Disease-associated Sequence Variations Congregate in a Polyanion Recognition Patch on Human Factor H Revealed in Three-dimensional Structure*

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Mutations and polymorphisms in the regulator of complement activation, factor H, have been linked to atypical hemolytic uremic syndrome (aHUS), membranoproliferative glomerulonephritis, and age-related macular degeneration. Many aHUS patients carry mutations in the two C-terminal modules of factor H, which normally confer upon this abundant 155-kDa plasma glycoprotein its ability to selectively bind self-surfaces and prevent them from inappropriately triggering the complement cascade via the alternative pathway. In the current study, the three-dimensional solution structure of the C-terminal module pair of factor H has been determined. A binding site for a fully sulfated heparin-derived tetrasaccharide has been delineated using chemical shift mapping and the C3d/C3b-binding site inferred from sequence comparisons and computational docking. The resultant information allows assessment of the likely consequences of aHUS-associated amino acid substitutions in this critical region of factor H. It is striking that, excepting those likely to perturb the three-dimensional structure, aHUS-associated missense mutations congregate in the polyanion-binding site delineated in this study, thus potentially disrupting a vital mechanism for control of complement on self-surfaces in the microvasculature of the kidney. It is intriguing that a single nucleotide polymorphism predisposing to age-related macular degeneration occupies another region of factor H that harbors a polyanion-binding site.

Inappropriate or disproportionate activation of the complement system underlies the debilitating symptoms of a long list of autoimmune, degenerative, and iatrogenic diseases (1). The role of the complement system is to rid the body of infectious agents and clear the bloodstream of immune complexes, apoptotic cells, and other debris (2, 3). Following its activation, deposition of complement proteins onto target surfaces ensues, marking the target for destruction and clearance, accompanied by release of mediators of inflammation and activators of the acquired immune response. Tight regulation and targeting of the complement system is thus essential to good health. The recent association of genetic variations in complement regulatory proteins with pathologies of the kidney (4, 5) and eye (6–9) is an important development. It not only offers additional insights into the role played by complement in pathophysiological mechanisms, but it opens up the possibility of tailoring prevention and intervention according to genotype. The ability to consider such sequence variations within the context of a three-dimensional structure in which key functional regions have been identified significantly enhances these opportunities. In this report, we describe the structure of a region of factor H implicated in atypical hemolytic uremic syndrome (HUS).2

Hemolytic uremic syndrome is a thrombotic microangiopathy that occurs primarily in the kidneys. It is characterized by hemolytic anemia and thrombocytopenia and is a leading cause of acute renal failure in children. Typical HUS is associated with infection by Escherichia coli (10) and has a good prognosis (11). The rarer atypical variant of HUS (aHUS) (12) is recurrent and displays high morbidity and mortality even following renal transplantation (13). It is triggered by a range of factors (14) but generally presents in the absence of infections. Many aHUS patients carry mutations in the regulators of complement activation (RCA) gene cluster of chromosome 1q32. Between 10 and 30% of aHUS patients are heterozygous for mutations in the complement regulatory protein, factor H (fH) (4, 5), most of which map to its C-terminal region (15–17). Many of the remaining patients carry mutations elsewhere in the RCA gene cluster (18). All such mutations should be considered against a background of single nucleotide polymorphisms in the RCA gene cluster that appear to further enhance disease susceptibility (14). A different single nucleotide polymorphism in the factor H gene predisposes to age-related macular degeneration (6–9) and to membranoproliferative glomerulonephritis (7), both of which are characterized by formation of fatty plaques, or deposits, rich in immune molecules.

Factor H is an abundant plasma glycoprotein (155 kDa) (19) that controls the alternative pathway of complement activation (20). The alternative pathway of complement is triggered on any surface (self or foreign) not protected by specialized regulatory proteins. Self-cells carry membrane-associated regulators to prevent complement activation via the alternative pathway, most of which, like fH, are encoded by the RCA gene cluster (21). Factor H is a fluid phase protein that normally prevents complement amplification on all self-surfaces. It is, therefore, important for blocking complement at host surfaces not enclosed by a cell membrane carrying other RCAs. Factor H acts on a key bimolecular enzymatic complex in the complement cascade, C3 convertase (C3b.Bb). Factor H competes with C3b for binding of factor B, is a...
cocrystal for proteolytic cleavage of C3b, and accelerates the decay of the C3 convertase into its components (22, 23).

