The Human Factor H-related Protein 4 (FHR-4)

A NOVEL SHORT CONSENSUS REPEAT-CONTAINING PROTEIN IS ASSOCIATED WITH HUMAN TRIGLYCERIDE-RICH LIPOPROTEINS

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Christine Skerka, Jens Hellwage, Wilfried Weber‡, Anne Tilkorn‡, Friedrich Buck§, Thomas Marti, Eva Kampen, Ulrike Beisiegel‡, and Peter F. Zipfel¶

From the Bernhard Nocht Institute for Tropical Medicine, Bernhard-Nocht-Strasse 74, 20359 Hamburg, the Medical Clinic, University Hospital Eppendorf, Martinistrasse 52, 20246 Hamburg, and the Institute for Cell Biology and Clinical Neurobiology, University of Hamburg, Suderfeldstrasse 24, 20246 Hamburg, Germany

A novel apoprotein of an apparent molecular mass of 86 kDa in its unreduced form was identified in human triglyceride-rich lipoproteins. This protein was purified and the amino acid sequence of six proteolytic fragments was found to overlap with that of the factor H-related proteins. In parallel we identified the cDNA encoding a new complement factor H-related protein, termed FHR-4. The sequences of the new apoprotein overlapped with that of the FHR-4 protein. Similar to the previously described factor H-related proteins, FHR-4 contains a hydrophobic signal sequence followed by a stretch of five repetitive elements termed short consensus repeats. Recombinant FHR-4 protein was expressed in the baculovirus system and has an apparent molecular mass of 42 kDa. In addition a 84-kDa dimeric form of the recombinant FHR-4 was detected. Using an immunoaffinity column with antibodies raised against the recombinant FHR-4, we isolated a 86-kDa protein from human plasma. The different molecular mass of the recombinant FHR-4 and the dimeric FHR-4 in plasma is due to different carbohydrate moieties. The 86-kDa plasma protein and the novel apolipoprotein had identical mobility on SDS-polyacrylamide gel electrophoresis analysis and reacted with antiserum raised against the recombinant FHR-4 and the purified apoprotein. In conclusion, we have identified a novel factor H-related protein, FHR-4, in human plasma and demonstrate that this protein is present in triglyceride-rich lipoproteins in a dimeric form. This observation provides an intriguing new aspect on possible function(s) of this novel protein and the other factor H-related proteins.

Several human factor H-related plasma proteins have been identified recently that represent a family of structurally and immunoologically related proteins and that are termed factor H-related proteins 1–3 (FHR-1 to FHR-3).1 Similar to human complement factor H, these proteins are exclusively composed of repetitive elements termed short consensus repeats (SCRs). Factor H-related molecules of human and mouse origin have been isolated on the cDNA, the protein, and the genomic level (1, 2). Three distinct human factor H-related cDNA clones, termed H36 (or pFH1.4), DDESK59, and DOWN16 have been isolated, and the corresponding plasma proteins are termed factor H-related proteins 1–3 (FHR-1 to FHR-3) (2–6). The H36 cDNA encodes two human plasma proteins of 37 and 42 kDa, which represent the differently glycosylated forms FHR-1a and FHR-1c (7). FHR-1b has two carbohydrate side chains attached, and the FHR-1a protein has one carbohydrate side chain attached. Similarly the DDESK59 cDNA encodes a glycosylated 29-kDa (FHR-2a) and a nonglycosylated 24-kDa plasma protein (FHR-2). The product of the DOWN16 cDNA, the FHR-3 protein, has been described as a 55-kDa plasma protein.7 Similarly, four factor H-related cDNAs, termed 13G1, 23L1, 3A4, and 9C4, have been isolated from a mouse liver cDNA library (8). Genomic analysis has demonstrated that the human FHR-2 gene and the mouse FHR transcripts are derived from loci that are distinct from the factor H gene (9, 10). Factor H, FHL-1, and all FHR proteins are structurally related, and Northern blot analyses confirmed that all identified molecules of human and mouse origin are synthesized in the liver. Individual SCRs of these proteins display a significant identity to each other and to SCRs of factor H. The proteins are also immunologically related: all human proteins react with antiserum that was raised against human factor H (2).

