The synthesis and secretion of factor H, a regulatory protein of the complement system, were studied in skin fibroblasts from an H-deficient child who has chronic hypocomplementemic renal disease. In normal fibroblasts, factor H transcripts of 4.3 and 1.8 kilobase pairs (kb) encode a 155-kDa protein containing short consensus repeat (SCR) domains 1–20 and a 45-kDa protein which contains SCRs 1–7, respectively. The patient's fibroblasts expressed normal amounts of the 4.3- and 1.8-kb messages constitutively and after tumor necrosis factor-α/interferon-γ stimulation. Lysates of [35S]methionine-labeled fibroblasts from the patient contained the 155- and 45-kDa H polypeptides, but secretion of the 155-kDa protein was blocked; the 45-kDa protein was secreted with normal kinetics. The patient's plasma lacked the 155-kDa protein but contained the small form of H. Moreover, in fibroblasts the retained 155-kDa factor H protein was not degraded, even after 12 h. Immunofluorescent staining and confocal microscopic imaging of the patient's fibroblasts indicated that factor H was retained in the endoplasmic reticulum. Sequence analysis of reverse transcription-polymerase chain reaction products (the entire coding region) and genomic DNA revealed a T1679C substitution on one allele and a G2949A substitution on the other (C518R mutation in SCR 16, respectively). Both mutations affect conserved cysteine residues characteristic of SCR modules and therefore predict profound changes in the higher order structure of the 155-kDa factor H protein. These data provide the first description of a molecular mechanism for factor H deficiency and yield important insights into the normal secretory pathway for this and other plasma proteins with SCR motifs.

Proteins of the complement system are powerful effectors of innate host defense against infection and of immunopathological responses (reviewed in Colten and Gitlin (1)). The biological effects of the complement cascade are under the control of membrane-bound and fluid phase proteins (e.g. complement receptor 1, decay accelerating factor, C4 binding protein, and factor H) that limit complement activation and/or activities.

Complement protein factor H (2, 3) inhibits the formation and accelerates the decay of the alternative pathway enzyme of complement activation (C3bBb), an enzyme that cleaves the third component of complement, C3. Factor H also serves as a cofactor for the factor I-mediated cleavage of one of the biologically active forms of C3, C3b. These activities depend on interaction with the C3b molecule via at least one of three binding sites in the factor H protein (4). Other less well defined functions of factor H have been suggested by the presence in factor H protein of at least two heparin binding sites (4, 5) that could facilitate interaction with extracellular matrix, and by the interaction of factor H with lymphocytes and other leukocytes. Primary sequence analysis of factor H revealed a tandem array of 20 homologous units, called short consensus repeats (SCRs) each about 60-amino acid residues long (6). These structures have been recognized in 12 complement proteins and a growing number of non-complement proteins including blood clotting factor XIIIa, the α chain of human interleukin-2 receptor, and cell adhesion molecules such as ELAM-1 and MEL-14. These SCR motifs define a protein superfamily (7). Conserved amino acid residues in the SCR unit include four cysteines that form disulfide bonds in a Cys4–Cys8 and Cys5–Cys9 linkage. These disulfide bridges are important in maintaining the characteristic structure of the SCR module (8, 9). The SCR modules are joined by short (3–8-amino acid) linkers yielding the elongated shape of the intact H protein which has been visualized as a string of beads (10). The approximately 100-kb factor H gene is located in the RCA gene cluster relatively near (97 megabases) genes coding for other regulators of complement activation (membrane cofactor protein, complement receptor 1 and 2, decay accelerating factor, and C4 binding protein) on chromosome 1q (11) and is most closely linked to factor XIIb of the coagulation cascade (12) and the factor H-related gene 2 (13). Two mRNAs are generated from the factor H gene, a 4.3-kb species that includes all 20 SCRs from which the 155-kDa protein H is derived and a 1.8-kb mRNA species that includes the first 7 SCRs plus an alternate 3'-untranslated region (5, 14). The latter gives rise to a 45-kDa factor H protein. Both mRNA species are present in the liver, the major site of factor H expression, and in several extrahepatic tissues (15). Both isoforms of factor H mRNA and
protein are expressed in primary cultures of human monocytes, umbilical vein endothelial cells, and fibroblasts (16–18).

