Conformation of Apolipoprotein B-100 in the Low Density Lipoproteins of Tangier Disease

IDENTIFICATION OF LOCALIZED CONFORMATIONAL RESPONSE TO TRIGLYCERIDE CONTENT*

(Received for publication, May 4, 1990)

Steven T. Kunitake‡‡‡, Stephen G. Young‡‡‡, G. Chi Chen‡, Clive R. Pullinger‡, Shan Zhu‡, Richard J. Pease‡‡, James Scott‡‡, Phillip Hass‡‡, James Schilling‡‡, and John P. Kane‡‡‡‡

From the Cardiovascular Research Institute, Gladstone Foundation Laboratories, and the Departments of Medicine, and Biochemistry and Biophysics, University of California, San Francisco, California 94143 and the Medical Research Council Clinical Research Centre, Harrow HA1 3UJ, United Kingdom, Genentech, South San Francisco, California 94080 and the California Biotechnology, Inc., Mountain View, California 94043

The low density lipoproteins (LDL) from patients with Tangier disease are enriched in triglycerides, 27% of LDL mass versus 7% for normal LDL. To study whether this unique LDL core lipid composition affects the surface disposition of apolipoprotein (apo) B-100, we analyzed the LDL by protease digestion and in competitive radioimmunoassays. Limited proteolytic digestion of Tangier LDL by Staphylococcus aureus V8 protease generated a prominent fragment of 120 kDa (cleavage site at residue 1076), which was not visible in similarly digested normal LDL. In competitive radioimmunoassay, Tangier LDL bound weakly to the apoB-specific monoclonal antibody MB20, compared with control LDL. We localized the MB20 epitope between residues 1031 and 1084 of apoB-100, probably near residue 1076. DNA sequencing of exon 21 of apoB genomic clones (coding for residues 1014-1084) from a Tangier patient revealed no difference from the normal DNA sequence, thus eliminating a protein polymorphism as a basis for the altered protease sensitivity and antibody binding. When the triglyceride contents of Tangier LDL were reduced to 10% of mass by incubation with normal high density lipoproteins, production of the 120-kDa fragment by proteolysis decreased and MB20 binding increased in affinity, implying a change toward normal conformation of apoB-100. Thus, using two independent techniques, proteolytic digestion and antibody binding of monoclonal antibodies, we have demonstrated an alternative conformation of apoB-100 in the vicinity of residue 1076, which reflects the content of triglycerides in the LDL particle.

Tangier disease is metabolically characterized by a nearly complete absence of high density lipoproteins (HDL) in the plasma of homozygous patients (1, 2). The reason for the virtual absence of HDL in these subjects is not completely known. The gene for apolipoprotein (apo) A-I in these patients appears to be normal (3, 4); however, there appears to be abnormal uptake of lipoproteins by macrophages and other scavenger cells, causing the HDL to be degraded at an increased rate (5). In many subjects with Tangier disease, the concentrations of both total cholesterol and low density lipoprotein (LDL) cholesterol are decreased as well (6). The LDL from Tangier patients are enriched in triglyceride at the expense of cholesteryl esters (7), which is thought to be a consequence of diminished transfer of cholesteryl esters to LDL from HDL. These unique LDL from the plasma of Tangier patients provide an excellent opportunity to address the question of whether the core lipid composition of LDL can affect the conformation of apoB-100 on the surface of the lipoprotein.

apoB-100 is known to assume different conformations, depending on the type of lipoprotein upon which it resides. In prior studies, apoB-100 conformational changes appeared to accompany differences in core lipid composition when assessed by the binding of apoB-specific monoclonal antibodies to the lipoproteins (8-14), by the differential accessibility to limited proteolytic digestion of apoB-100 (15), or by measuring the disposition of the lysine groups of apoB-100 (16) on the lipoproteins studied. Conformational differences were documented when comparing very low density lipoproteins (VLDL) to LDL from one subject (8-12), LDL from normal subjects to those from hypertriglyceridemic subjects (13, 14), and LDL before and after modulating core lipid contents (14, 16). However, in the studies where size was measured, changes in core composition were linked with changes in the size of the lipoproteins studied. Therefore, the observed differences in conformation of apoB could reflect either changes in core lipid composition or particle size.