Several apoproteins have been described to be important structural and functional components of human triglyceride-rich lipoproteins (TG-Lp), as there are chylomicrons (CM) and very low density lipoproteins (VLDL) (11–13). The main structural apoproteins are apoB-48 in TG-Lp and apoB-100 in VLDL. Both lipoproteins contain apoE, which serves as ligand for lipoprotein receptors (14), apoC-II as a cofactor for lipoprotein lipase activity (15), and apoC-III, known to modulate the receptor binding affinity (16). ApoA-I and ApoA-IV are further apoproteins associated with these TG-Lp, and both are known to activate the enzyme lecithin-cholesteryl acyltransferase (17, 18). However, detailed studies on further possible human apoproteins of plasma CM or VLDL have not been performed.

In our experiments with iodinated human TG-Lp, we detected density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; ChR, chylomicron remnants; CM, chylomicrons; TG-Lp, triglyceride-rich lipoproteins; PBS, phosphate-buffered saline; PAGE, polyacrylamide gel electrophoresis; FPLC, fast protein liquid chromatography; HPLC, high performance liquid chromatography.

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ected one additional major apoprotein that has not yet been characterized. This novel apoprotein was purified and found to be identical to the factor H-related protein 4, which was cloned and recombinantly expressed in parallel. The recombinant FHR-4 protein, as expressed in insect cells, is of amphipathic nature and the native protein is detected as a homodimer in human plasma. The FHR-4 plasma protein is found free and associated with TG-Lp and other lipoproteins.

EXPERIMENTAL PROCEDURES

Preparation of Human Lipoproteins—Human TG-Lp were obtained from plasma of patients with lipoprotein lipase deficiency, a genetic disorder that leads to massive accumulation of TG-Lp, due to lack of the hydrolytic activity in plasma (19). The TG-Lp were isolated from 36 ml of plasma by ultracentrifugation. The plasma was adjusted to 10% sucrose, and 6 ml were layered under 6 ml of PBS. The first separation was performed in the SW 42 rotor (Beckmann) for 16 h at 4°C, 88,000 rpm. The TG-Lp were isolated from the top of the tube and again adjusted to 10% sucrose. 2 ml were layered under 10 ml of PBS and centrifuged under the same conditions. The TG-Lp were isolated from the top, and the protein concentration was determined by a modification of the Lowry method (20). The protein content of the isolated TG-Lp were typically around 0.46 mg/ml. The TG-Lp were iodinated with Na125I by the iodine-monochloride method (21). The specific activity reached was 20–80 cpm/ng of protein. For the SDS-PAGE 100 µl of iodinated TG-Lp were delipidated in chloroform/methanol (8:5) and directly applied to an SDS-PAGE for protein analysis. The bottom (B1) was also delipidated and analyzed on the SDS-PAGE. For a further separation the lipoproteins were again adjusted to the density 1.21 g/ml with KBr and underlayered in the SW 42 rotor (Beckmann). The top (B1) and the middle (B2) fraction was taken from the top of the tubes and either used for the lipoprotein separation in a density gradient ultracentrifugation or were delipidated (chloroform/methanol, 8:5) and directly applied to an SDS-PAGE for protein analysis. The bottom (B1) was also delipidated and analyzed on the SDS-PAGE. For a further separation the lipoproteins were again adjusted to the density 1.21 g/ml with KBr and underlayered in the SW 42 rotor (Beckmann). The top (B1) and middle (B2) fraction was taken from the top of the tubes and either used for the lipoprotein separation in a density gradient ultracentrifugation or were delipidated (chloroform/methanol, 8:5) and directly applied to an SDS-PAGE for protein analysis. The bottom (B1) was also delipidated and analyzed on the SDS-PAGE. For a further separation the lipoproteins were again adjusted to the density 1.21 g/ml with KBr and underlayered in the SW 42 rotor (Beckmann). The top (B1) and the middle (B2) fraction was taken from the top of the tubes and either used for the lipoprotein separation in a density gradient ultracentrifugation or were delipidated (chloroform/methanol, 8:5) and directly applied to an SDS-PAGE for protein analysis. The bottom (B1) was also delipidated and analyzed on the SDS-PAGE.

Separation with FPLC—Plasma lipoproteins were separated on a Superose 6 column (10 × 300 mm; Pharmacia Biotech Inc.). The column was equilibrated with PBS. 200 µl of plasma were applied to the column, and the run was performed at room temperature with a flow rate of 0.3 ml/min. Protein was detected with the absorbance of 280 nm. Fractions of 0.5 ml were collected and prepared for SDS-PAGE. For the TG-Lp the whole fractions were delipidated with chloroform/methanol, 1:1 (v/v), while for the protein peak, corresponding to the bottom fraction of the ultracentrifugation step, only 5% of the material was used for gel electrophoresis.