Genetic deficiencies of the approximately 30 proteins that constitute the effector and control proteins of the complement system have been described in humans and other species. Some, such as C9 deficiency, have no significant clinical consequences, whereas most of the others either have profound effects on susceptibility to infection or are associated with manifestations of “autoimmune” disorders including systemic lupus erythematosus and chronic glomerulonephritis (19). Genetic deficiency of factor H has been described in domesticated animals (20) and in a relatively small number of human kindreds (21–28). In general, patients with homozygous factor H deficiency suffer recurrent bacterial infections (including Neisseria spp.), vasculitis, and/or glomerulonephritis (29). In none of these cases has the molecular basis for factor H deficiency been determined.

The availability of skin fibroblasts from a patient with factor H deficiency and hypocomplementemic glomerulonephritis (28) made it possible to investigate the molecular and cellular biology of H deficiency in this kindred. In studies of this patient we demonstrated a compound heterozygous deficiency with mutations of conserved cysteine residues in SCR 9 and 16. These mutations disrupt intrachain disulfide bridges, thereby perturbing the higher order structure of factor H. This leads in turn to a profound selective block in secretion of the 155-kDa factor H protein. Because both mutations are downstream of these conserved cysteine residues in SCR 9 and 16. These mutations make it possible to investigate the molecular and cellular biology of H deficiency in this kindred.

MATERIALS AND METHODS

Protein Blot Analysis—1–μl plasma samples and 1 μg of recombinant human factor H (Sigma) were run on 8.5% SDS-PAGE under nonreducing conditions, then transferred to a nitrocellulose membrane (Hybond ECL, Amersham Corp.). The membrane was blocked and developed with goat anti-human factor H IgG (Incstar, Stillwater, MN) and rabbit anti-goat IgG-peroxidase (Rockland, Gilbertsville, PA) using 3,3′-diaminobenzidine/urea peroxide substrate (Sigma). The blots were digitized and processed by Adobe Photoshop software (version 4.0, Adobe, San Jose, CA).

Fibroblast Cultures—A punch skin biopsy was obtained under local anesthesia after obtaining informed consent according to the institutional review board guidelines at the University of Minnesota. Primary cultures of skin fibroblasts were established from either collagenase dispersed cells or from an explant. Normal primary adult human fibroblasts (GMS39B) were obtained from the human genetic repository (NIH General Medical Sciences, National Institutes of Health). These adherent cultures were maintained in growth medium (Dulbecco’s modified Eagle’s medium with HEPES, t-glutamine, and 10% heat-inactivated fetal bovine serum (Life Technologies, Inc.) at 37°C in humidified air with 5% CO2. Fibroblasts at passage 10 or lower were used for all experiments.

Cytokine Stimulation/Treatment—Cells were grown to confluence, washed twice with Hanks’ balanced salt solution (HBSS, Life Technologies, Inc.), and incubated overnight in medium containing HEPES, t-glutamine, penicillin, streptomycin, and 0.1% bovine serum albumin (cell culture tested, Sigma) alone or with 20 ng/ml TNF-α (Genentech, Inc., San Francisco, CA) and/or 1000 units/ml IFN-γ (R&D Systems, Minneapolis, MN). After 24 h cell monolayers were washed twice with HBSS, and RNA was harvested, or the cells were incubated in medium containing radiolabeled methionine for biosynthetic labeling. RNA Blot Analysis—Total RNA was prepared from the patient’s fibroblasts by cesium chloride centrifugation of guanidinium thiocyanate (Fluka Chemical Company, Ronkonkoma, NY) lysates (30). Ten micromoles of total RNA samples were subjected to electrophoresis in a formaldehyde-containing 1% agarose gel with modifications as described by Barnett et al. (31). Following transfer to a nitrocellulose sheet (Bio-Rad), hybridization was performed using a 1.4-kb cDNA probe (H-19, gift from Dr. Dennis Hourcade, Washington University, St. Louis, MO), which spans the nucleotide sequence from the first half of SCR 1 to the end of SCR 7 (33). The probe was radiolabeled with [α-32P]dCTP (DuPont NEN) using the Random Primed DNA labeling kit (Boehringer Mannheim) according to the manufacturer’s instructions. The filter was washed and dried, and autoradiography was developed on Kodak XAR-3 film (Eastman Kodak Co.).