In this study, we compared the conformation of apoB-100 on triglyceride-rich Tangier LDL with that of normal LDL, assessing differences by determining their binding to several apoB-specific monoclonal antibodies and their accessibility to limited proteolytic digestion. Dramatic differences in protease accessibility and antibody binding localized to a specific region of apoB-100 were found between Tangier and normal LDL despite minimal size differences in particle diameter, suggesting that the core lipid composition influences the conformation of apoB-100. Further support of this hypothesis comes from the observation that these differences in antibody binding and protease accessibility are reversed after decreasing the triglyceride content of Tangier LDL in vitro.
Isoelectric focusing—Venous blood was drawn from two siblings (T.M. and P.M.) with Tangier disease and seven normolipidemic male siblings (T.M. and P.M.) with Tangier disease and seven normolipidemic

Isolation of Lipoproteins—Venous blood was drawn from two siblings (T.M. and P.M.) with Tangier disease and seven normolipidemic control subjects, and the following inhibitors and preservatives were added: EDTA (0.1% w/v), caproic acid (0.13%), benzamidine (0.3 mg/ml), phenylmethylsulfonyl fluoride (1 mM), gentamicin (10 µg/ml), and α-macroglobulin (10 µg/ml). The final concentration of each additive is stated in parentheses. The plasma was obtained by centrifugation of the blood at 1000 x g for 30 min at 4 °C. The LDL, d < 1.055 g/ml, were isolated by sequential ultracentrifugation. The isolation involved two centrifugational runs at a density of 1.055 g/ml for 18 h at 36,000 rpm and 4 °C in a Beckman 40.3 ultracentrifuge rotor, followed by two more runs at a density of 1.055 g/ml under the same conditions. HDL were isolated in the same manner except that the density interval was 1.07–1.21 g/ml. The 1.07 g/ml bottom was generated by adjusting plasma to 1.07 g/ml with KBr and centrifuging as above. The supernatant layer was removed and the intratranant fluid reincubated to insure removal of all apoB-containing lipoproteins. Each lipoprotein fraction was dialyzed into 5 mM Tris, pH 7.4, and preservatives were added for storage at 4 °C, as above.

Lipoprotein and Apolipoprotein Analysis—The chemical compositions of normal and LDL were measured as follows. The concentration of protein was determined by the method of Lowry et al. (17), with and without tetramethyl urea pretreatment (18), and employing a correction for difference in the chromogenicity between apoB and the bovine serum albumin standard. Cholesterol and cholesteryl ester were measured by a fluorometric enzymatic method (19), phospholipid was determined from the measurement of lipid-associated phosphorus content (20), and triglycerides were measured by the enzymatic determination of the glycerol content (Sigma) (21). ApoA-I and apoB contents of plasma samples were measured by radial immunodiffusion (TAGO, Burlingame, CA).

Particle diameters of LDL were measured from electron micrographs. The LDL were negatively stained with 2% potassium phosphtostate and photographed with a Siemens 101 electron microscope. The radii of 200 individual free-standing particles were measured by a computer-linked digitization pad (22).

ApoB-specific Antibodies—ApoB-specific monoclonal antibodies MB3, MB20, and MB47 were obtained from Drs. L. Curtiss and J. Witrum of the La Jolla Arteriosclerosis SCOR. Focal polyclonal antisera 890, a rabbit antiserum to a synthetic peptide corresponded to residues 890–908 of apoB, was generously provided by Dr. T. L. Innerarity of the Gladstone Foundation Laboratories.

Radioimmunoassay of ApoB —The ability of normal LDL and Tangier LDL to bind to the apoB-specific monoclonal antibodies MB3, MB20, and MB47 was tested in a solid-phase, competitive radioimmunoassay using 96-well polyvinyl chloride microtiter plates (Dynatech, Chantilly, VA), as previously described (23). The standard curve for the RIA was constructed from serial dilutions of a freshly prepared normal LDL sample. Serial dilutions of additional normal LDLs and the two Tangier LDL preparations were tested as "unknowns" in the assay. Both the LDL standard and the unknown LDLs were added to the assay on the basis of their total protein concentrations. The results were plotted as Fl/Bo versus log protein concentration, where B and Bo represent the specific counts bound in the presence and absence of competitor, respectively. In order to compare simply and easily the reactivity of different LDL samples in the assay, the protein concentration of each LDL preparation yielding 50% displacement on the competition curve (B/Bo = 0.5) was calculated. This protein concentration was designated the ED50 value.

The apparent apoB content of plasma samples was also determined in RIAs using antibodies MB3, MB20, and MB47. In these assays, several dilutions of each plasma sample were included in the assay. The apparent apoB content of each plasma sample was calculated from the LDL standard curve using the plasma dilution that fell within the midportion of the LDL standard curve (B/Bo = 0.5–0.7). We observed that the apparent plasma apoB concentration for Tangier plasma was low in the MB20 RIA compared with values obtained in RIAs using antibodies MB3 and MB47 in contrast to normal LDL. This systematic difference was not observed with the monoclonal antibody MB20 divided by the apoB content determined in an RIA using monoclonal antibody MB3 or MB47.

Immunosassays were performed on delipidated and denatured LDL samples. The LDL samples, 1 ml of LDL (200 times protein mass) and 1 ml of the d > 1.07 g/ml fraction of plasma (original plasma concentration, as a source of cholesteryl ester transfer protein) or 8 µg of purified cholesteryl ester transfer protein to a final volume of 6.0 ml. The mixture was incubated at 37 °C for 5 h. After incubation the 1.07 g/ml LDL samples were ultracentrifuged at a density of 1.063 g/ml. Following dialysis into Tris-buffered saline, the incubated LDL (iLDL) were analyzed as above. As a control, Tangier LDL were incubated alone; no changes in chemical composition, oxidation of lipids measured by the production of thiobarbituric acid reactive substances, or breakdown of protein visible by SDS PAGE were observed (data not shown).

