Identification of the Proteoglycan Binding Site in Apolipoprotein B48*

Christofer Flood‡§, Maria Gustafsson‡§, Paul E. Richardson¶, Stephen C. Harvey¶, Jere P. Segrest¶, and Jan Börn‡**

From the ¤Wallenberg Laboratory for Cardiovascular Research, The Sahlgrenska Academy at Göteborg University, S-413 45 Göteborg, Sweden and the ¶Department of Biochemistry and Molecular Genetics and the ‡Department of Medicine and Atherosclerosis Research Unit, University of Alabama, Birmingham, Alabama 35294

Received for publication, April 25, 2002, and in revised form, June 6, 2002 Published, JBC Papers in Press, June 17, 2002, DOI 10.1074/jbc.M204053200

An initial event in atherosclerosis is the retention of lipoproteins within the intima of the vessel wall. Previously we identified Site B (residues 3359–3369) in apolipoprotein (apo) B100 as the proteoglycan binding sequence in low density lipoproteins (LDLs) and showed that the atherogenicity of apoB-containing lipoproteins is linked to their affinity for artery wall proteoglycans. However, both apoB100- and apoB48-containing lipoproteins are equally atherogenic even though Site B lies in the carboxyl-terminal half of apoB100 and is absent in apoB48. If binding to proteoglycans is a key step in atherogenesis, apoB48-containing lipoproteins must bind to proteoglycans via other proteoglycan binding sites in the amino-terminal 48% of apoB. In vitro studies have identified five clusters of basic amino acids in de-lipidated apoB48 that bind negatively charged glycosaminoglycans. To determine which of these sites is functional on LDL particles, we analyzed the proteoglycan binding activity of recombinant human LDLs from transgenic mice or rat hepatoma cells. Substitution of neutral amino acids for the basic amino acids in Site B—Ib (residues 84–94) abolished the proteoglycan binding activity of recombinant apoB53. Carboxyl-truncated apoB80 bound biglycan with higher affinity than apoB100 and apoB48.apoB80 in which Site B was mutated had the same affinity for proteoglycans as apoB48.

These data support the hypothesis that the carboxyl terminus of apoB100 “masks” Site B—Ib, the amino-terminal proteoglycan binding site, and that this site is exposed in carboxyl-truncated forms of apoB. The presence of a proteoglycan binding site in the amino-terminal region of apoB may explain why apoB48- and apoB100-containing lipoproteins are equally atherogenic.

Elevated levels of low density lipoproteins (LDLs)1 and other lipoproteins containing apolipoprotein (apo) B cause increased atherosclerosis, but the molecular and cellular mechanisms for the pathobiological changes that lead to the disease are still poorly understood. Accumulating evidence during recent years has led to the concept that subendothelial retention of apoB100-containing lipoproteins is the initiating event in atherogenesis (1, 2). Subsequently, a series of biological responses to this retained material leads to specific molecular and cellular processes that promote lesion formation (1).

Previously we identified Site B (residues 3359–3369) in apoB100 as the principal proteoglycan binding sequence (3) and presented direct experimental evidence that the atherogenicity of apoB-containing lipoproteins is linked to their affinity for artery wall proteoglycans (2). Mice expressing proteoglycan binding-defective LDLs developed significantly less atherosclerosis than mice expressing wild-type control LDLs (2). However, Site B lies in the carboxyl-terminal half of apoB100 and is absent in apoB48. This finding presented a paradox because numerous studies have demonstrated that apoE-deficient (ApoE−/−) mice develop severe atherosclerosis (4–6). What is less appreciated is that these animals contain mainly apoB48-containing lipoproteins, which must therefore be the type of lipoprotein that causes their atherosclerosis. In addition, at similarly high cholesterol levels, gene-targeted mice expressing only apoB48 or only apoB100 developed an equivalent degree of atherosclerosis (7). If binding to artery wall proteoglycans is a key step in atherogenesis, these apoB48-containing lipoproteins must bind to proteoglycans at other proteoglycan binding sites in the amino-terminal 48% of the apoB molecule.

apoB48 interacts with heparin (8, 9), but its interaction with artery wall proteoglycans has not been evaluated. In this study, we analyzed the interaction between apoB48-containing lipoproteins and artery wall proteoglycans, identified the proteoglycan binding site of apoB48, and elucidated why this site is nonfunctional in full-length apoB100.