Isolation of a Novel Apolipoprotein—For the isolation of water-soluble apolipoproteins the TG-Lp were delipidated as described above, and the apoproteins were extracted by PBS. Thereby the novel protein could be separated from the water-insoluble apolipoprotein B. The extraction was repeated twice, and the samples were concentrated by precipitation in chloroform/methanol (8:5) before SDS-PAGE. A 10% SDS-PAGE was performed according to Neville (22) with reduced samples. The proteins were electroblotted onto nitrocellulose and stained by Coomassie on both edges. The 106-kDa band of the novel apoprotein was cut out and used for immunization.

Protein Digest and Sequence Analysis—In order to obtain sequence information the 106-kDa protein was electroeluted from SDS-PAGE in a Biotrap elution chamber (Schleicher & Schuell, Dassel, Germany). The elution buffer was 25 mM Tris, 192 mM glycine, and 0.025% SDS, pH 8.6. The eluted protein was precipitated with chloroform/methanol (8:5) to remove the detergent and then digested with trypsin or V8 protease (protease sequencing grade, Boehringer, Mannheim, Germany). Digestions were performed in 100 µl of the respective standard buffers (0.1 M Tris/HCl, pH 8.5, 2 mM CaCl2 for trypsin; 0.1 M Tris/HCl, pH 8.0, for protease V8) at 37°C for 12 h at an estimated substrate/protease ratio of 5:1. The proteolytic fragments were again further purified by a second HPLC run (Nucleosil C8, 5 µm, 1.6 × 125 mm, gradient as above). Protein sequences were determined by standard Edman degradation on an automatic peptide sequencer (473A, Applied Biosystems).

Labeling of Oligonucleotide Probes and Screening—A human liver oligo(dT)-primed cDNA library in ZAP (Stratagene) was screened according to standard procedures (25) with a cDNA fragment (DOWN16) representing the previously described FHR-3 protein (5).

Characterization of Isolated Plasmids and Sequence Analysis of cDNA Clones—Several plasmids, which showed a restriction pattern distinct from the FHR-3 cDNA, were further analyzed. Their cDNA inserts were sequenced in double-stranded form by the dyeoxy chain termination method (24) using a32P-labeled Peptide Sequencing II (U. S. Biochemical Corp.). Several oligonucleotide primers were synthesized, and the sequence of the cDNA was determined in both orientations.

RNA Isolation and Northern Blot Analysis—Total cellular human RNA was extracted with guanidinium thiocyanate and isolated by centrifugation over CsCl (25). 8 µg of RNA were separated by electrophoresis in a formaldehyde-agarose gel and subsequently transferred to a nylon membrane (FALL).

SDS-PAGE and Western Blot Analysis—Recombinant FHR-4, delipidated lipoprotein fractions, and fractions obtained by Nickel chromatography were separated by SDS-PAGE using either 10 or 12% gels according to Neville (22) using unstained broad range markers (Bio-Rad) or a 12% SDS-PAGE according to Laemmli (23, 26) using prestained low range markers (Bio-Rad) as standards. Proteins were visualized either by Coomassie staining, silver staining, or were electroblotted. Proteins separated according to the Neville method were transferred to nitrocellulose in buffer chambers, while proteins separated by Laemmli SDS-PAGE were transferred by semidry blotting (27). Membranes were blocked for 30 min using either 5% (w/v) dried milk in PBS or 5% bovine serum albumin in Tris/HCl, pH 8.6. Incubations with the specific antibody were performed overnight and at the indicated times. Dilutions of the polyclonal rabbit antibodies used in the incubations were 1:500 for the antibody raised against the novel apolipoprotein and 1:1000 against the recombinant FHR-4. After washing in PBS for five times membranes were incubated with peroxidase-conjugated goat anti-rabbit antibody (Daco or Jackson) for 2–3 h. Protein bands were visualized by the addition of 0.3% (w/v) 4-chloro-1-naphthol in 10% (v/v) ethanol in methanol in PBS.