Results

Two male siblings were diagnosed as having Tangier disease. They had typical carotenoid pigmentation of the rectal mucosa and tonsillar tabs, and one had peripheral neuropathy. The corneas were free of infiltrates. Both parents had abnormally low levels of IDL cholesterol in plasma. The plasmas from these siblings were found to contain very low levels of...
apoA-I (3.1 and 2.9 mg/dl) and almost no HDL cholesterol (1.0 mg/dl for each) (Table I). In addition, each of these patients had low levels of both total plasma cholesterol (23 and 30 mg/dl) and apoB (36 and 24 mg/dl).

The chemical composition of LDL (1.03-1.055 g/ml) from the Tangier patients differed from LDL of normal subjects. Tangier LDL contained much more triglyceride than normal LDL (26.2% of mass compared with an average of 7.6% of mass) (Table II). This increased triglyceride content came primarily at the expense of cholesteryl ester mass. Tangier and normal LDL had similar protein contents, attributable primarily to apoB (greater than 95% of protein mass as determined by tetramethyl urea-insoluble protein). The Tangier LDL were similar in size to normal LDL; average radii were 10.0 and 10.8 nm, respectively. In fact, the mean radii of some normal LDL samples differed from Tangier LDL by only 0.1 nm.

The binding of three monoclonal antibodies, MB3, MB20, and MB47, to both Tangier LDL and normal LDL was measured in solid-phase, competitive, radioimmunoassays. The Tangier LDL bound only 50% as well to antibody MB20 compared with seven normal LDL samples (Fig. 1). In contrast, Tangier LDL and normal LDL possessed similar binding to monoclonal antibodies MB3 and MB47. These results were verified on two separate occasions with both Tangier subjects. The results of all the competitive RIAs were presented as the ratios of the EDso (the protein concentration that results in 50% displacement of the standard curve; B/B0 = 0.5) obtained for the different LDL samples in parallel assays using different monoclonal antibodies (i.e. the EDso obtained in the MB20 assay divided by the EDso from the MB3 assay) (Table III). Calculating the ratios provided a simple means of comparing the reactivity of the LDLs and effectively eliminating error in the measurement of protein mass of the LDL by the Lowry assay. The MB20/MB3 ratio for the Tangier LDL was 1.92, compared with 1.02 for a group of five samples of normal LDL. Similarly, the MB20/MB47 ratio was 1.68 for the Tangier LDL and 0.90 for the normal LDL. These results demonstrate that the MB20 epitope is less reactive on Tangier LDL compared with normal LDL. Whether this finding is explained by a decreased affinity of MB20 for an epitope that is uniformly expressed on all LDL or because of the complete absence of MB20 epitope expression on a significant subpopulation of Tangier LDL particles, is not known. In any case, the difference in immunoreactivity is present in Tangier LDL in spite of the fact that the diameters of these particles were close to those of some normal LDL samples.

To eliminate the possibility that the difference in immunoreactivity between Tangier and normal LDL in the MB20 RIA was an artifact of the isolation of LDLs, we assessed the reactivities of the three monoclonal antibodies to the apoB in the fresh plasma of Tangier and normal subjects (Table IV). The apparent apoB concentration of each plasma was determined from the standard curve obtained as described under "Experimental Procedures." The MB20/MB3 ratio (the ratio of the apparent plasma apoB concentration measured in the MB20 assay divided by the apparent plasma apoB concentration measured in the MB3 assay) was 0.93 for Tangier LDL compared with 1.65 for normal plasma. The MB20/MB47 ratio for Tangier plasma was 0.88, substantially different from that for normal plasma, 1.53. Thus, the altered MB20 immunoreactivity was just as apparent in plasma as in the LDL fractions prepared by ultracentrifugation. We have noted that the MB20/MB3 and MB20/MB47 ratios for the normal plasma are greater than 1.0. While the use of antibodies MB3 and MB47 to quantitate plasma apoB levels has been verified, evidence exists which implies that MB20 cannot be used for this purpose (9). Our sole intent in performing these competitive RIAs was to establish the differential reactivity of MB20 toward Tangier and normal LDL.

