepared a series of apo B cDNA constructs designed to express carboxy-terminally truncated forms of apo B. The expression in vitro of the short constructs has been studied after both transient and stable transfection into the human and rat liver tumor cell lines HepG2 and McArdle 7777, respectively.

Experimental Procedures

Plasmid Constructs. Fragments of apo B cDNA were subcloned from plasmids pABF and pA6c (Knott et al., 1986) into the eukaryotic expression vector pSV7d (Truett et al., 1985) which carries SV40 early promoter and polyadenylation signals. All constructs lack the first 14 nucleotides of the apo B 5' untranslated region. The apo B coding sequence extends as far as the *Eco*RV site at nucleotide 1947 in apo B13, the *Bam*HI site at nucleotide 2551 in apo B17, the *Taq*I site at nucleotide 4124 in apo B29, and the *Cla*I site at nucleotide 5847 in apo B41. Apo B23 was 3460 nucleotides and corresponded to the previously described cDNA A6C (Knott et al., 1986). Termination signals in three frames stop translation after the end of each cloned cDNA. The predicted mRNA and apo B primary translation product sizes for the constructs respectively are the following: apo B13, 2.3 kb, 583 residues; apo B17, 2.9 kb, 784 residues; apo B23, 3.8 kb, 1084 residues; apo B29, 4.5 kb, 1306 residues; apo B39, 6.2 kb, 1880 residues (Figure 1). Plasmid DNAs were prepared by the alkaline lysis method and banded twice in CsCl before transfection (Sambrook et al., 1989).

Cell Culture. HepG2 cells supplied by Dr. B. B. Knowles (Wistar Institute of Anatomy and Biology, Philadelphia, PA) and McArdle 7777 cells (McARH 7777, ATCC-CR1.1601) were grown in Dulbecco's modified Eagle's medium (DMEM)[2] supplemented with 10% heat-inactivated fetal calf serum/100 units/mL penicillin/100 μg/mL streptomycin/2 mM L-glutamine and nonessential amino acids as previously described (Pullinger et al., 1989).

Transfection. Cells were transfected by using the calcium phosphate precipitation method of Chen and Okayama (1987). Briefly, exponentially growing cells were trypsinized, seeded at 1×10^6 cells per 9-cm diameter plate, and incubated overnight in 10 mL of growth medium. Twenty micrograms of plasmid DNA was mixed with 0.5 mL of 0.25 M $CaCl_2$ and 0.5 mL of 2 × BES [50 mM 2-[bis(2-hydroxyethyl)amino]-ethanesulfonic acid (pH 6.95)/280 mM NaCl/1.5 mM Na_2HPO_4], and the mixture was incubated for 30 min at room temperature. The calcium phosphate/DNA solution (1 mL) was added dropwise to the plate of cells and incubated for 16–18 h at 37 °C under 2% CO_2. The medium was removed, and cells were incubated with 10 mL of growth medium for 8–24 h at 37 °C under 5% CO_2. McArdle 7777 cells were cotransfected with 2 μg of pSV2Neo (linearized with *Bam*HI) and 20 μg of apo B plasmid DNA (linearized with *Pvu*I) coding for apo B41 and apo B23. After selection for G418 resistance, individual colonies were grown and screened for expression of truncated apo B constructs first by Northern blotting as previously described (Pullinger et al., 1989) and subsequently by SDS–PAGE analysis of immunoprecipitated [^{35}S]methionine-labeled secretory proteins. Transfection efficiency was routinely 25% as assessed by using the β-galactosidase expression plasmid pCH110 (Pharmacia/PL), and was linear with increasing plasmid concentration up to 40 μg of DNA.

^{35}S Labeling. Transiently transfected HepG2 cells were allowed to recover for 24 h after transfection and then incubated with 1 mCi per 9-cm dish of >1000 Ci/mmol [^{35}S]-methionine (Amersham, U.K.) in 4 mL of serum-free, methionine-free growth medium supplemented with 50 μM methionine for 4 h. Protease inhibitors were added as described by Cardin et al. (1984), and the conditioned medium was removed and centrifuged at 1000*g* to remove cell debris. The mixture was dialyzed extensively against 150 mM NaCl/1 mM EDTA (pH 7.2) containing inhibitors at 4 °C to remove unincorporated [^{35}S]methionine.