EXPERIMENTAL PROCEDURES

Materials—The QuikChange mutagenesis kit was from Stratagene (La Jolla, CA), and primers were from Oligos Etc. (Wilsonville, OR). Tfx 50 was from Promega (Madison, WI). Genitin, and immunoprecipitation from Invitrogen. Eagle's minimal essential medium was from BioRad. A horseradish peroxidase-conjugated polyclonal antibody against human apoB was from The Binding Site (Birmingham, UK). 1-Step Turbo TMB-ELISA was from Pierce.

Generation of Mutant P1 Clones Encoding ApoB80 with Mutations in Site B—The 95-kb apoB P1 plasmid p158 (10) was prepared and mod-

* This work was supported by The Swedish Research Council, The Swedish Foundation for Strategic Research, and The Swedish Heart-Lung Foundation. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ Both authors contributed equally to this work and should be considered first authors.

** To whom correspondence should be addressed: Wallenberg Laboratory, Sahlgrenska University Hospital, S-413 45 Göteborg, Sweden and J.B. E-mail: Jan.Boren@wlab. wall.gu.se.

1 The abbreviations used are: LDL, low density lipoprotein; apo, apolipoprotein; GBSM, glycosaminoglycan binding sites mutated; CHD, cyclohexanone; ApoE−/−, apoE-deficient.
ifed by RecA-assisted restriction endonuclease cleavage as described previously (11). A 4.2-kb fragment containing the RK3359–3369SA mutation (3) was excised from the P1 plasmid RK3359–3369SA apoB100 (3) using oligomers EcoRI-35763 and EcoRI-39948 as described previously (3) and inserted into the pZErO 5.7-kb plasmid containing a stop codon at residue 3369 (d) in apoB80 (3). In the SDS-polyacrylamide gel filtration with a Superose 6 HR 10/30 column, the basic residues in Site B were converted to neutral amino acids. The 5.7-kb fragment was excised from the plasmid using oligomers EcoRI-35763 and EcoRI-41496 (3) and ligated into a 5.7-kb deleted P1 plasmid (3). The resulting P1 plasmid encodes apoB80 with Site B mutated (i.e. RK3359–3369SA apoB80).

**Generation of Mutant P1 Clones Encoding ApoB48 and 3-GBSM ApoB.** A 6-GBSM plasmid encoding apoB48 was generated by substitution of the Q2153L mutation at the editing site in the P1 apoB100 (3) with a translational termination codon (TA). The Q2153L mutation effectively abolishes the formation of apoB48, which is formed by an editing mechanism in mouse liver (12). The pZErO 5.7-kb plasmid was subjected to site-directed mutagenesis (QuikChange mutagenesis kit). The mutation was inserted into the 5.7-kb fragment using oligomer 5′-GCTGAGGAG-3′; and 5′-GCCAGTACACATACAACTAT-3′. Mutant colonies were screened for the lack of a PstI cleavage site. The same approach was used to generate the apoB P1 plasmid 3-GBSM (i.e. three glycosaminoglycan binding sites mutated) pZErO48 from the P1 apoB plasmid 6-GBSM apoB100 (2). In 6-GBSM apoB100, the six carboxyl-terminal sequences of the eight glycosaminoglycan binding sequences identified in delipidated apoB100 have been mutated (8, 13, 14).

**Binding studies of the Heparin Binding Sites on the Amino Terminal of ApoB.** A 2.45-kb EcoRI-HindIII fragment was excised from pb100L-1 and inserted into the pZErO1 vector (Invitrogen) to generate a subclone designated pZErO-2.45kb such as described previously (15). The pZErO-2.45kb was then subjected to site-directed mutagenesis. Different oligonucleotides were used to convert the basic amino acids arginine and lysines in the amino terminal to the neutral amino acids serine and glutamines, respectively: RK15–255Q, 5′-agctccgacctcctgcagatcacagctcagagccagcatgtggc-cttgaggag-3′; RK84–94Q, 5′-gcaagcttccagcacctca-ctctgaggag-3′; and RK221–231Q, 5′-cactggtcagacctgctttgctggaagacgg-3′. The three mutated 2.45-kb constructs were religated into human apoB53 Q2153L in a pCMV vector (15).