Tangier and normal LDL were subjected to limited proteolytic digestion by SP. Following timed digestions ranging from 0 to 90 min, the apoB-100 on normal LDL (Fig. 2, lanes 1-5) was cleaved into three fragments similar in size to the T2, T3, and T4 fragments generated by thrombin cleavage of LDL (28) (SP was found to cleave apoB-100 at the carboxyl sides of residues 1287 and 3198 (15) rather than residues 1297 and 3249 (29), the points of thrombin attack). These fragments were designated SP2, SP3, and SP4. Proteolysis of Tangier LDL generated two apoB-100 fragments of approximately 120 and 260 kDa (Fig. 2, lanes 6-10) that were not visible in the digests of normal LDL samples (Fig. 2, lanes 1-5 and 11-15). These observations were true and reproducible for the LDL from both Tangier patients and for every normal LDL tested. Some normal LDL (Fig. 2, lanes 11-15) as well as Tangier LDL have a protease-sensitive region that creates an apoB fragment of approximately 115 kDa. These two amino-terminal fragments of apoB (120 and 115 kDa) are associated with complementary fragments, which are larger than the SP3 proteolytic fragment (see below). The fragment complementary to the 120-kDa fragment was the 260-kDa fragment, designated SP3' (denoted by arrow), and the fragment complementary to the 115-kDa fragment was designated SP3" (denoted by arrow).

The amino terminus of several proteolytic fragments was determined by sequencing the fragments after they were transferred to polyvinylidene difluoride membranes. Both the 115- and 120-kDa fragments began with residue 7 of apoB, while the SP4 fragment began with residue 1. The SP3 fragment began with residue 1288, while the SP3' fragment

---

**Table I**

| Lipid and apolipoprotein contents of plasma from subjects with Tangier disease |
|---------------------------------|--------|--------|--------|--------|
|                                | Total cholesterol | Total triglyceride | HDL cholesterol | ApoA-I |
| **TM**                         | 23     | 40     | 1.0    | 3.1 ± 0.5 | 36 ± 1.0 |
| **PM**                         | 30     | 78     | 1.0    | 2.9 ± 0.5 | 24 ± 1.0 |

---

**Table II**

| Percent chemical composition of LDL |
|-----------------------------------|--------|--------|--------|--------|
| Protein                          | Phospholipid | Cholesteryl ester | Cholesterol | Triglyceride | Radius (nm) |
| **TM**                           | 30.8    | 21.7    | 18.6   | 5.5     | 24.8       | 9.97 ± 0.8 |
| **PM**                           | 27.6    | 13.7    | 24.1   | 7.0     | 27.6       | 10.1 ± 1.3 |
| Normal (n = 7)                   | 27.3 ± 1.2 | 20.9 ± 4.7 | 32.6 ± 6.2 | 12.3 ± 1.0 | 7.0 ± 1.4 | 10.8 ± 0.9 (10.1-11.5) |
FIG. 1. Binding of the apoB-specific monoclonal antibodies MB20, MB3, and MB47 to Tangier and normal LDL. The $B/B_0$ values were plotted as a function of the log (LDL concentration) for the competition of Tangier LDL (●), and the average of seven normal LDL (□) samples against a control normal LDL for the binding to monoclonal antibodies MB20 (a), MB3 (b), and MB47 (c).

TABLE III

Immunoreactivities of Tangier and normal LDL to apoB-specific monoclonal antibodies MB20, MB3, and MB47

| Subject | MB20/MB3 | MB20/MB47 |
|---------|----------|-----------|
| TM      | 1.92     | 1.68      |
| Normals |          |           |
| 1       | 0.67     | 0.80      |
| 2       | 0.97     | 0.64      |
| 3       | 1.06     | 0.88      |
| 4       | 1.09     | 1.00      |
| 5       | 1.32     | 1.17      |
| Average | 1.02 ± 0.2 | 0.90 ± 0.2 |

*The MB20/MB3 ratio represents the ratio of the protein concentration resulting in 50% competition (ED$_{50}$) in the MB20 RIA divided by the ED$_{50}$ in the MB3 RIA. Similarly, the MB20/MB47 ratio represents the ratio of the ED$_{50}$ in the MB20 RIA divided by the ED$_{50}$ in the MB47 RIA.

began with residue 1077. The amino terminus of SP3'' was not determined.

The location of the MB20 epitope on apoB was determined by binding to the proteolytic fragments generated by SP degradation and to several fusion proteins containing segments of apoB-100. The SP proteolytic fragments were transferred to nitrocellulose membranes and immunoblotted with MB20, MB3, and antiserum 890 (Fig. 3). MB3 and antiserum 890 bound to all three apoB amino-terminal fragments (SP4 and the 115 and 120-kDa fragments) (Fig. 3, B and C); in contrast, MB20 bound only to the SP4 fragment (Fig. 3A).