Permanently transfected McArdle 7777 cells were labeled by using a pulse–chase protocol: cell monolayers were grown until 90% confluent on 9-cm plates, prepulsed for 2 h with 4 mL of methionine-free and serum-free DMEM, pulsed for 30 min with 4 mL of methionine-free and serum-free DMEM supplemented with 0.5 mCi of [^{35}S]methionine per plate, and then chased for 5 h with complete serum-free DMEM (Figures 3–5). The chase medium was treated exactly as described for HepG2 clutured medium, but was not dialyzed.

Lipoprotein Preparation. The culture medium density was adjusted to d = 1.25 g/mL with solid KBr and then centrifuged for ≥40 h at 150000g (Beckman 70.1 Ti rotor) at 15 °C (Havel et al., 1955). Lipoprotein and the entire infranatant fractions were dialyzed against 150 mM NaCl/1 mM EDTA (pH 7.2) at 4 °C and then concentrated by ultrafiltration using Centricon C30 cartridges (Amicon, Stonehouse, U.K.) to approximately 300–500 μL. For subfractionation into VLDL, LDL, and HDL, the chase media densities were adjusted to d = 1.006 g/mL, d = 1.063 g/mL, or d = 1.235 g/mL, respectively, using solid KBr as previously described (Collins et al., 1988).

Immunoprecipitation. [^{35}S]Methionine-labeled human apo B was immunoprecipitated with sheep anti-human apo B antiserum (Boehringer, FRG) essentially according to Bamberger and Lane (1988). This anti-human apo B antiserum was found

[2] Abbreviations: DMEM, Dulbecco's modified Eagle's medium; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

to immunoprecipitate rat apo B with comparable efficiency. Concentrated lipoprotein and infranatant fractions were added to 1 mL of immunoprecipitation buffer [1% w/v sodium deoxycholate/100 mM HEPES[1] (pH 7.5)/40 mM EDTA/150 mM NaCl/0.5% (w/v) fatty acid free BSA]; 100 μL of a 0.6% (w/v) suspension of protein A–Sepharoase in 150 mM NaCl/1% (w/v) sodium deoxycholate/0.7% (w/v) Nonidet P-40/50 mM HEPES (pH 7.5)/0.5% fatty acid free BSA was added, and samples were precleared by mixing for 1 h at room temperature. The protein A–Sepharose was removed by centrifugation, and 5 μL of anti-apo B antiserum was added to the supernatants which were incubated with gentle mixing for 2 h at room temperature; 100 μL of 0.6% (w/v) protein A–Sepharose suspension was added, and the tubes were incubated overnight at 4 °C with gentle mixing. The protein A–Sepharose pellets were washed 4 times with 1 mL of 150 mM NaCl/1% (w/v) sodium deoxycholate/0.1% (w/v) SDS/1% (v/v) Triton X-100/50 mM HEPES (pH 7.5)/0.5% (w/v) fatty acid free BSA and then once with phosphate-buffered saline. Quantitative recovery of apo B was verified by reprecipitating medium fractions and washes.

Electrophoresis and Autoradiography. Immunoprecipitates were denatured for 5 min at 100 °C in 0.5 M Tris-HCl (pH 6.8)/2% (w/v) SDS/20% (v/v) glycerol/10% (v/v) mercaptoethanol/1 mM EDTA/0.01% (w/v) bromophenol blue. ^{35}S-Labeled proteins were resolved by electrophoresis in 3–15% polyacrylamide gradient slab gels with a 3% stacking gel (Laemmli, 1970). Gels were fixed in 25% (v/v) methanol/10% (v/v) glacial acetic acid, impregnated with AMPLIFY autoradiography enhancer fluor (Amersham, U.K.), then dried, and exposed to Kodak X-AR5 film for 3–10 days at 70 °C.

Northern Blots. Total RNA from cells and Northern blots were prepared exactly as previously described (Pullinger et al., 1989). The probe was apo B13 cDNA.

Densitometer Scanning of Autoradiographs. Autoradiographs were scanned with a Shimadzu dual-wavelength scanner (Model CS-930). The results from scanning the [^{35}S]methionine-labeled apo B constructs were corrected for the number of methionine residues in the protein.

Results

Transient Expression in HepG2 Cells. To investigate the regions of apo B required for buoyant lipoprotein assembly and secretion, truncated constructs of apo B were transiently expressed in HepG2 human hepatoblastoma cells. HepG2 cells were cotransfected with equimolar amounts of the apo B41 plasmid and one of the other constructs, apo B29, apo B23, apo B17, or apo B13 (Figure 1). RNA was isolated from the cells after recovery and analyzed by Northern hybridization. After metabolic labeling with [^{35}S]methionine, total lipoprotein and infrantant fractions were prepared from conditioned medium by density gradient ultracentrifugation.