Factor H consists of 20 tandem, ~60-residue-long, complement control protein modules (CCPs). The cofactor and decay acceleration activities of fH map to the N-terminal four CCPs (24, 25). There are three C3b-binding sites in fH (26): CCPs 1–4, CCPs 8–15, and finally, CCPs 19–20, which also binds C3d generated during complement activation (27). Factor H interacts with polyanions via three sites, in CCPs 7, 9, or 13 and in CCP 20 (28–30). Indeed, the capacity of fH to distinguish self from non-self depends upon a sophisticated recognition capability for specific polyanionic markers on host surfaces (31). The primary region for host recognition has been mapped to the four C-terminal modules (32). Thus, it is noteworthy that the two C-terminal modules of fH harbor a binding site for polyanions and one for C3b/C3d, in addition to being a hotspot of aHUS-associated mutations (15). This polyanion-binding site appears to also represent a site for attachment to endothelial cell surfaces (33). Therefore one hypothesis is that aHUS-linked mutations induce pathogenesis by interfering with a recognition site on fH for polyanionic self-markers. Additional explanations are that the mutations disrupt structure, that they interfere with the C3b/C3d-binding site, or that they compromise the ability of fH to form quaternary structure. Affinity chromatography and related techniques, utilizing for the most part commercially available preparations of heparin, have been employed previously to provide an overall assessment of how well mutant forms of fH bind polyanionic carbohydrates, but they do not provide a clear picture of the potential contribution of individual amino acid residues to the more sophisticated in vivo recognition process. Thus, as described below, elucidation of the three-dimensional structure of the CCPs 19 and 20 (fH−19–20) and experimental identification of the residues that comprise a polyanion recognition site represent a powerful means of investigating these hypotheses further.

**EXPERIMENTAL PROCEDURES**

**Protein Production**—The fragment containing human fH residues 1107–1231 (native sequence numbering) was cloned into the *Pichia pastoris* expression vector pPICZaA. The expressed fH−19–20 was directed to the secretory pathway by placing the coding sequence behind the *Saccharomyces cerevisiae α*-factor secretion sequence. Inefficient cleavage of this secretion signal combined with cloning artifacts resulted in the additional sequence EAEF at the N terminus. Cation-exchange chromatography was used to purify the fH−19−20 collected from the culture supernatant.

**NMR Data Collection**—NMR spectra were acquired on Bruker AVANCE 600 and 800 MHz spectrometers using 5-mm triple resonance probes. Data were collected at 37 °C on a 1 mM sample of 15N fH−19−20 in 20 mM sodium acetate (deuterated), 200 mM NaCl at pH 4.0. 15N-edited and 13C-edited NOE-SQHC experiments were acquired with mixing times of 100 ms. NMR data were processed using the AZARA suite of programs, provided by Wayne Boucher and the Department of Biochemistry, University of Cambridge with maximum entropy processing used for the F1 and F2 dimensions of the three-dimensional experiments. Heteronuclear (1H−15N) nuclear Overhauser effects (NOEs), at 600 MHz, were calculated from the ratio of the intensities of the cross-peaks in the reference spectra to those recorded with saturation of the 1H signal.

**Resonance Assignment and Structure Calculation**—Processed spectra were viewed and nuclei were assigned using ANSIG (34). Resolved peaks in 15N-NOESY and 13C-NOESY spectra were picked and, where possible, assigned unambiguously. Ambiguous restraints were generated by matching the chemical shifts of NOESY cross-peaks with the
resonance assignments. The “connect” routine within the AZARA suite was used to convert normalized intensities into four distance categories: <2.7 Å, <3.3 Å, <5 Å, and <6 Å. Relatively slowly exchanging amide protons were identified by analysis of two-dimensional heteronuclear water exchange experiments. Structure calculations were carried out using the crystallography and NMR system (CNS)-based protocols (35). The first round of structure calculations was performed using only the unambiguous restraints list, whereas all subsequent rounds were performed using both unambiguous and ambiguous restraints (36–38). The potential H-bond acceptors for slowly exchanging amide protons were elucidated from inspection of well converged initial structures calculated in the absence of H-bond based restraints and on the basis of supporting nuclear Overhauser effect (NOE) data. Hydrogen bonds inferred in this way were represented in the calculation by an appropriate set of distance restraints that were included during subsequent rounds of structure calculations. Four “filtering” steps were performed on the ambiguous restraints between successive rounds of structure calculations (38); the first three removed restraints contributing <1% of the total NOE, and the final one eliminated restraints contributing <2%. An ensemble of 26 lowest NOE-energy structures was selected.
**RESULTS**

**Structure Determination of fH~19–20**—There were no NOEs, nor was there other evidence, in support of non-transient self-association of fH~19–20, despite the relatively high sample concentration in the NMR tube. Two disulfide linkages in each module were inferred from the proximity and geometry of Cys side chains. All proline residues in fH~19–20 were defined as trans on the basis of chemical shift differences between C\textsubscript{n} and C\textsubscript{m} resonances and the observation of appropriate NOEs.