Using fusion proteins generated by Pease and colleagues (30), we found that antibody MB20 interacted with fusion proteins T4/5 (residues 781-1084) and T4/6 (residues 994-1328) (not shown), indicating that the MB20 epitope was between residues 994 and 1084. Because MB3 was found to bind to the 11.5-kDa fragment while MB20 bound to its complementary fragment, SP3'', this indicated that the MB20 epitope is on the carboxyl-terminal side of the MB3 epitope. Since it has been determined that MB3 binds between residues 1022-1031 of apoB (30), our results would narrow the location of the MB20 epitope to the carboxyl-terminal side of residue 1031 (Fig. 4). Furthermore, since MB20 appears to bind to neither the 120-kDa fragment nor its complementary fragment, SP3'', this implies that the cleavage of apoB at residue 1076 destroys the MB20-binding epitope (i.e. that the SP cleavage site and the MB20 epitope are very close to each other). Our results, therefore, localize both the SP cleavage site and the MB20 epitope between residues 1031 and 1084, and the 115 and 120-kDa fragments (Fig. 3, B and C); in contrast, MB20 bound only to the SP4 fragment (Fig. 3A).
were due to differences in conformation or due to a primary sequence polymorphism in the apoB of the Tangier subjects, we conducted several further experiments. First, in a direct binding RIA, we tested the ability of monoclonal antibodies MB20, MB3, MB47 to bind to Tangier and normal LDL binding RIA, we tested the ability of monoclonal antibodies MB20, MB3, MB47 to bind to Tangier and normal LDL. Ten clones were sequenced (five from the coding strand and five from the noncoding strand) and found to have identical sequence to that published for normal apoB (29, 31-36). To test the effect of altering the core lipid composition on the conformation of apoB on Tangier LDL, the triglyceride content of the Tangier LDL was lowered by incubation with HDL (HDL/LDL protein ratio 200:1) and cholesteryl ester transfer protein for a period of 5 h at 37 °C (Table VI). After this incubation, the triglyceride content of the LDL decreased from 27 to 10% of mass. At the same time the cholesteryl ester content increased from 22 to 33%. The incubation did not degrade apoB and did not appreciably increase the content of non-apoB apoproteins on LDL, as judged by SDS-PAGE (not shown). Furthermore, the diameters of the LDL following incubation did not change appreciably, when measured by electron microscopy (Table VI).

The immunoreactivity of normal LDL (nLDL), Tangier LDL prior to incubation (tLDL), and Tangier LDL after incubation (iLDL) was assessed in RIAs using the three monoclonal antibodies. The incubation virtually abolished the abnormal pattern of MB20 immunoreactivity observed with the Tangier LDL prior to incubation (Table VII). Next, the nLDL, tLDL, and iLDL were subjected to limited digestion by SP for 90 min (Fig. 5). The SP digestion of the iLDL resulted in the production of a minor amount of the 120-kDa fragment but significantly less than that produced by the digestion of tLDL. Digestion of tLDL also produced less SP4 than did digestion of iLDL or nLDL, which would be expected if apoB-100 fragmentation were diverted to the production of the 120-kDa fragment. The quantity of the 120-kDa fragment was determined by separating fragments by SDS-PAGE, immunoblotting with antisera 890, isolating the 120-kDa, SP4, and B-100 bands from the nitrocellulose membrane, and measuring the associated radioactivity. In this way we could assess the relative amounts of the 120-kDa, SP4, and B-100 proteins in the various digested LDL samples (Table VIII).

and probably very close to residue 1076. This portion of apoB is encoded entirely by exon 21 and the apoB gene (exon 21 codes for residues 1014-1084). Interestingly, this alternative conformation of apoB appears to be very localized in that the conformation of apoB appears to be very localized in that the epitope for MB3, which is within 50 amino acid residues from the apparent MB20 epitope and the SP cleavage site, appears at most to be modestly affected in competition curves (Fig. 1B) and has no effect on the determination of the apparent ED50 for MB3.

To determine whether these differences in MB20 binding and protease accessibility between Tangier and normal LDL were due to differences in conformation or due to a primary sequence polymorphism in the apoB of the Tangier subjects, we conducted several further experiments. First, in a direct binding RIA, we tested the ability of monoclonal antibodies MB3, MB20, and MB47 to bind to Tangier and normal LDL that had been delipidated and then denatured in 6 M guanidinium hydrochloride. The results of these studies indicated that MB20 bound to delipidated and denatured Tangier LDL as well as to normal LDL (Table V). These results cast some doubt on the possibility that a protein polymorphism was responsible for the altered immunoreactivity and protease accessibility, but they were not definitive. To exclude this possibility, we cloned and sequenced exon 21 of the apoB gene from one of the Tangier subjects. Ten clones were sequenced (five from the coding strand and five from the noncoding strand) and found to have identical sequence to that published for normal apoB (29, 31-36).