Apo B was immunoprecipitated from the lipoprotein ($d <$ 1.25 g/mL) and infranatant ($d >$ 1.25 g/mL) fractions and analyzed on SDS–polyacrylamide gels. The quantity of apo B secreted was estimated by densitometry and corrected for the number of methionine residues in the protein. The percentage of apo B species in the lipoprotein and infranatant fractions is given in parentheses and is the mean of data taken from two separate experiments. The amount of apo B100 secreted relative to the transient by expressed constructs was very low (Figure 2A). Apo B41 (100%), apo B29 (48%), and apo B23 (20%) were clearly visible in the lipoprotein fraction. A smaller amount of B17 (18%) was also discernible in the lipoproteins. Apo B13 was not detected in the lipoprotein fraction (Figure 2A). The infranatant fraction contained no

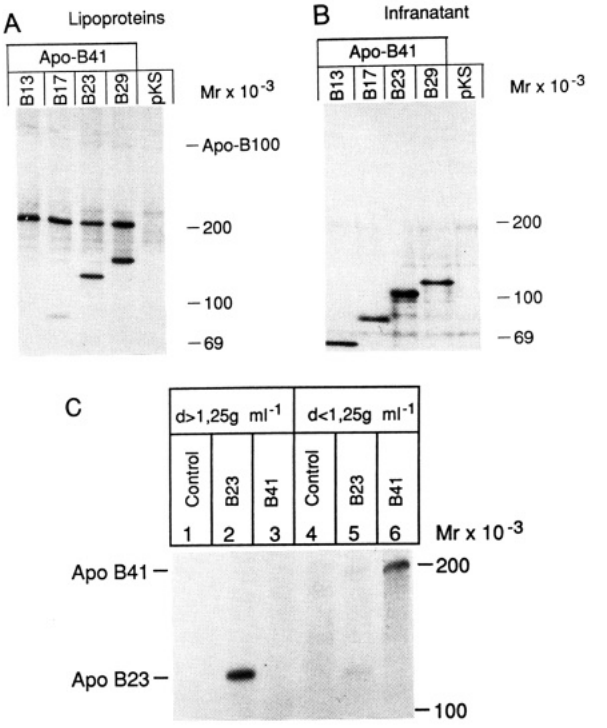

FIGURE 2: Secreted ^{35}S-labeled apo B. (A) Lipoprotein and (B) infranatant fractions of conditioned medium from transiently transfected HepG2 cells. Apo B41 was contransfected with one of the other short constructs. The plasmid pKS was used as a nonexpressing transfection control. (C) ^{35}S-Labeled apo B23 and apo B41 in lipoprotein and infranatant fractions of conditioned medium from permanently transfected McArdle 7777 cells. The control lanes are untransfected McArdle 7777 cells. Immunoprecipitated apo B was resolved by 3–15% SDS–PAGE and analyzed by fluorography.

apo B100 or apo B41, but apo B29 (51%), apo B23 (75%), apo B17 (82%), and apo B13 (100%) were all present (Figure 2B). The distribution of truncated apo B proteins between the lipoprotein and infranatant fractions therefore showed a gradual shift toward the infranatant with decreasing length, suggesting that the ability to bind sufficient lipid to form a $d <$ 1.25 g/mL lipoprotein particle is progressively lost as sequences between the carboxyl terminal of apo B41 and apo B13 are deleted. There were no substantial differences between constructs in levels of mRNA (data not shown) or of secreted proteins (Figure 2).

Stable Expression in McArdle 7777 Cells. The level of apo B100 mRNA in HepG2 cells was low compared to that derived from the transfected apo B constructs (data not shown). This presumably reflects the high plasmid copy number in the transfected cells and the greater strength of the SV40 than apo B promoter. HepG2 cells may not be able to assemble the large amounts of truncated apo B proteins produced by transient expression into normal lipoprotein particles, and competition may occur with apo B100 for lipoprotein assembly. In addition, HepG2 cells secrete apo B as a triglyceride-rich LDL particle and do not produce a particle in the VLDL density range (Thrift et al., 1986).

To obtain independent confirmation of the results from transient expression in HepG2 cells, stable cell lines containing either apo B41 or apo B23 were prepared in McArdle 7777 cells. This rat hepatoma secretes apo B100 as a VLDL (Tanabe et al., 1989; Blackhart et al., 1990). In McArdle 7777 cells, apo B41 was found exclusively in the lipoprotein fraction; 90–98% of apo B23 was in the infranatant (Figure 2C).