Labeling and Hybridization—For library screening a full-length FHR-3 fragment was used. A specific fragment of FHR-4, representing mainly SCR 5 and part of the 3′-untranslated region was used as a probe for Northern blot analysis. The insert was excised, purified on low melt agarose gels, and after labeling with 32P by random priming (Amersham Corp.) was used for hybridization at 47°C (10 × Denhardt′s, 5 × SET: 1 × SET: 150 mM NaCl, 30 mM Tris, 2 mM EDTA, pH 8.0, 0.1% SDS, 0.1% sodium pyrogenophosphate, and 250 mg/ml denatured salmon sperm DNA). Following hybridization for 14–18 h the filters were washed at a final stringency of 0.1 × SSC, 0.1% SDS at 47°C. The filters were exposed at −70°C using intensifying screens (Quant III, DuPont).

Immunopurification of the Native FHR-4 Protein from Human Plasma—Antiserum raised against rFHR-4 (10 µl) was coupled to 200 µl of protein A-Sepharose (Pharmacia) by agitation for 1 h at room temperature. After two washing steps in 10-fold volume of 0.2 mM sodium borate, pH 9.0, protein A was resuspended in the same buffer and mixed with the cross-linker dimethyl pemiliminate (Pierce) (20 mM) for an additional 1 h at room temperature. Subsequent washing steps were performed in 0.2 mM ethanalamine, pH 8.0, under agitation for 2 h at room temperature followed by PBS. The beads were stored in the same buffer at 4°C.

Human serum or supernatant of infected insect cells was preclared

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PBS. After washing three times in PBS and once in 10 mM potassium
buffer, pH 8.0, the bound protein was eluted with triethanolamine, pH 11.5. After neutralization with 1 M sodium phosphate, pH 6.8, the eluted protein was analyzed by SDS-PAGE and by Western
blotting.

RESULTS

Identification of a Novel Apolipoprotein—In studies designed to understand the catabolism of human TG-Lp, the apoprotein composition of these lipoproteins was analyzed after iodination. In addition to the known apoproteins apoB-100, apoB-48, apoA-I, apoA-IV, apoE, and apoC, a protein of approximately
106 kDa was found as a major labeled band in reduced samples separated by SDS-PAGE (Fig. 1A). This protein was found associated with the TG-Lp also after repeated recentrifugation (see below, Fig. 9) and immunodetection with a polyclonal antiserum raised against the purified 106-kDa protein (lane 3). Peroxidase labeled goat anti-rabbit was used for visualization.

by incubation for 1 h at room temperature with protein A-Sepharose. Sepharose beads were discarded, and the serum was incubated over-
night at 4 °C with 20 μl of FHR-4-protein A-Sepharose and 20 μl of PBS. After washing three times in PBS and once in 10 mM potassium phosphate buffer, pH 8.0, the bound protein was eluted with triethanolamine, pH 11.5. After neutralization with 1 M sodium phosphate, pH 6.8, the eluted protein was analyzed by SDS-PAGE and by Western
blotting.

A New Factor H-related Protein—In order to identify se-
quences coding for additional factor H-related proteins an oli-
got(DT)-primed human liver cDNA library was screened with a full-length cDNA clone (DOWN16), which encodes the FHR-3 protein. One clone showed a restriction pattern distinct from the FHR-3 coding cDNA, and this cDNA was used to rescreen the same library (data not shown). The nucleotide sequence of the longest clone isolated termed SAC6 is shown in Fig. 3. This clone is 1315 nucleotides long and has a poly(A) tail. The motif TCT AAC ATG (position 80–88) shows a good match (six out of nine, including the ATG) with the consensus sequence of initiation sites GCC ACC ATG (28). There is a poly(A) signal “AATAAA” at position 1285–1288.

Protein Structure of FHR-4—The nucleotide sequence of the longest clone isolated termed SAC6 is shown in Fig. 3. This clone is 1315 nucleotides long and has a poly(A) tail. The motif TCT AAC ATG (position 80–88) shows a good match (six out of nine, including the ATG) with the consensus sequence of initiation sites GCC ACC ATG (28). There is a poly(A) signal “AATAAA” at position 1285–1288.
Structural Analysis of FHR-4 and Homologies in the FHR Family—Structural alignment of the protein encoded by the SAC6 cDNA indicated a protein composed of an NH₂-terminal signal peptide followed by five SCR (Fig. 4). Each of the five SCRs includes the essential four Cys (C) residues (boxed with double lines in Fig. 4) and additional conserved amino acids such as a Pro (P), an Asn (N), a Gly (G), a Leu (L), 2 Tyr (Y), 2 Gly (G), a Trp (W), and a Pro (P) residue (Fig. 4). The signal peptide and the individual SCRs display identity to SCRs of FHR-3 (DOWN16 cDNA), FHR-2 (DESK59 cDNA), FHR-1 (H36 cDNA), and factor H, respectively (Fig. 5 and Table I). This comparison demonstrates that the COOH-terminal ends of FHR-4 and FHR-3 are highly related with an identity of 98.4% for SCR 4 and 93.8% for SCR 5, respectively. The relatedness between FHR-4 and FHR-3 is underlined by the identical amino acids of their signal peptides.