The immunoreactivity of normal LDL (nLDL), Tangier LDL prior to incubation (tLDL), and Tangier LDL after incubation (iLDL) was assessed in RIAs using the three monoclonal antibodies. The incubation virtually abolished the abnormal pattern of MB20 immunoreactivity observed with the Tangier LDL prior to incubation (Table VII). Next, the nLDL, tLDL, and iLDL were subjected to limited digestion by SP for 90 min (Fig. 5). The SP digestion of the iLDL resulted in the production of a minor amount of the 120-kDa fragment but significantly less than that produced by the digestion of tLDL. Digestion of tLDL also produced less SP4 than did digestion of iLDL or nLDL, which would be expected if apoB-100 fragmentation were diverted to the production of the 120-kDa fragment. The quantity of the 120-kDa fragment was determined by separating fragments by SDS-PAGE, immunoblotting with antisera 890, isolating the 120-kDa, SP4, and B-100 bands from the nitrocellulose membrane, and measuring the associated radioactivity. In this way we could assess the relative amounts of the 120-kDa, SP4, and B-100 proteins in the various digested LDL samples (Table VIII).

**FIG. 3.** Immunoblots of antibodies to proteolytic fragments of apoB. After limited digestion for 90 min, both Tangier LDL (lanes 1, 3, 5, 7) and normal LDL (lanes 2, 4, 6, 8) were immunoblotted with MB20 (A), MB3 (B), and antiserum 890 (C). A Coomassie blue-stained gel of the digestion fragments is shown for comparison (D). The nomenclature used was explained in Fig. 2 and under "Results."

**FIG. 4.** Schematic diagram demonstrating the apoB fragments generated by SP digestion of Tangier and normal LDL. The fragments that are bound by MB20 and MB3 are identified by immunoblotting. Also shown are the fusion proteins containing portions of apoB that bind MB20 and MB3. The MB20 epitope resides slightly to the carboxyl-terminal side of the MB3 epitope (1022-1031) and appears to be destroyed by SP cleavage at residue 1076. *, denotes MB20 binding; **, denotes MB3 binding.

**TABLE V**

| Subject | MB20/MB3 | MB20/MB47 |
|---------|----------|-----------|
| TM      | 0.30     | 0.82      |
| PM      | 0.24     | 0.81      |

* Tangier and normal LDL samples were delipidated, denatured in 6 M guanidinium hydrochloride, and coated onto microtiter wells as described under "Experimental Procedures." Following incubation with a fixed and limited concentration of each monoclonal antibody the plates were washed and subsequently incubated with a 125I anti-mouse secondary antibody. The radioactivity bound to the wells was measured; these counts represented a measurement of monoclonal antibody binding to the denatured apoB. To eliminate errors due to differences in apoB binding to the microtiter plate wells and errors due to protein measurements, we calculated the ratio of MB20/MB3 binding and the ratio of MB20/MB47 binding for each apoB preparation.
ApoB Conformation in Tangier LDL

DISCUSSION

Tangier LDL provide an excellent opportunity to study the effect of core lipid contents on the conformation of apoB-100 in LDL because they are naturally enriched in triglycerides. In this study, we investigated the immunoreactivity and the accessibility to protease attack of the apoB-100 on the surface of these particles. Our data indicate the presence of a local conformational difference between normal LDL and Tangier LDL. This difference is manifested in the impaired recognition of apoB-100 on Tangier LDL by monoclonal antibody MB20 and its increased accessibility to cleavage by S. aureus V8 protease at residue 1076.

The differences between normal and Tangier LDL in antibody binding and protease accessibility could be due to polymorphisms in the primary sequences of apoB-100. Several polymorphisms in the sequence of the human apoB gene leading to differences in primary amino acid structure of apoB have been documented (31, 37), and several of these apoB polymorphisms can be recognized by monoclonal antibodies (23, 38, 39). However, the altered binding of antibody MB20 to Tangier LDL was not caused by a primary sequence polymorphism. We have found that the reactivity of denatured apoB from Tangier LDL to the monoclonal antibody MB20 was similar to that of denatured apoB from normal subjects. In contrast, the MB19 polymorphism, which is due to an amino acid sequence polymorphism, is easily detectable on denatured apoB (23). In order to establish this point unequivocally, we sequenced exon 21 of the apoB gene from one of the Tangier subjects. This exon encodes the region encompassing the conformationally variable site. We found that this sequence was identical to that reported for the apoB gene, ruling out a mutation of apoB.

Conformational differences in apoB that are dependent upon the type of lipoprotein on which the apoB resides have been well documented. VLDL, intermediate density lipoproteins, LDL, and their subfractions interact with different affinities to selected monoclonal antibodies (8-12). These differences occur among lipoprotein classes from a single individual and only with certain monoclonal antibodies. Also, antibodies whose binding to apoB is dependent on the presence of associated lipid and others insensitive to lipid have been documented (40). The LDL of hypertriglyceridemic subjects, approximately 9.5% triglyceride by mass, possessed different epitope expression from normal LDL (14); also, LDL of varying triglyceride mass, ranging from 4 to 10%, were found to vary in their expression of specific epitopes, apparently correlating with triglyceride content (13). Drug treatment resulting in the lowering of the triglyceride content of LDL also altered the epitope expression of apoB (14). Again, alteration of the triglyceride contents of LDL by incubation with triglyceride-rich particles or by lipolysis caused an alteration in the exposure of lysine groups on apoB (16). These findings imply that the core composition of the lipoprotein, i.e. the triglyceride/cholesterol ester ratio, may affect the conformation of apoB on the particles. However, in the studies where particle size was measured, the difference in composition was linked with differences in diameters of the particles. Thus, the conformational changes observed could have reflected either changes in radius of curvature of particles or direct effects from altered lipoprotein composition.