Biochemical and Pulse-Chase Experiments—Fibroblasts were grown to near confluence in 24-well tissue culture plates (Corning, Corning, NY), then washed twice with warm HBSS and incubated for 1 h in Dulbecco’s modified Eagle’s medium (Life Technologies, Inc.) containing t-glutamine, HEPES, penicillin-streptomycin, and 10% dialyzed fetal bovine serum (Life Technologies, Inc.), supplemented with 250 μCi/ml [35S]methionine (specific activity, 1199 Ci/mmol, ICN Radiochemical, Irvine, CA). At the end of the pulse period, the labeling medium was removed, and cells were washed once with warm HBSS and incubated for 0–12 h in growth medium. At timed intervals the medium was removed and the cell monolayer washed once with phosphate-buffered saline (PBS) and lysed by one freeze-thaw cycle in PBS containing 0.5% sodium deoxycholate (Fisher), 1% w/v Triton X-100 (Sigma), 10 mM EDTA (Sigma), 2 mM phenylmethylsulfonyl fluoride (Sigma), and 100 μg/ml leupeptin (Boehringer Mannheim). Cell lysates and media were clarified by centrifugation (13,000 × g for 10 min). Total protein synthesis was measured by trichloroacetic acid (Sigma) precipitation. SDS (Bio-Rad) was added to each sample to a final concentration of 1%.

Immunoprecipitation and SDS-PAGE—Immunoprecipitation and SDS-PAGE were performed as described previously (34). Briefly, samples were precleared with heat-killed protein A-containing Staphylococcus aureus Immunoprecipitin, Life Technologies, Inc.) and incubated overnight with excess goat antibody to human factor H (Quidel Corporation, San Diego, CA) at 4°C. Immunocomplexes were collected with 0.1% Triton X-100 (Sigma) in PBS and 100% methanol, blocked with 10 mM glycine in PBS, and then washed with antibody dilution solution (PBS with 3% PBS (Life Technologies, Inc.), 3% bovine serum albumin (ICN, Irvine, CA), and 3% skim milk powder). The monolayers were incubated overnight with rabbit anti-calnexin (DP-23, IgG fraction, kind gift of Dr. David Paller) and/or goat anti-human factor H (Incstar, Stillwater, MN) conjugated with fluorescein isothiocyanate, then washed three times with 1% Triton X-100 in PBS and incubated with goat anti-rabbit IgG Texas Red conjugate for 2 h. Following four washes, cells were fixed with 4% paraformaldehyde in PBS for 20 min, incubated with 10 mM glycine, washed with PBS, and mounted with 100 μM propyl gallate in 90% glycerol. Slides were examined in a Zeiss epifluorescence microscope. Slides were assembled with a Bio-Rad MRC laser confocal microscope adaptor using a 63× immersion oil objective. The collected confocal images were processed using Adobe Photoshop software (version 4.0, Adobe, San Jose, CA). IgG controls (primary and secondary antibody) were also run and were found to be not significantly different from the unstained background. The fluorochromes (fluorescein isothiocyanate and Texas Red) were also negative for emittance at the filter setting used for the other. The factor H staining could be completely blocked by recombinant factor H (Sigma).