Expression Analysis—Expression of the FHR-4 mRNA in human liver was demonstrated by Northern blot analyses. A fragment specific for FHR-4 cDNA hybridized to several mRNA species with an estimated size of 1.4, 2.2, and 3.5 kilobases (Fig. 6). As the SAC6 cDNA sequence represents an almost full-length clone, the 1.4-kilobase transcript encodes the FHR-4 protein. The identification of several transcripts suggested the existence of additional closely related, but yet unidentified factor H-related proteins (Fig. 5 and Table I). This comparison demonstrates that the COOH-terminal ends of FHR-4 and FHR-3 are highly related with an identity of 98.4% for SCR 4 and 93.8% for SCR 5, respectively. The relatedness between FHR-4 and FHR-3 is underlined by the identical amino acids of their signal peptides.

Immunopurification of FHR-4 from Human Serum—Having demonstrated the existence of a hydrophobic NH₂-terminal region and expression of the FHR-4 mRNA in human liver, we asked for the existence of this novel factor H-related protein in human plasma. By immunopurification and SDS-PAGE analysis according to Laemmli a single protein of approximately 86 kDa was isolated from human serum with the specific FHR-4 antiserum (Fig. 7A, lane 1). The mobility of the human plasma protein isolated by immunopurification with FHR-4 antisem is distinct from that of the monomeric and the dimeric recombinant FHR-4 (Fig. 7A, compare lanes 1 and 2), but identical to the apoprotein (Fig. 8). The specificity of the immunopurification procedure was demonstrated by immunopurification of the reFHR-4 protein from the culture medium of infected insect cells. Both the 42- and 84-kDa form of the reFHR-4 protein were isolated (Fig. 7B, lane 3). To confirm that the purified human plasma protein represents a dimeric form of native FHR-4, the isolated plasma protein was reduced by extensive boiling in the presence of dithiothreitol and analyzed by SDS-PAGE. This treatment resulted in a predominant band with an apparent molecular mass of 63 kDa, which appears to represent the reduced monomeric form (Fig. 7B, lane 3). The additional band of weaker intensity was not further characterized.

Fig. 3. Nucleotide sequence of cDNA clone SAC6 and derived protein sequence. The nucleotide sequence and the amino acid sequence of the predicted FHR-4 protein is shown below in the single letter amino acid code. The numbers referring to the nucleotide acid sequence are indicated on the right (boxed with double lines in Fig. 4). The signal peptide and the individual SCRs display identity to SCRs of FHR-3 (DOWN16 cDNA), FHR-2 (DESK59 cDNA), FHR-1 (H36 cDNA), and factor H, respectively (Fig. 5 and Table I). This comparison demonstrates that the COOH-terminal ends of FHR-4 and FHR-3 are highly related with an identity of 98.4% for SCR 4 and 93.8% for SCR 5, respectively. The relatedness between FHR-4 and FHR-3 is underlined by the identical amino acids of their signal peptides.

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Identity of the New Apoprotein and the FHR-4—Given the overlap in sequence between the proteolytic fragments obtained from the newly identified apolipoprotein and the predicted FHR-4 protein, we asked whether the two proteins might even be identical. To this end, the mobility and immunoreactivity of the novel human apoprotein, the immunopurified human plasma protein, and the recombinant FHR-4 protein were compared. Both human proteins had identical mobility, when separated on SDS-PAGE in their nonreduced and reduced forms (Fig. 8A, lanes 1 and 2). In unreduced gels, both proteins of human origin migrate at a molecular mass of 86 kDa. However, the mobility of the human proteins differs from that of the recombinantly expressed protein (Fig. 8A, lanes 3 and 6). Upon reduction with dithioerythritol, a decrease in mobility (increase of apparent molecular mass) was observed, both human proteins migrate with an apparent molecular mass of 106 kDa. This treatment seems to cause an unfolding of the SCRs, while the two subunits remain attached to each other. The two monomeric forms can be separated by extensive boiling in the presence of dithiothreitol, resulting in a protein of 63 kDa, which represents the reduced unfolded monomeric form (Fig. 7B, lane 3). In addition, immunological analysis revealed that all three proteins react with specific antisera raised against the reduced purified apolipoprotein or with antisera raised against the nonreduced purified recombinant FHR-4 protein (Fig. 8). The apoprotein can be isolated with the immunoaffinity column from the soluble apoprotein fraction of TG-Lp. This direct comparison of the mobility and the immunoreactivity with specific antisera revealed that the novel human apolipoprotein and the native form of the...
plasma FHR-4 protein are highly related and may even be identical.