**Creation of Human ApoB Transgenic Mice.** Transgenic mice were generated at MouseCamp (Karolinska Institute, Stockholm, Sweden) by microinjection of P1 DNA into fertilized mouse eggs (B6CBA). Transgenic mice were identified with a tga47 apoB80-specific immunoblot using antibodies ID1 (16) and Bsd22 (16). The two founders with the highest levels of plasma apoB were selected for breeding and further analysis.

**Stable Transformation of Mutated ApoB53 into McArdle 7777 Cells.** McArdle 7777 cells were cultured as described previously (17). One day before transfection, the cells were seeded in a six-well plate (25 × 10^6) cells/well. The P1 plasmid apoB53 was transfected with pCDNA3.1 (+) (+) using Tfx 50 reagent at a 3:1 ratio. Two days after the transfection, Geneticin (600 μg/ml) was added to the culture medium. Colonies were picked after 2 weeks and screened for human apoB expression by Western blot with a horseradish peroxidase-conjugated polyclonal antibody against human apoB.

**Assembly and Secretion of Mutated Human ApoB53 in McArdle 7777 Cells.** Two clones of each construct were labeled with [35S]methionine as described previously (17). Cellular apoB and apoB in the medium were recovered by immunoprecipitation with a polyclonal anti-apoB antibody. After separation by electrophoresis on a 3–15% polyacrylamide gel containing 0.1% SDS, apoB was visualized by autoradiography.

**Production and Concentration of Human ApoB53.** Clones 36 and 58 expressing apoB53 and clones 18 and 20 expressing K84–94Q apoB53 were cultivated in 32 bottles (150 cm²) each, containing 50 ml of medium. After 3 days, the medium was collected, and 1 mM methylsulfonyl fluoride and 10 units/ml aprotinin were added. The medium was centrifuged for 5 min at 600 × g and concentrated in a stirred ultrafiltration cell (Amicon) with a Millipore membrane (molecular weight, 100,000). The d < 1.07 g/ml supernatant was isolated by sequential ultracentrifugation (TI 70 rotor) and dialyzed against 150 mM NaCl and 0.01% EDTA, pH 7.4, and endogenous rat apoB and apoE were removed by immunoaffinity chromatography (3). After ultracentrifugation (d = 1.07 g/ml) at 657,000 × g for 4 h in a TLA 110 rotor, the top 1 ml was recovered and separated by fast performance liquid chromatography gel filtration (AKTA Explorer, Amersham Biosciences) with a Superose 6 HR 10/30 column. Aliquots of 500 μl were injected onto the column and separated with phosphate-buffered saline buffer, pH 7.4, at a flow rate of 0.2 ml/min. The fractions corresponding to LDL size were pooled and subjected to binding studies.

**Isolation of Recombinant Lipoproteins.** Recombinant LDLs (d = 1.02–1.05 g/ml) were isolated by sequential ultracentrifugation and dialyzed against 150 mM NaCl and 0.01% EDTA, pH 7.4. Mouse apoE and apoB or rat apoE and apoB were removed by immunoaffinity chromatography with rabbit polyclonal antibodies (3).

**Competition of Mouse ApoE with Chemically Modified Human ApoE.** ApoE–ApoB48-containing lipoproteins (d = 1.02–1.05 g/ml) isolated from transgenic mice (3) were incubated with a 200-fold molar excess of cyclohexanedione (CHD)-modified apoE from human very low density lipoproteins as described previously (2). After ultracentrifugation (d = 1.07 g/ml) at 657,000 × g for 4 h in a TLA 110 rotor, the top 1 ml was recovered and separated by fast performance liquid chromatography gel filtration with a Superose 6 HR 10/30 column to eliminate contaminating free apoE. Fractions corresponding to LDL size were pooled and subjected to binding studies. The incubation with CHD-modified apoE was performed when not all endogenous apoE was removed by immunoaffinity chromatography.

**Solid-phase Assays of LDL Binding to Biglycan or Decorin.** Maxisorp immunoplates (NUNC) were coated with biglycan or decorin (10 μg/ml) in HBS buffer (20 mM HEPES, 150 mM NaCl, pH 7.4) overnight at room temperature and blocked with HBS buffer with 1% bovine serum albumin for 1 h at room temperature. LDL samples in HBS buffer with 2 mM CaCl₂ and 2 mM MgCl₂ were added to the wells and incubated for 1 h at room temperature. The plates were then incubated with the same buffer supplemented with lipoprotein-deficient serum (diluted 1:50) for 30 min. To each well, 100 μl of a horseradish peroxidase-conjugated polyclonal antibody against human apoB (The Binding Site; diluted 1:750 in HBS buffer with 0.1% bovine serum albumin and 0.05% Tween 20) was added. After 1 h of incubation at 1.5 h. Finally, 100 μl of 1-Step Turbo TMB-ELISA (Pierce) substrate was added and incubated for 5 min. The reactions were stopped with an equal volume of 2 M H₂SO₄ and measured at 450 nm in a spectrophotometer.