Generation of Factor H cDNA Clones for Sequence Analysis—RNA isolated from the patient’s fibroblasts served as template for synthesizing the RT-PCR fragments shown in Fig. 1. The Invitrogen cDNA cycle kit (Invitrogen Corporation, San Diego, CA) was used for generating the RT-PCR fragments 1–6 (Fig. 1) using the following oligonucleotides as primers for the reverse transcription at 68°C annealing temperature: 5′-CAT ACT CGA GCC ACC GCT AGT ACC TTA-3′ (3903–3929, antisense) and 5′-TCT GAC TCG AGG TGT GCA CTT TAT GAT TGA-3′ (3772–3801, antisense; engineered XhoI restriction sites are underlined). Annealing temperature was 68°C. Segments 7 (Fig. 1) was synthesized using a SUPERSCRIPT II RT-PCR kit (Life Technologies, Inc.) with the kit’s oligo(dT) primer for reverse transcription. Annealing temperature was 70°C. The PCR reactions were performed using AmpliTaq polymerase (Perkin-Elmer) for segment 1–6 and the KlenTaq LA (Wayne Barnes, St. Louis, MO) for segment 7 using the oligonucleotides listed in Table 1 with a 65°C annealing temperature. Segment 1–3 and 6–7 were gel-purified either by trough elution or by
Cysteine Mutations in Factor H Deficiency

**Results**

Case Report—A detailed report of the presentation, clinical course, and renal pathology in the patient with factor H deficiency has previously been published (28). Briefly, a 13-month-old Native American (Sioux) boy presented with hypocomplementemic hypertensive renal disease. Renal biopsy showed changes consistent with membranoproliferative glomerulonephritis, deposition of type III collagen, and segmental complement C3 deposition in capillary loops. A serum complement profile revealed decreased levels of C3 and factor B but normal levels of C4 and factor I; factor H was undetectable by radial immunodiffusion analysis. Slightly depressed levels of factor H were present in both parents. The child underwent renal transplantation in February 1996. Serum C3 concentrations have remained low, as have factor H levels. Western blot analysis of the patient's plasma before and after renal transplantation (Fig. 2) revealed slightly increased concentration of the 45-kDa factor H and no detectable 150-kDa factor H when compared with seven normal plasma samples. There was no significant difference between the pre- and post-transplantation samples.

Northern Analysis—RNA blot analysis of skin fibroblasts from the factor H-deficient patient revealed 4.4- and 1.8-kb products were purified by trough elution on agarose gels and subjected to DNA sequencing in the manner described above, utilizing the oligonucleotides used for the PCR as primers. Both strands were sequenced.

## RESULTS

**Sequence Analysis**—DNA sequencing was performed using a model 373A automated DNA sequencer (Applied Biosystems, Foster City, CA) using the standard protocol for the TaqDyeDeoxy Terminator cycle sequencing kit from Applied Biosystems. For PCR fragments cloned into Bluescript, M13 and M13 reverse primers were used, in addition to paired internal 20-mer oligonucleotides designed on the basis of the published factor H sequence (6). All oligonucleotides were synthesized with seven normal plasma samples. There was no significant difference between the pre- and post-transplantation samples.

**Direct Sequencing of Genomic DNA**—To confirm the mutations found in the cDNA clones, high molecular weight DNA was isolated (37) from the peripheral blood leukocytes of the patient and his parents.

300 ng of genomic DNA were amplified by oligonucleotides within SCR 9 (Table 1) to confirm the mutation found at position 1679. The resulting PCR product was gel-purified by elution onto DEAE paper and directly sequenced using internal sequencing oligonucleotides (5’-CCC AGT ATT TAT GAA TGC CAG-3’ (1606–1626, sense), 5’-CCA ACC ATT GTA ACC ACA CAC-3’ (1727–1747, antisense)).

**Table I**

The oligonucleotides listed were used to generate the PCR fragments shown in Fig. 1.