These results confirm the findings obtained by transient expression in HepG2 cells of apo B41 exclusively in the li-

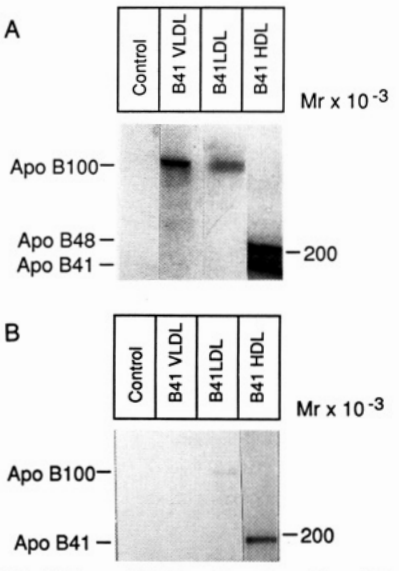

FIGURE 3: VLDL, LDL, and HDL subfractions of conditioned medium from permanently transfected McArdle 7777 and transiently transfected HepG2 cells. (A) VLDL, LDL, and HDL fractions of the apo B 41 stably transfected the McArdle 7777 cells. The control lane was untransfected cells. (B) VLDL, LDL, and HDL fractions secreted from HepG2 cells transiently expressing apo B41. The control lane was cells transfected with the pKS plasmid. Ultracentrifugation to prepare lipoproteins was performed sequentially after adjustment of the conditioned medium to an appropriate density. Apo B was immunoprecipitated from all the fractions and analyzed by 3–15% SDS–PAGE and fluorography.

poprotein fraction and apo B23 in the infranatant fraction.

Lipoprotein Fractionation. To examine the distribution of apo B41 between lipoprotein fractions in the conditioned medium of McArdle 7777 cells permanently transfected with these constructs, conditioned medium was adjusted to density $d = 1.006$, (VLDL), $d = 1.063$ (LDL), and $d = 1.25$ g/mL (HDL) with solid KBr, and after ultracentrifugation, apo B was immunoprecipitated from the lipoprotein fraction and analyzed by SDS–PAGE (Figure 3A). Endogenous rat apo B100 was present solely in the VLDL and LDL fractions, whereas rat apo B48 was found only in the HDL fraction. Human apo B41 was found with rat apo B48 in the HDL fraction in equimolar amounts. The finding of apo B48 and apo B41 in the HDL fraction of McArdle 7777 raised the possibility that apo B41 and apo B48 may be assembled into a lipoprotein particle containing one or more molecules of each. However, in HepG2 cells transiently transfected with apo B41, apo B100 was found exclusively in the LDL, and apo B41 in the HDL fraction (Figure 3B). Moreover, there was a considerable molar excess of apo B41 compared to apo B100 secreted from HepG2 cells. Together these results suggest that apo B41 does not assemble in the same lipoprotein particle as apo B100 or apo B48.

Discussion

These studies were designed to establish whether truncated forms of apo B can be secreted, and to identify the structural regions of apo B that are required for the assembly and secretion of buoyant lipoproteins. The results show that stepwise truncation of the region from apo B41 (amino acid residue 1880) to apo B13 (residue 583) leads to the gradual loss of the ability of cultured human and rat liver cells to form and secrete buoyant lipoproteins.

Previous studies have identified mutations of the apo B gene in persons with hypobetalipoproteinemia that predict truncated variants of apo B. Low levels of these proteins in plasma (or the inability to detect the predicted protein) might be due to impaired secretion. Defective secretion of some variants of α_1-antitrypsin and of the LDL receptor has been previously reported (Yamamoto et al., 1986; Sifers et al., 1988). We therefore investigated whether the short forms of apo B were secreted as failure to do so could contribute to the phenotype in hypobetalipoproteinemia. Our results from the transfection studies show that the truncated proteins are all secreted into the culture medium whether overexpressed in HepG2 cells or expressed at levels close to that of endogenous B100 in McArdle 7777 cells.