Plasma Distribution of the FHR-4 Dimer—The observed association of the FHR-4 protein with TG-Lp led us to study the plasma distribution of this protein in more detail. To this end lipoproteins were separated from plasma proteins by ultracentrifugation and the distribution of the FHR-4 protein in plasma was analyzed. We found that a relatively large fraction of the FHR-4 was associated with lipoproteins (Fig. 9A, lane 2). Due to the delipidation of the lipoproteins and the lack of a quantification method, we were unable to precisely determine the exact distribution of FHR-4. Separation of the different lipoprotein fractions by a second density gradient ultracentrifugation showed that most of the protein was associated with VLDL, and relatively smaller amounts were detected in the LDL and HDL fractions (Fig. 9B). The more TG-Lp were present in the plasma the more FHR-4 could be detected in this fraction. The appearance of FHR-4 protein in the bottom fraction (B2) after the second centrifugation is due to a partial loss of the protein from the lipoproteins during separation. This phenomenon is also observed for other apoproteins. A very similar distribution was observed in lipoproteins isolated from a series of normal probands and hyperlipidemic patients. From these results we conclude that the FHR-4 dimer is present in human plasma as free protein and also in TG-Lp, such as CM and VLDL, as well as other lipoproteins. The high molecular mass band of about

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Lipid Association of a Novel Factor H-related Protein

The dimeric form of FHR-4 is present in plasma and in the lipid-fraction of plasma. A, Detection of the FHR-4 protein in lipoprotein fractions. Lipoproteins were isolated from human plasma by ultracentrifugation and equal amounts (80 μg) of the bottom (B1) and the lipoprotein fraction were separated by SDS-PAGE and analyzed by Western blotting using anti-FHR-4 antisera. B, Identification of FHR-4 and factor H in lipoprotein particles of different density. The individual fractions, obtained after a second ultracentrifugation of the isolated lipoproteins (as shown in A) are indicated. The fraction designated B2 shows the bottom fraction obtained after the second ultracentrifugation and represents proteins that were dissociated from the isolated lipoproteins. High density lipoproteins (HDL), low density lipoproteins (LDL), intermediate density lipoproteins (IDL), and very low density lipoproteins (VLDL) were delipidated and 50 μg of each applied to a 10% SDS-PAGE (Neville system). Proteins were transferred to a nylon membrane and subjected to immunoblotting using antisera against FHR-4 (lanes 1, 3, 5, 7, and 9) or against the factor H-like protein 1 (FHL-1) (lanes 2, 4, 6, 8, and 10). The band detected with FHL-1 antisera in lanes 2, 6, and 10 might represent the 150-kDa factor H-like protein. The mobility of the size markers is indicated on the left.

and is closely related to the previously described FHR-3 protein (5). While recombinant FHR-4 protein, expressed in the baculovirus system, exists mainly as a monomer, the native protein present in plasma and in TG-Lp exists predominantly in a dimeric form. The immunopurified plasma form of FHR-4 and the protein isolated from TG-Lp have identical mobility and cross-react with the specific antisera. The apparent differences between the plasma forms and the recombinant FHR-4 protein are explained by differences in glycosylation.