**RESULTS**

**Proteoglycan Binding of ApoE-free ApoB48-containing Lipoproteins.** To determine whether lipoproteins containing apoB48 bind to artery wall proteoglycans, we performed binding studies with LDLs from Apo⁻/⁻ gene-targeted mice. Scanning of the SDS-polyacrylamide gels revealed that the d = 1.02–1.05 g/ml fraction contained greater than 95% apoB48. The binding studies revealed that apoB48 LDLs bind to biglycan (Fig. 1). To verify that the binding of apoB48 LDLs to proteoglycans was mediated by apoB48 and not by the lipids of the lipoprotein, we treated the lipoproteins with CHD or acetic anhydride (not shown) to modify the arginines and lysines, respectively. After chemical modification, the apoB48 LDLs had greatly reduced ability to interact with biglycan (Fig. 1). These results were verified in binding experiments with decorin and the total proteoglycan fraction from aortic smooth muscle cells (not shown). Thus, proteoglycan binding sites other than those on normal LDLs are exposed and physiologically important on apoB48 LDLs.
The Proteoglycan Binding Site in Apolipoprotein B48

**TABLE I**

Amino acid sequences of eight putative glycosaminoglycan binding regions of apoB

| Site (amino acids) | Amino acid sequence |
|-------------------|---------------------|
| B-Ia (15–25)      | Asp Ala Thr Arg Phe Lys His Leu Arg Lys Tyr |
| B-Ib (84–94)      | Ala Leu Leu Lys Thr Lys Asn Ser Glu Glu |
| B-II (222–232)    | Leu Asp Ala Lys Arg Lys His Val Ala Glu Ala |
| B-III (900–910)   | Pro Ser Pro Lys Arg Pro Val Lys Leu Leu Ser |
| B-IV (2079–2089)  | Gln Phe Val Arg Lys Tyr Arg Ala Ala Leu Gly |
| B-V (2117–2127)   | Ala Leu Thr Lys Lys Tyr Arg Ile Thr Glu Asn |
| B-VI (3148–3158)  | Ala Gln Tyr Lys Lys Asn Lys His Arg His Ser |
| B-VII (3359–3369) | Arg Leu Thr Arg Lys Arg Gly Leu Lys Leu Ala |
| B-VIII (3686–3678) | Ser Ile Gly Arg Arg Gln His Leu Arg Val Ser |

*The first putative glycosaminoglycan binding region was divided into B-Ia (15–25) and B-Ib (84–94).*

**Fig. 2.** Position of the putative glycosaminoglycan binding sites in apoB48 and apoB100. The sites that were subjected to site-directed mutagenesis in apoB48-containing 3-GBSM LDLs and apoB100-containing 6-GBSM LDLs are marked.

**Generation and Binding Studies of Recombinant ApoB48**—In vitro studies of delipidated apoB100 have identified eight clusters of basic amino acids in the apoB moiety of LDLs that bind to the negatively charged glycosaminoglycans (8, 13, 14) (Table I). However, only one of these putative glycosaminoglycan binding sites (i.e. Site B-VII at residues 3359–3369) is functionally exposed on the surface of apoB100 LDLs (18). Five of the sites (B-I–B-V) are present in apoB48. To elucidate the role of these sites in the binding of proteoglycans by apoB48 LDLs, we generated transgenic mice expressing wild-type apoB48 or 3-GBSM apoB48, a mutant form of apoB48 in which the basic amino acids in Sites B-I–B-V were converted to neutral amino acids (arginines to serines and lysines to alanines) (Fig. 2). Because of the apparent importance of the amino-terminal domain of apoB100 in the formation of nascent very low density lipoproteins, we did not mutate the two heparin binding sites in the extreme amino terminus of the human apoB gene.