We next investigated whether the constructs could direct the assembly and secretion of apo B containing lipoproteins. Transient expression of apo B in HepG2 cells and stable expression in McArdle 7777 rat hepatoma cells show qualitatively similar secretion of the short forms of apo B in lipoprotein particles, thus supporting each system as a model for lipoprotein assembly. Apo B41 was quantitatively recovered in the HDL fraction in both transfection systems. In McArdle 7777 cells, endogenous B48 was also recovered in HDL, and B100 in VLDL and LDL. Apo B100 was recovered in LDL secreted from HepG2 cells. In normal plasma, apo B48 is recovered at the density of VLDL, and apo B100 is present in both VLDL and LDL. In subjects with hypobetalipoproteinemia, apo B39 was found in VLDL and LDL, and apo B46, apo B40, and apo B37 were found in VLDL, LDL, and HDL. Thus, while there are differences in the densities of the apo B41 and apo B48 particles secreted from cultured cells and the density of apo B48, apo B46, apo B40, apo B39, and apo B37 in plasma, it is clear that truncated proteins in this size range are competent to direct lipoprotein assembly. The secretion of apo B48 in the HDL fraction has been previously reported both for McArdle 7777 cells and for primary rat hepatocytes in culture (Tanabe et al., 1989; Yao et al., 1989).

Apo B29 was only studied in transiently transfected cells where it was recovered in both the lipoprotein and the infranatant fractions. This is qualitatively similar to the distribution of the natural variant apo B31, which is found both in the lipoprotein and in the infranatant fractions of plasma (Young et al., 1990). In contrast, the natural variant apo B29 was not detected by us in plasma lipoproteins or infranatant (Collins et al., 1988). Similarly, the natural variant apo B25 could not be detected in plasma (Huang et al., 1989). Since we observed decreased lipoprotein assembly in cells which expressed apo B29, we studied the shorter proteins apo B23, apo B17, and apo B13. Carboxyl-terminal truncation leads to a progressive shift from the lipoproteins to infranatant with decreasing length. This result indicates that the finding with apo B29 in cells was not particular to that protein, but fits a trend of decreased lipid binding as the protein is truncated. Although it is possible that truncated forms of apo B are secreted as lipoproteins but dissociate during ultracentrifugation, these data demonstrate that the unique character of apo B, a protein found exclusively in the lipoprotein fraction of plasma, is changed by carboxyl-terminal truncation to a protein that appears with other water-soluble plasma proteins in the infranatant fraction of conditioned medium from cells.

What are the structural features of the region of apo B between residues 1880 and 583, the loss of which accounts for the appearance of the short forms of apo B in the nonlipoprotein infranatant fraction? Deletion of the region between residues 1306 (apo B29) and 1880 (apo B41) causes apo B29 to appear in the infranatant fraction of conditioned medium. The deleted region contains many hydrophobic structures, some of which are extensive, alternating with hydrophilic

sequences. This region is largely inaccessible to digestion by the proteases trypsin and staphylococcus aureus V8 (Yang et al., 1989; Pease et al., 1990). It is likely that this protease-inaccessible region of apo B is not exposed to water in which trypsin can act, and may therefore be lipid-associated. In addition, this region contains several short hydrophobic peptides, but with insufficient amino acids to generate a classical 20-residue transmembrane sequence (Knott et al., 1986; Olofsson et al., 1987). Prominent throughout this region is a proline-rich cluster (residues 900–1324) having high probability of forming an amphipathic β-sheet (Knott et al., 1986; Yang et al., 1986, 1989). Further deletion of the sequences between apo B29 (1306 amino acids) and apo B13 (583 amino acids) leads to progressive loss of the ability to form buoyant lipoproteins. Since potential lipid binding structures exist throughout the region between 1880 and 583, it appears that it is the overall loss of lipid binding mass that causes the short forms of apo B to appear in the infranatant. If it is assumed that apo B is distributed evenly around the surface of near-spherical lipoprotein particles, stabilizing a hydrophobic lipid core, any decrease in apo B length would cause a proportionately greater decrease in the volume of the core, and result in an overall increase in density.