We believe the alternative conformation of apoB-100 that we have observed is due to a difference in the core composition of LDL rather than changes in particle diameter. The conformational difference was detected between Tangier and normal LDL even though the mean diameters of some samples of normal LDL were very similar to those of Tangier LDL. Furthermore, incubation of Tangier LDL with HDL and cholesteryl ester transfer protein, which reduced the triglyc-
The amino acid sequence of residues 1041–1100 of apoB is presented according to the analysis of Chou and Fasman (42) (Fig. 6). Amino acid residues 1074–1083, encompassing the SP cleavage site at residue 1076, possess the propensity to assume either LY helix or β sheet structure. It is attractive to speculate that triglyceride in the lipid core may favor one of these conformations and cholesteryl esters the other. That at least some part of apoB-100 is intimately related to hydrophobic properties of the lipoprotein core is inferred by results of studies employing β carotene as an optical probe (43, 44).

The production of low density lipoproteins from their precursor, the very low density lipoproteins, appears to involve a concatenation of biochemical events: lipolysis, the acquisition of triglyceride and of small apolipoproteins, enrichment with cholesteryl esters, and the organization of at least one receptor ligand in apoB-100. It is unlikely that the conformationally responsive locus we have identified plays a role in the formation of the ligand for the LDL receptor because monoclonal antibodies that map to this region do not appear to inhibit binding (45). It is possible, however, that this locus serves as a conformational switch in cueing some step in the final processing of VLDL remnants to LDL.

Acknowledgments.—We would like to express our thanks to Dr. H. Hobbs for supplying material from the Tangier subjects, Drs. L. Curtiss and J. Witztum for their gift of MB20, MB3, and MB47, Dr. T. Innerarity for his gift of antiserum 890, and Drs. E. Krul and G. Schonfeld for their gift of several monoclonal antibodies.