Position of each oligonucleotide in the factor H gene is given in parentheses.

| 5’ primer Product size (bp) | 3’ primer |
|-----------------------------|-----------|
| Segment 1 5’-GCT ATC TAG AGG AGA ACT GGA CGT TGT GAA-3’ (4–33) | 866 | 5’-ATC TCT CTA GAG ATG AAG GCA AGC GAC GCCA-3’ (839–869) |
| Segment 2 5’-GCG CTC TAG ATC TCC TAT ATC TCA GAA GAT-3’ (718–747) | 1008 | 5’-CTG ACT CGA GTG TGT CTT CCA GTA TTG CTT-3’ (1696–1725) |
| Segment 3 5’-GGG CTC TAG ATG GTC AAA CAT CAG GAT CAA-3’ (1518–1547) | 1010 | 5’-GGA ATC TCG AGT GGA GGT GAG CAA GGA ATC TCT CGA GAC ATG AAG GCA ACG TTC TCA TCT AGA GAG CCA CCG GTC TCA GCT TAT AAT-3’ (3899–3931) |
| Segment 4 5’-AAT CAT GTG GTG CAC CTC CT-3’ (1956–1975) | 839 | 5’-TAT CCT GAA ACC ACC CTC AC-3’ (2775–2794) |
| Segment 5 5’-CTC AGA TAG AAC AGG GAA CC-3’ (2694–2713) | 893 | 5’-TGG GGA TAT TAC ACA CGG AT-3’ (3567–3586) |
| Segment 6 5’-GAT GTC TAG AAG GCG GGT GAG CAA GTG ACT TAC A-3’ (3089–3122) | 841 | 5’-CAT ACT CGA GCC ACC GGT CTC AGC TTA-3’ (3903–3929) |
| Segment 7 first amplification 5’-GCG CAC ACC ACA GTT ACA TGT ATG-3’ (1346–1369) | 2582 | 5’-GAA GAG AGC CAC CGG TCT CAG CCT TT-3’ (3904–3927) |
| Segment 7 reamplification 5’-CCA CTC GAC CAT GTA TGG AGA ATG GCT GG-3’ (1353–1381) | 2579 | 5’-TTA TCT AGA GAG CCA CCG GTG TCA GCT TAT AAT-3’ (3899–3931) |
| SCR 9 genomic 5’-GGG GCG TCT AGA TAT CCG ATT TAT GAA TGC C-3’ (1373–1391) | 173 | 5’-GAC GGG TAC TCG AGA CCA ACC ATT GTA ACC-3’ (1733–1762) |
| SCR 16 genomic 5’-ACC TGA GAT TTC TCA TGG TGT TG-3’ (2875–2897) | 137 | 5’-GAC ACC ATT TTG CTT CTA AGC AT-3’ (2989–3011) |
factor H transcripts in cells incubated in medium alone. As in normal cells (17), both transcripts were up-regulated by TNF-α or the combination of TNF-α and IFN-γ (Fig. 3). The two-factor H mRNA species from the H-deficient cells were similar in size and amount to the corresponding H transcripts in wild type cells.

Biosynthetic Analysis—A preliminary experiment showed that the 155-kDa factor H was synthesized but not secreted in fibroblasts from the homozygous H-deficient patient. During a prolonged pulse, a small quantity of 155-kDa factor H (apparent size of the intracellular polypeptide) was detected in medium from the patient’s cells. Accordingly, a pulse-chase experiment was performed. The patient’s fibroblasts synthesized 155- and 45-kDa forms of factor H in quantities and sizes similar to normal fibroblasts (Fig. 4). The 45-kDa protein was secreted with similar kinetics in the patient’s and in normal fibroblasts (t1/2; 45 min). Factor H (molecular mass, 155 kDa) disappeared from the intracellular compartment accompanied by the appearance of the 160-kDa H protein in the extracellular medium from normal fibroblast cultures with a half-time of 40–60 min. In contrast, for H-deficient cells the 155-kDa factor H protein was retained within cell lysates. Although a small quantity of the 155-kDa H protein was detected in the medium at the 12-h chase time point, little or no secreted 160-kDa protein was detected. Moreover, as estimated from direct scintillation spectrometry of the radiolabeled bands, less than 10% of cell-associated 155-kDa factor H was lost at 12-h chase; i.e. little or no degradation of retained factor H was apparent in the deficient cells.