To what extent do these results help to explain the low levels of truncated apo B in hypobetalipoproteinemia? For apo B29 expressed in vitro and from the finding of apo B31 in the infranatant in vivo, it is clear these proteins do show decreased lipid binding. Although with B41 we are unable to show a defect in secretion from the in vitro studies, the results in vivo showing that while B48 is in the VLDL fraction, apo B37, apo B40, and apo B46 were found in VLDL, LDL, and HDL might suggest an abnormality in lipid binding. However, the different origin of the lipoproteins containing apo B48 (intestinal) and the truncated proteins (presumably liver and intestinal) could contribute to these findings. Also, apo B37, some B40, and B46 are present in HDL, but apo B39 was found only in VLDL and LDL, showing that chain length is not the only determinant of lipoprotein density in vivo. For apo B39, where we have no evidence for impaired secretion or assembly, we do not know the major factors causing low levels of this protein to be present in the blood. Whether there is a difference in genetic background of the different subjects with hypobetalipoproteinemia affecting the expression of these alleles or whether there are intrinsic features of the structures of these proteins that cause their different assembly and metabolism is unknown. We cannot therefore exclude that there are subtle differences in assembly of the lipoprotein particles that decrease lipid binding in vivo and contribute to the phenotype. More generally, hypobetalipoproteinemia can also be caused by much longer truncated alleles such as apo B90 and apo B87, which might be predicted to show near-normal lipid binding similar to B100 (Young et al., 1990; Krul et al., 1989). This suggests that other factors may also be involved, such as rapid clearance from the circulation or low mRNA levels due to mutations that affect transcription of mRNA stability. Indeed, low levels of apo B mRNA have been demonstrated in the liver of persons with hypobetalipoproteinemia (Ross et al., 1988).

In conclusion, this work suggests that truncated forms of apo B of less than 1306 residues have a reduced capacity to form buoyant lipoproteins. This may contribute, in conjunction with other factors, to the absence of detectable truncated apo B in patients with hypobetalipoproteinemia alleles of apo B29 or smaller.

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Structural Studies of Gangliosides by Fast Atom Bombardment Ionization, Low-Energy Collision-Activated Dissociation, and Tandem Mass Spectrometry†

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ABSTRACT: Negative ion fast atom bombardment, low-energy collision-activated dissociation, and tandem mass spectrometry techniques were applied for the structural elucidation of gangliosides. The mass spectra were simplified by selecting a single molecular ion or fragment ion in the analysis of mixtures, and interference by background signals from the liquid matrix could be avoided. Introduction of collision-activated dissociation produced abundant fragment ions convenient for structural analysis. In the daughter scan mode, ions were produced by cleavage of the glycosidic bonds, and not by cleavage at the sugar ring. These ions all contain ceramide moieties, except the sialic acid fragment ion. In the parent scan mode, product ions resulting from cleavage at the sugar ring were detected beside the ions resulting from cleavage at the glycosidic bonds, and ions of oligosaccharide fragments were also detected. In parent scan mode spectra of gangliosides based on the sialic acid ion, all ions contained a sialic acid residue, and the observed ions were similar to those obtained in the high-energy collision-activated dissociation daughter scan mode. These results indicate the usefulness of low-energy collision-activated dissociation tandem mass spectrometry in the daughter and parent scan modes for the analysis of ganglioside structure, in combination with fast atom bombardment mass spectrometry and high-energy collision-activated dissociation mass spectrometry.

Glycolipids are components of the cell membrane and exhibit characteristic distribution patterns depending on the species, organ, cell type, and developmental stage (Hakomori, 1981). They have vital functions on the cell surface, as antigens (Clausen & Hakomori, 1989), binding sites for microbes (Karlsson, 1989), cell–cell recognition sites (Kojima & Hakomori, 1989), and signals for cell growth (Bremer et al., 1986) and differentiation (Nojiri et al., 1986). For studies in all these areas, characterization of the chemical structure of the glycolipids is a fundamental requirement. In many cases, the content of the glycolipids is low, and the purification is difficult and time-consuming. The development of new techniques which require only a minute amount of the sample and give detailed information is therefore essential. Nuclear magnetic resonance spectroscopy is a powerful method especially for stereochemical and linkage analysis (Sweeley & Nunez, 1985), but it requires relatively large amounts of samples (at least 100 nmol for two-dimensional nuclear magnetic resonance spectrometry) and is almost impossible to apply for the analysis of mixtures. The use of tandem mass spectrometry (MS/MS)[1] has been reported to overcome these difficulties, and it can give valuable information for structural studies. Domon and Costello reported the application of fast atom bombardment high-performance tandem mass spectrometry (MS/MS) for the analysis of glycolipids using high-energy (kiloelectron volts) collision to obtain collision-activated dissociation (CAD) (Domon & Costello, 1988a).

In this report, we show that another type of structural information can be obtained by using MS/MS with low-energy (tens of electron volts) CAD in the daughter scan mode and in the parent scan mode, and we compare the results with those

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[1] Abbreviations: MS, mass spectrometry; MS/MS, tandem mass spectrometry; CAD, collision-activated dissociation; FAB, fast atom bombardment; sialyl2-3nLc$_4$Cer, NeuAcα2-3Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ1-ceramide; Cer, ceramide.

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