REFERENCES

1. Hoffman, H. N., and Fredrickson, D. S. (1965) Am. J. Med. 39, 682–683
2. Fredrickson, D. S. (1964) J. Clin. Invest. 43, 228–237
3. Law, S. W., and Brewer, H. B., Jr. (1985) J. Biol. Chem. 260, 12610–12614
4. Makrides, S. C., Ruiz-Opazo, N., Hayden, M., Nussbaum, A. L., Breslow, J. L., and Zannis, V. I. (1987) Eur. J. Biochem. 173, 460–471
5. Assmann, G., Schmitz, G., and Brewer, H. B., Jr. (1990) Familial HDL Deficiency: Tangier Disease, pp. 1267–1282, McGraw-Hill, New York
6. Schaefer, E. J., Zech, L. A., Schwartz, D. E., and Brewer, H. B., Jr. (1980) Annu. Intern. Med. 93, 261–266
7. Gretchen, H., Hannemann, T., Cusak, W., and Vivell, O. (1974) N. Engl. J. Med. 291, 548–552
8. Curtiss, L. K., and Edgington, T. S. (1982) J. Biol. Chem. 257, 15222–15228
9. Tsao, B. P., Curtiss, L. K., and Edgington, T. S. (1982) J. Biol. Chem. 257, 15222–15228
10. Salmon, S., Goldstein, S., Pastier, D., Theron, L., Bertheret, M., Ayrault-Jarrier, M., Dubarry, M., Reboucret, R., and Pau, B. (1984) Biochem. Biophys. Res. Commun. 125, 704–711
11. Tikkanen, M. J., Cole, T. G., Hahn, K.-S., Krul, E. S., and Schonfeld, G. (1984) Arteriosclerosis 4, 135–146
12. Krul, E. S., Kleinman, Y., Kinoshita, M., Pfleger, B., Oida, K., Law, A., Scott, J., Pease, R., and Schonfeld, G. (1988) J. Lipid Res. 29, 937–947
13. Tikkanen, M. J., Cole, T. G., and Schonfeld, G. (1988) J. Lipid Res. 29, 1494–1499
14. Kleinman, Y., Schonfeld, G., Gavish, D., Oshry, Y., and Eisenberg, E. (1987) J. Lipid Res. 29, 540–546
15. Chen, G. C., Zhu, S., Hardman, D. A., Schilling, J. W., Lau, K., and Kane, J. P. (1989) J. Biol. Chem. 264, 14369–14375
16. Aviram, M., Lund-Katz, S., Phillips, M. C., and Chait, A. (1988) J. Biol. Chem. 263, 16842–16848
17. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275
18. Kane, J. P., Sata, T., Hamilton, R. L., and Havel, R. J. (1975) J. Clin. Invest. 56, 1622–1634
19. Huang, H. J., Kuan, J. W., and Guibault, G. G. (1975) Clin. Chem. 21, 1605–1608
20. Steward, C. P., and Hendry, F. R. (1983) Biochem. J. 209, 1683–1689
21. Bucolo, G., and David, H. (1973) Clin. Chem. 19, 476–482
22. Chen, G. C., Kane, J. P., and Hamilton, R. L. (1984) Biochemistry 23, 1119–1124
23. Young, S. G., Berties, S. J., Curtis, L. K., Casal, D. C., and Witztum, J. L. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 1101–1105
24. Toshin, H., Stuehelin, T., and Gordon, J. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 4390–4394
25. Mutsudaira, P. (1987) J. Biol. Chem. 262, 10035–10038
26. Hunkapiller, M. W., and Hood, L. E. (1983) Methods Enzymol. 91, 486–493
27. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
28. Cardin, A. D., Witt, K. R., Chao, J., Margolius, H. S., Donaldson, V. H., and Jackson, R. I. (1984) J. Biol. Chem. 259, 8529–8528
29. Knott, T. J., Wallis, S. C., RaiI, S. C., Jr., Innerarity, T. L., Blackhart, B., Taylor, W. H., Marech, Y., Milne, R., Johnson, D., Fuller, M., Lusis, A. J., McCarthy, B. J., Mahley, R. W., Levy-Wilson, B., and Scott, J. (1986) Nature 323, 734–738
30. Pease, R. J., Milne, R. W., Jessup, W. K., Law, A., Provost, P., Fruchtman, J. C., Dean, R. T., Marsh, L. Y., and Scott, J. (1990) J. Biol. Chem. 265, 553–568
31. Ludwig, E. H., Blackhart, B. D., Pierotto, V. K., Casati, L., Fortier, C., Knott, T., Scott, J., Mahley, R. W., Levy-Wilson, B., and McCarthy, B. J. (1987) DNA 6, 363–372
32. Proctor, A. A., Hardman, D. A., Schilling, J. W., Miller, J., Appleby, V., Chen, G. C., Kirshner, S. W., McEnrooe, G., and Kane, J. P. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 5678–5682
33. Carlson, P., Darnfors, C., Olofsson, S.-O., and Bjursell, G. (1986) Gene (Amst.) 49, 29–51
34. Cladaras, C., Hadziopoulu-Cladaras, M., Nolte, R. T., and guns, L. E. (1986) EMBO J. 5, 3495–3507
35. Law, S. W., Grant, S. M., and Higuchi, K. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 8142–8146
36. Chen, S.-H., Yang, C.-Y., Chen, P.-F., Setzer, D., Tanimura, M., Li, W.-H., Oschi, A. M., Jr., and Chan, L. (1988) J. Biol. Chem. 263, 12918–12921
37. Yang, C.-Y., Gu, Z.-W., Weng, S.-A., Kim, T.-W., Chen, S.-H., Pownall, H. J., Sharp, P. M., Liu, S.-W., Li, W.-H., Goto, A. M., Jr., and Chan, L. (1989) Arteriosclerosis 9, 96–108
38. Schumaker, V. N., Robinson, M. T., Curtiss, L. K., Butler, R., and Sparkes, R. S. (1984) J. Biol. Chem. 259, 6499–6500.
39. Dunning, A. M., Duriez, P., Vu Dac, N., Fruchart, J. C., and Humphries, S. E. (1988) Clin. Genet. 33, 181–188.
40. Milne, R. W., Blanchette, L., Theolis, R., Jr., Weech, P. K., and Marcel, Y. L. (1987) Mol. Immunol. 24, 435–447.
41. Weisgraber, K. H., and Rall, S. C., Jr., (1976) J. Biol. Chem. 262, 11097–11103.
42. Chou, P. Y., and Fasman, G. D. (1978) Annu. Rev. Biochem. 47, 251–276.
43. Chen, G. C., and Kane, J. P. (1975) Biochemistry 14, 3357–3362.
44. Chen, G. C., Chapman, M. J., and Kane, J. P. (1983) Biochim. Biophys. Acta 754, 451–456.
45. Milne, R., Theolis, R., Jr., Maurice, R., Pease, R. J., Weech, P. K., Rassart, E., Fruchart, J.-C., Scott, J., and Marcel, Y. L. (1989) J. Biol. Chem. 264, 19754–19760.
Conformation of apolipoprotein B-100 in the low density lipoproteins of tangier disease. Identification of localized conformational response to triglyceride content. S T Kunitake, S G Young, G C Chen, C R Pullinger, S Zhu, R J Pease, J Scott, P Hass, J Schilling and J P Kane

J. Biol. Chem. 1990, 265:20739-20746.

Access the most updated version of this article at http://www.jbc.org/content/265/34/20739

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/265/34/20739.full.html#ref-list-1