**Solid-phase Assays of Recombinant LDLs with Biglycan**—To determine the ability of the recombinant LDLs to interact with proteoglycans, recombinant control apoB48 LDLs, apoB48 3-GBSM LDLs, recombinant apoB100 control LDLs, and apoB100 6-GBSM LDLs were isolated from transgenic mice (2, 3). In apoB100-containing 6-GBSM LDLs, the basic amino acids in the six putative carboxyl-terminal glycosaminoglycan binding sequences (Table I) in apoB100 were converted to neutral amino acids (2) (Fig. 2). Previously we showed that apoB100-containing 6-GBSM LDLs do not interact with artery wall proteoglycans (2).

In addition to the direct interaction between LDL protein and proteoglycans, retention of LDLs in the extracellular matrix of the artery wall also appears to involve indirect binding facilitated by apoE (1, 19, 20). Thus, it was important to deplete the lipoproteins of apoE. This was especially important for apoB48 LDLs, which are more enriched in apoE than apoB100 LDLs. Immunoaffinity chromatography removed all of the apoE from recombinant apoB100 LDLs but only 32 ± 5% of the apoE from apoB48 LDLs. Therefore, we incubated the recombinant apoB48 LDLs with a 200-fold molar excess of CHD-modified apoE isolated from human very low density lipoproteins to replace the endogenous apoE with CHD-modified human apoE, which does not interact with proteoglycans (2). Endogenous mouse apoB was removed by immunoaffinity chromatography (3). Control experiments showed that the competition with CHD-modified apoE does not affect the ability of apoB to interact with glycosaminoglycans (not shown).

The purified recombinant LDLs, containing recombinant apoB48 or apoB100 only and no contaminating proteins (not shown), were subjected to solid-phase assays with artery wall proteoglycans. In two independent experiments, recombinant control apoB48 LDLs and recombinant control apoB100 LDLs bound biglycan with high affinity, whereas recombinant apoB100 6-GBSM LDLs displayed severely impaired binding to biglycan (Fig. 3). Of particular interest, recombinant apoB48 3-GBSM LDLs interacted normally with biglycan (Fig. 3). These results were verified in binding experiments with decorin and the total proteoglycan fraction from aortic smooth muscle cells (not shown). These results indicate that one or both of the putative glycosaminoglycan binding sequences that are not mutated in apoB48 3-GBSM LDLs (i.e. Sites B-I and B-II) are exposed and functional in apoB48 LDLs. These data also indicate that these site(s) are nonfunctional in apoB100.

**Molecular Model of Glycosaminoglycan Binding Sites B-I and B-II**—To better understand how Sites B-I and B-II are located in the lipoproteins, we performed molecular modeling studies of the amino-terminal 6.6% of apoB. Regions of multiple local sequence homology between vertebrate lipovitellin and mammalian apoB were identified with the program MACAW (21, 22). The regions of local sequence homology identified in the first 1000 residues of human apoB were then used to orient the sequence for molecular modeling by the Modeler-4 program.
FIG. 3. Binding of recombinant apoB48 and apoB100 LDLs to biglycan. The binding of recombinant control apoB100 LDLs (○), recombinant control apoB48 LDLs (□), apoB100 6-GBSM LDLs (○), and apoB48 3-GBSM LDLs (■) to biglycan is shown. Endogenous apoE was removed by immunoaffinity techniques from the apoB100-containing LDLs and replaced with CHD-modified human apoE on the apoB48-containing LDLs. Endogenous mouse apoB was removed from all lipoproteins by immunoaffinity chromatography. The results represent mean values (±S.D.) from two independent experiments, each performed with freshly isolated LDLs from 25 mice of each group (n = 4 in each experiment).

A molecular model for the first 300 amino acids of human apoB created with the molecular graphics program RIBBONS (24) is shown in Fig. 4. The model shows that Site B-I consists of two separate domains, Sites B-Ia (residues 15–94) and B-Ib (residues 84–94) (Table I) and that Sites B-Ia and B-II form a single contiguous positively charged domain. Site B-Ib is at the other end of the C domain (25).

The extent of homology (i.e. sequence identity) in the model was 19.3%. Furthermore, the similarity (i.e. conserved substitutions) was 38.0%. These values are similar to those of the apoB model reported by Mann et al. (26). Those values were 19.4% and 35.5%, respectively.