Visualization of Factor H in Fibroblasts by Immunofluorescence—When investigated with a standard epifluorescent microscope, factor H showed perinuclear staining similar to staining obtained with antibody to calnexin. Therefore confocal images were taken of fibroblasts stained for both factor H and calnexin in several focal planes. Perinuclear distribution of H staining (green fluorescence, Fig. 5A) also showed a similar pattern using antibody to calnexin (red fluorescence, Fig. 5B), a marker for the endoplasmic reticulum compartment (32). Overlaying the two patterns revealed colocalization of the signals (Fig. 5C).

Sequence Analysis of Factor H cDNA Clones—Nucleotide sequencing of the factor H-deficient patient’s entire cDNA re-

vealed 6 base substitutions which deviated from the published normal H sequence (6,GenBank™ HSH.GB.PR, accession no. Y00716). Only changes found on both strands in two or more clones are reported to exclude RT-PCR artifacts.

Two mutations affecting the codons for conserved cysteine residues, one in SCR 9 and the other in SCR 16 were noted, one on each allele (Fig. 6). That is, in the patient’s cDNA a T to C mutation at position 1679 changes cysteine 2 in SCR 9 to arginine (Cys318 → Arg) and a G to A substitution at base 2949

FIG. 2. Protein blot analysis. Plasma samples of the H-deficient patient (lane 1, prior to renal transplantation; lane 2, post renal transplantation), normal controls (lanes 4–10), and recombinant factor H (lane 3) were analyzed. A blot was developed using a goat anti-human factor H IgG fraction. Note that in patient’s plasma the large H protein is undetectable, and slightly elevated levels of the small form are present in the patient’s plasma. The lower panel shows the gel segment with low molecular mass H bands at increased gain. Quantitation of the low molecular mass H band in the digitized image (of another blot) revealed that patient’s plasma had 2.5 times the average amount in six normal control samples (range 55–157% of average value).

FIG. 3. RNA blot analysis of normal control and factor H-deficient fibroblasts: unstimulated (lanes 1 and 5), stimulated with 20 μg/ml TNF-α (lanes 2 and 6), 1000 units/ml IFN-γ (lane 3), and the combination of both (lanes 4 and 7). 10 μg of RNA/lane.

FIG. 4. Immunoprecipitation of factor H from a pulse-chase experiment in normal (upper panel) and factor H-deficient (lower panel) fibroblasts stimulated with TNF-α and IFN-γ (1-h pulse) (24-h exposure, inset of upper panel, 25 days of exposure).
converts cysteine 2 in SCR 16 to tyrosine (Cys941 → Tyr). Each of these affect conserved cysteine residues that form disulfide bridges (9) critical for maintenance of higher order structure of the SCR module.

In addition four other substitutions were detected: (a) G to A change at nucleotide 257 causes a valine to isoleucine change in SCR 1 (Val44 → Ile), (b) T to C at nucleotide 1277 changes a tyrosine to histidine in SCR 7 (Tyr384 → His), (c) G to A at position 1492 causes no change in amino acid sequence (both GCG and GCA encode for Ala455 in SCR 8), and (d) G to C at position 1551 changes an arginine to a threonine in SCR 8 (Arg475 → Thr).

Sequence Analysis of Genomic DNA of the Patient and the Family Members—Sequencing of genomic DNA derived from the patient showed T and C peaks of equal heights at position 1679 (SCR 9), indicating that the T → C mutation is on only one allele. The father’s genomic DNA was also heterozygous for this mutation while the mother’s DNA was homozygous wild type at nucleotide 1679 (Fig. 7). Equal G and A peaks were found at base 2949 in the patient’s genomic DNA showing that he is heterozygous for this mutation (SCR 16). The mother was also heterozygous for this mutation, while only a G peak was found in the father’s genomic DNA, showing that he was homozygous for the wild type (Fig. 7).