The FHR-4 molecule is a member of the factor H gene family. Similar to factor H and to the other factor H-related molecules, the processed FHR-4 protein is exclusively composed of SCRs (2). The FHR-4 protein of human plasma exists as a 86-kDa dimer and is glycosylated. In addition to its presence in plasma as free FHR-4 protein, it is also identified as a constituent of lipoprotein particles. It can be detected in all lipoproteins, namely HDL, LDL, and TG-Lp. The recombinant FHR-4 protein, expressed in the baculovirus system, has a molecular mass of 42 kDa, is glycosylated and can form a homodimer of 84 kDa. The different molecular mass of the dimeric native protein (86 kDa) and the dimeric recombinant protein (84 kDa) is due to attachment of different carbohydrate moieties. The reduced protein has an apparent molecular mass of 106 kDa in SDS-PAGE, indicating unfolding of the SCRs before monomerization.

The amino acid sequences of the predicted FHR-4 protein and of the proteolytic fragments obtained from the purified native apoprotein are highly related but not identical. Five of the protein fragments (fragments II–VI) display a match of 94.9%; however, fragment I shows only an identity of 68.2%. Although polymorphic variants have been described for other members of the FHR gene family (9, 30, 31), the reason(s) for the observed differences are yet unclear. However, the identical mobility of the protein isolated from human TG-Lp and of the native plasma protein immunopurified using antisera raised against the recombinant FHR-4 protein in SDS-PAGE and the cross-reactivity of the two proteins with the corresponding specific antisera indicates that both proteins are highly related or even identical.

The two factor H-related proteins FHR-4 and FHR-3 show a similar structural organization, both are organized in five SCRs and show a striking identity on the amino acid level. However the two proteins are distinct, as only the FHR-4 protein, but not the FHR-3 protein, has a domain related to SCR 9 of factor H. This domain is also represented by peptides II and III obtained from the 106-kDa protein isolated from TG-Lp (Fig. 5), again highlighting an identical feature of the two proteins. The individual SCRs of FHR-4 show a high degree of identity to SCRs of factor H and to the FHR-3 protein (Table I). In particular SCRs 2, 4, and 5 of FHR-4 have over 93% identity to the corresponding SCRs of FHR-3. The relatedness of the two proteins is also indicated by the amino acid identity of their signal peptides. SCRs 4 and 5 of FHR-4 show also significant identity to SCRs 19 and 20 of factor H. The characterized biological functions of factor H (inactivation of the alternative pathway convertases and cofactor activity for the cleavage of C3b, as well as polyanion/heparin binding) have been mapped to the NH2-terminal SCRs 1–4 and to SCR 13, respectively (2, 32–34). The SCRs present in FHR-4, FHR-3, FHR-2, FHR-1, and homologous SCRs of factor H exclude these functionally characterized protein domains. Using recombinant FHR-4 protein for functional analysis demonstrates that this protein lacks the complement regulatory functions of factor H. The function of the newly identified FHR-4 protein seems, therefore, distinct from the complement regulatory role of factor H. A distinct biological function has, however, not yet been described for these members of the family of factor H-related proteins. Here we demonstrate that the FHR-4 protein is associated with lipoproteins and TG-Lp, thus indicating a potential function of this protein in lipid metabolism. Although FHR-4 is identified in all analyzed lipoprotein particles, the distribution of this protein is not uniform. Semiquantitative analyses suggest that in relation to other apoproteins the majority of lipid-associated FHR-4 was found in TG-Lp, such as CMs and VLDL. The amount of FHR-4 in these lipoproteins in relation to the free form is dependent on the level of these lipoproteins in plasma.

Similar as described here for the FHR-4 protein, an association with lipoprotein particles has recently been demonstrated for two additional members of this protein family, i.e. FHR-1 and FHR-2 (35). Thus suggesting for the FHR proteins a general role as constituents of lipoproteins.

At present also three complement regulatory proteins have been found associated with lipoproteins. C4-binding protein (C4BP), the glycoprophatidylinositol lipid-anchored membrane protein CD59 (protectin), and the soluble human complement lysis inhibitor (clusterin) are also associated with lipid particles (36–40). While C4BP is associated mainly with VLDL and LDL proteins, the other two complement regulatory proteins, which inhibit formation or insertion of the membrane attack complex are part of human HDL particles. Clusterin is incorporated into lipoproteins due to amphipathic α-helical structures, and the similar biochemical properties qualify FHR-4 as an apolipoprotein. Lipid-free proteins and highly concentrated fractions of the two proteins show a tendency to aggregate and are poorly soluble in water.

The association of FHR-4, a new member of the FHR family with lipoproteins, suggests either a role for these proteins in

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lipid transport, a functional interaction of lipids or lipoproteins with the complement system, or the use of lipoproteins as transport vehicles for these amphipathic proteins.

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