Expression of Mutated ApoB53 by McA-RH7777 Cells—To identify the proteoglycan binding sequence in apoB48, the basic amino acids in regions B-Ia, B-Ib, and B-II were converted to neutral amino acids (arginines to serines and lysines to glutamines). Stably transfected McA-RH7777 cells expressing recombinant control apoB53, RK15–25SQ apoB53, K84–94Q apoB53, or RK221–231SQ apoB53 were generated. Analysis of two clones per construct showed that recombinant control apoB53 and K84–94Q apoB53 were secreted from the cells (Fig. 5). RK15–25SQ and RK221–231SQ apoB53 were expressed in the cells but were not secreted (Fig. 5).

Solid-phase Assays of Recombinant ApoB53 LDLs with Biglycan—To determine the importance of Site B-Ib (residues 84–94) in the interaction with artery wall proteoglycans, recombinant control apoB53 LDLs and K84–94Q apoB53 LDLs were isolated from the cell culture medium by ultracentrifugation and size-exclusion chromatography. Approximately 84 ± 5% of the endogenous rat apoB and 74 ± 6% of the apoE were removed by immunoaffinity chromatography techniques. However, in contrast to highly apoE-enriched recombinant apoB48 LDLs isolated from transgenic mice, recombinant apoB53 LDLs isolated from McA-RH7777 cells contain significantly less apoE. Therefore, only trace amounts of apoE were detected in purified apoB53 LDLs after immunoaffinity chromatography (not shown).

In two independent experiments, recombinant control apoB53 bound biglycan with high affinity, whereas K84–94Q apoB53 displayed severely impaired binding to biglycan (Fig. 6). These results were obtained with two different lines of each construct and verified in binding experiments with decorin and the total proteoglycan fraction from aortic smooth muscle cells (not shown). These data indicate that Site B-Ib in apoB53 interacts with proteoglycans.

Generation of Transgenic Mice Expressing ApoB80 and RK3359–3369SA apoB80—The first 89% of apoB100 enwraps the LDL particle like a belt, and the carboxyl-terminal 11% constitutes a “bow” that crosses over the belt and brings the carboxyl tail of apoB100 close to amino acid 3500 (Fig. 7). Thus, the epitopes recognized by the monoclonal antibodies MB19 (residue 71; apoB2) (27, 28) and MB43 (residues 4027–4081; apoB89) (29) are very close to or even on top of each other (30). Site B-Ib (residues 84–94) is located at apoB2. To test the hypothesis that the carboxyl terminus of full-length apoB100 masks this site in apoB100 LDLs, we generated human apoB transgenic mice expressing apoB80 or RK3359–3369SA apoB80 (i.e. apoB80 with Site B mutated). Recombinant LDLs containing control apoB100, apoB80, RK3359–3369SA apoB80, and apoB48 were isolated, and endogenous mouse apoB was removed by immunoaffinity techniques. Endogenous apoE was removed from the apoB100- and apoB80-containing lipoproteins by immunoaffinity chromatography and replaced with CHD-modified human apoE on the apoB48 LDLs.

In two independent experiments, apoB80 bound biglycan and decorin (not shown) with higher affinity than apoB100 (Fig. 8). RK3359–3369SA apoB80 (Site B mutated) had the same affinity for the proteoglycans as apoB48 (Fig. 8). These data support the hypothesis that the carboxyl terminus of apoB100 masks Site B-Ib and that this site is exposed in carboxyl-truncated forms of apoB. Thus, apoB80 binds to proteoglycans using both the amino-terminal (i.e. Site B-Ib) and the carboxyl-terminal (i.e. Site B-VII) proteoglycan binding sites in apoB. ApoB80 in which Site B has been mutated interacts with the amino-terminal proteoglycan binding site only and displays the same affinity as apoB48 for artery wall proteoglycans.

DISCUSSION

In this study, we investigated the molecular mechanism underlying the interaction of apoB48-containing lipoproteins and artery wall proteoglycans. The K84–94Q substitution in apoB53 abolished the interaction with artery wall proteoglycans. These results identified Site B-Ib (residues 84–94) as the proteoglycan binding site of apoB48. This site is located at the other end of the B domain where Sites B-Ia and B-II form a single contiguous positively charged domain. Thus, it is less likely that Site B-Ib acts cooperatively with Sites B-Ia and B-II in the association with proteoglycans. This possibility could not be tested since RK15–25SQ and RK221–231SQ apoB53 were sorted to posttranslational degradation and not secreted.