Direct sequencing of PCR products derived from patient genomic DNA confirmed all of the other mutations found in the

Fig. 5. Confocal images of the patient's fibroblasts stained for factor H and calnexin. The two images were taken in the same focal plane using filters for the green fluorescence corresponding to factor H (panel A) and the red fluorescence corresponding to calnexin (panel B). The obtained gray scale images were colorized and overlaid to produce panel C, which shows the colocalization of the two signals (producing orange). Colocalization was established in all focal planes in many fields in several experiments. The cell in the lower right portion of the field shows only very faint H staining, but it is colocalized with the much stronger signal for calnexin.
Cysteine Mutations in Factor H Deficiency

The investigation of the cellular and molecular basis of factor H deficiency in a patient with chronic kidney disease revealed a striking block in secretion of the large (155 kDa) isoform of factor H and a defect in degradation of the retained protein in primary cultures of the patient’s fibroblasts. Colocalization of the retained factor H with calnexin indicated that the block in secretion prevents egress from the endoplasmic reticulum. Staining for factor H was relatively weak compared with staining for calnexin, possibly due to complexing of factor H with endoplasmic reticulum proteins. This block in secretion of the 155-kDa factor H was selective inasmuch as synthesis and secretion of the small (45 kDa) factor H isoform was normal. The selective block in secretion of the 155-kDa H protein was also reflected in the absence of the corresponding high molecular mass factor H in the patient’s plasma and the presence of the low molecular mass H protein. Even though the low molecular mass H has factor I cofactor activity and has the binding site for C3b (39), its decay accelerating activity is only 1% of the large H form (40). This provides an explanation for H deficiency, low serum C3 protein level, and kidney disease even in the presence of the 45-kDa H protein.

Expression of both H isoforms was increased by cytokines (TNF-α and IFN-γ) at the RNA and protein levels, indicating that constitutive and regulated control of H transcription and translation were unaffected by the mutations accounting for the deficiency. The 45-kDa factor H protein is derived from an alternatively spliced transcript of the single factor H gene which also gives rise to the 155-kDa H. The former contains the first seven SCR units of the 20 that comprise the 155-kDa factor H protein (Fig. 6). Since the 45-kDa factor H protein was secreted with kinetics indistinguishable from normal, we considered it likely that the mutation(s) responsible for the secretory defect was (were) 3’ to sequences encoding the first seven SCR units. To test this hypothesis sequencing of the patient’s factor H was undertaken.

Generation of RT-PCR fragments and analysis of the entire factor H coding sequence identified two mutations (one on each allele) that could directly affect higher order structure. The first, Cys618 → Arg in SCR 9, is the consequence of a T → C substitution at nucleotide 1679. Thus disruption of a disulfide

cDNA generated by RT-PCR. One of these, the T to C change at nucleotide 1277 (Tyr384 → His) is a common polymorphism reported previously (38).
bridge critical for the characteristic structure of an SCR and a charge change was imposed by this mutation. To analyze the other allele, CDNA clones were generated spanning a segment between the 3‘ end of SCR 7 and the 3‘-untranslated region. Clones that did not contain the mutation at position 1679 in SCR 9 therefore must have originated from the other allele. On this allele we identified a mutation Cys1941 → Tyr in SCR 16 (caused by G → A change at nucleotide 2949) that also disrupted a disulfide bridge. The mutation in SCR 16 creates a new SauI restriction enzyme cleavage site. Both mutations were confirmed by sequencing the patient’s genomic DNA, and the mode of inheritance was established by sequencing parental genomic DNA.

Each of these mutations is downstream of nucleotides encoding the normally secreted 45-kDa protein. Hence, disruption of disulfide bridges in SCR 9 and 16 would affect proper folding only of the newly synthesized 155-kDa factor H. Presumably this abnormality in folding accounted for the endoplasmic reticulum retention of factor H in the H-deficient fibroblasts. Mutations resulting in substitution of critical cysteine residues have been identified in deficiencies of other proteins in association with clinical disease. For example, in Bernard-Soulier disease, a Cys → Ser in glycoprotein Ibα is associated with the absence of the protein on platelets (40). Factor IX deficiency has been associated with mutations that disrupt a critical disulfide bridge (Cys326-Cys322). Mutation of either conserved cysteine residue results in deficiency of factor IX (42). Similarly, disruption of an intrachain disulfide bridge by the Cys536 → Gly mutation in the μ heavy chain immunoglobulin gene was also associated with impaired IgM expression on the surface of the B cells of an immunodeficient patient (43). Mutation of a conserved cysteine residue (Cys115 → Arg) in the γ chain of interleukin 2 receptor resulted in the absence of this molecule from the surface of another patient’s B cells (44). Unfortunately, the cellular biology of these deficiencies has not been investigated. Hence, the potential for endoplasmic reticulum retention of these misfolded proteins has not been determined.

A comparable block in secretion has also been observed in a mutation of one of the framework cysteine residues in factor XIIIb, another protein with SCR domains (45). One major difference between the factor H and XIIIb deficiencies is the fact that the retained and presumably misfolded factor H apparently was not degraded during a 12-h chase, whereas the mutant XIIIb was. Two possibilities for this difference were considered. First, a unique structural feature of the abnormal H protein, for example, the size or charge (e.g., Arg) of residues substituted for the cysteine, might affect degradation pathways. However, on one of the affected alleles the size of the amino acid substituted (tyrosine and phenylalanine) is approximately the same for the factor H and the XIIIb deficiency. In the XIIIb deficient, the retained protein is rapidly catabolized in a transfected surrogate cell. The rate of catabolism of mutated XIIIb in the patient’s cells was not ascertained. The H-deficient patient may have inherited an independent mutation that limits efficiency of degradation of endoplasmic reticulum retained misfolded proteins (46). This mechanism has been identified in a subset of children with α1-antitrypsin deficiency with liver disease (32, 47). No evidence for liver disease was obtained in the factor H-deficient patient, but he was not subjected to liver biopsy so that minimal changes in liver structure or function cannot be completely ruled out. Total accumulation of the retained, misfolded protein would be a function of relative synthesis rate and age of the patient. The extent of liver injury might also be a function of specific cytopathic effects of a given protein. α1-Antitrypsin is one of the most abundant liver proteins, whereas factor H is less abundant, and the H-deficient patient reported here is young. These may provide explanations for the absence of obvious clinical liver disease at this time.

Four other mutations were identified in CDNA and genomic DNA from the factor H-deficient patient, but these are likely to be of lesser importance than the cysteine substitutions. The G1492A mutation does not produce an amino change. The T1277C mutation yields the Tyr354 → His change in SCR 7, a common polymorphism in the normal population (38). The G257A mutation causes a Val124 → Ile switch in SCR 1, and the G1551C mutation produces an Arg275 → Thr transition in SCR 8. Only the last of these mutations has any potential for perturbing higher order structure of the 155-kDa mutant H protein without affecting the small molecular mass factor H. This substitution in SCR 8 affects the residue immediately preceding cysteine 3. This position is not highly conserved in other SCRs of factor H. In fact, seven of the 20 SCRs contain threonine at this position, making it unlikely that the G1551C mutation accounts for a major change in the structure of the 155-kDa factor H. Thus, it is unlikely that any of these mutations contribute to the cellular pathophysiology of factor H deficiency.

Abundant data are now available that define the biochemical and cellular details of postsynthetic processing and transport of proteins (48–50). Mutations causing an arrest in the maturation process of the nascent polypeptide chain may cause the retention of the protein in the endoplasmic reticulum (51, 52). With these as background, it should be possible to use this genetic deficiency as a probe of H transport mechanisms for secretion of factor H and other SCR protein family members.

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