Proper folding of the amino-terminal globular domain of apoB is essential for the biosynthesis of apoB-containing lipoproteins, and two binding domains for microsomal triglyceride transfer protein and artery wall proteoglycans. The K84–94Q substitution in apoB53 abolished the interaction with artery wall proteoglycans. Therefore, apoB48 and the carboxyl-terminal 17% of apoB was functional in apoB48 (26, 31, 32). These domains correspond to apoB residues 1–152 (26) and residues 512–592 (31, 32). Thus, it is possible that substitution of neutral amino acids for the basic amino acids in the contiguous positively charged domain in the amino terminus of apoB directly or indirectly disrupts the interaction between microsomal triglyceride transfer protein and apoB. This would explain the abolished secretion of RK15–25SQ and RK221–231SQ apoB53. The identification of Site B-Ib as the proteoglycan binding site in apoB48 is in agreement with results reported by Goldberg et al. (9). These investigators analyzed the interaction between an artificial nonlipidized apoB fragment and proteoglycans and found that the amino-terminal 17% of apoB is sufficient for association with glycosaminoglycans (9). However, it was not clear whether any of the putative glycosaminoglycan binding sites present in the nonlipidized apoB fragment were functionally exposed on the surface of LDL particles since only one of the eight glycosaminoglycan binding sites that have been identified in delipidated apoB100 is functional in apoB100.
LDLs (18). Therefore, structure/function studies of apoB must be performed with native LDLs.

What is the biological significance of Site B-Ib in lipid metabolism and atherogenesis? In humans, apoB48-containing lipoproteins are enriched in apoE, a well-known heparin-binding protein. Thus, the importance of site B-Ib for interaction with glycosaminoglycans is unclear, and it is possible that all binding is mediated by apoE. However, Goldberg et al. (9) have argued against the hypothesis that apoB48-containing lipoproteins require additional proteins for retention, and they have stated that the hypothesis that apoE promotes atherosclerosis by causing retention of apoB48 remnants is not tenable. We recently showed that apoE can substitute for the defective proteoglycan binding of mutant forms of apoB100 (2), and we have found that apoE-containing apoB48 LDLs bind proteoglycans with higher affinity than apoE-depleted apoB48 LDLs (not shown). These data indicate that Site B-Ib and apoE on apoB48-containing lipoproteins act cooperatively in binding to
proteoglycans. The finding that apoB48 isolated from Apoe−/−
gene-targeted mice binds proteoglycans is important because it
is consistent with the response-to-retention hypothesis. How-
ever, direct experimental evidence for a role of Site B-Ib in
atherogenesis is lacking and has to be addressed in future
studies.

Previously we proposed that the carboxyl-terminal tail of
apoB100 serves as a modulator that can alter the affinity of the
receptor binding domain of apoB100 (3) and that the interac-
tion between arginine 3500 and tryptophan 4369 is essential
for correct conformation of the carboxyl-terminal tail of
apoB100 (33). LDLs containing apoB95 lack tryptophan 4369
and therefore have enhanced receptor binding; in contrast,
apoB97 LDLs contain tryptophan 4369 and bind normally (33, 34).
However, both apoB95 and apoB97 display the same bind-
ing affinity for both biglycan and decorin (not shown). There-
fore, we do not believe that this mechanism explains the in-
creased affinity of apoB80 for proteoglycans.

Instead we suggest a model that explains why apoB100- and
apoB48-containing lipoproteins are equally atherogenic. In
apoB100, Site B-Ib, the amino-terminal proteoglycan binding
site, is masked by the carboxyl terminus, whereas in carboxyl-
truncated forms of apoB, Site B-Ib is exposed. ApoB80 uses
both the amino-terminal and the carboxyl-terminal proteogly-
can binding sites to interact with proteoglycans. ApoB80 in
which Site B has been mutated uses only the amino-terminal
proteoglycan binding site and displays the same affinity for
artery wall proteoglycans as apoB48 (Fig. 8).

In summary, we have identified Site B-Ib as the principal
proteoglycan binding site in apoB48 LDLs and have proposed a
model that explains why this site is masked and nonfunctional
in full-length apoB100 LDLs. The presence of a proteoglycan
binding site in the amino-terminal region of apoB is consistent
with the response-to-retention hypothesis and may explain why
apoB48- and apoB100-containing lipoproteins are equally
atherogenic.

Acknowledgments—We thank Lisbeth Lindgren, Kristina Skålen,
Elin Björk, and Anita Lund for technical assistance and Stephen Ord-
way for editorial assistance.

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