It is apparent from the ensemble of lowest energy structures (Fig. 1) and the root mean square deviation values (Table 1) that the three-dimensional structure of each module is well defined by experimental data. Residues without long range NOEs occupy loops, and they have low \(^1\)H, \(^15\)N NOE values (Fig. 2), consistent with nanosecond time scale backbone flexibility. The numbers of medium and long range NOEs originating from linker residues (Leu\(^{1164}\text{–Pro}^{1166}\)) are similar to those from residues in well structured regions of the two modules (Fig. 2), and the sets of module-to-module and module-to-linker distance restraints dictate a well defined mutual orientation of the module pair (Fig. 1A). The \(^1\)H, \(^15\)N NOE values for linker residues indicate relatively little linker mobility on the nanosecond time scale. These two modules therefore form a structural unit with limited intermodular flexibility.

CVP 19 adopts a classic CCP module fold (Fig. 3A), elongated (~32 × 16 Å) with N and C termini at opposite ends. Two disulfide bonds, Cys\(^{1}\text{–Cys}^{11}\) Cys\(^{12}\text{–Cys}^{13}\), lie at opposite extremes of a hydrophobic core to which the consensus Trp (Trp\(^{1157}\)) contributes. Its structure resembles those of other CCPs found elsewhere in factor H (45, 46), and in other complement regulators, CD35 (47), CD46 (48), and CDS5 (49, 50).

CVP 20 (Fig. 3A) has the same disulfide pattern, but the overall structure of this module differs from those of all other CCPs solved to date.

**Determination of the Minimum Heparin Species Necessary for Binding**—Heparin-derived oligosaccharides were prepared from low molecular weight heparin as described (39). Fluorophore-labeled heparin species were produced by attachment of 2-aminoacridone to the oligosaccharide reducing end as described (40). The gel mobility shift assays were performed as described (41).

**Modeling the C3d-Factor H Complex**—The program HADDOCK (42) was employed to dock the C3d structure (43) onto the lowest energy example in the NMR-derived ensemble of fH~19–20 structures. Active residues were defined on the basis that they were both solvent-accessible and among those suggested by Hellwage et al. (44). Passive residues were defined as the solvent-exposed residues surrounding the active residues. Standard analyses were performed by HADDOCK, including an analysis of the energy contributions from buried surface area and electrostatic interactions. On this basis, the best 13 structures from 50 water-refined structures overlaid on all backbone atoms with a root mean square deviation value of 0.7 Å.

**Locating the Polyanion-binding Site by Chemical Shift Mapping**—A 10 μM sample of \(^{15}\)N-labeled fH~19–20 in 8 mM NaOAc (deuterated), 50 mM NaCl, pH 4.0, was used to acquire \(^1\)H, \(^15\)N HSQC spectra on the 800 MHz spectrometer, at 25 °C, in the presence of varying concentrations of homogeneous, fully sulfated, heparin-derived tetrasaccharide over the range 0–120 μM. Backbone amide resonance assignments for the free protein were reconfirmed under these buffer conditions. The polyanion-binding site was inferred from the location of amide resonances that exhibited significant changes in chemical shift (combined chemical shift difference ≥0.02 ppm) or line width, in spectra of the complex and that could be confidently assigned on the basis of assignments of the free protein.

Although adopting the CCP module fold, CVP 20 (~28 × ~20 Å) is shorter than CVP 19 and exhibits more prominent lateral bulges and a helical turn. Nearly all CCPs contain a structurally critical Trp residue between Cys\(^{13}\) and Cys\(^{14}\); in CVP 20, Trp\(^{1219}\) occupies such a position in the sequence but is solvent-exposed. A second Trp residue in CVP 20, Trp\(^{1183}\), is part of a prominent hypervariable loop (Fig. 3A, and see below).

The two modules are stacked in an elongated fashion (Fig. 3B), unlike the tilted arrangement observed in many other CCP module pairs (51), stabilized by interactions between the two modules and the bulky side chains of the short linker. Equivalent parts of the two modules are on the same face of the overall structure. An electrostatic surface representation (Fig. 3C) reveals that one face of fH~19–20 is positively charged, whereas the other has a predominantly negative charge. A band of mainly positive charge running up one face of CVP 19 is contiguous with a positively charged area of the CVP 20 surface, which stretches to the C terminus of the protein. The five-membered ring of the indole side chain of Trp\(^{1183}\) is wedged between Arg\(^{1182}\) and Lys\(^{1186}\) within the...
hypervariable loop, leaving the six-membered aromatic ring exposed to solvent at the center of the positively charged CCP 20 surface patch.

**Determination of the Binding Sites in fH⁻¹⁹–²⁰**—To characterize the binding of polyanions by fH⁻¹⁹–²⁰, sulfated heparin, a commonly used analogue of heparan sulfate (52, 53), was adopted. The shortest heparin fragment still able to bind with a realistic affinity to the double CCP module was required to minimize molecular weight-associated line broadening upon complex formation. In gelmobility shift assays (Fig. 4, A and B), it is clear that approximately equivalent quantities of fH⁻¹⁹,20 are required to retard the mobility of roughly equal proportions of a set amount of either a fully sulfated heparin tetrasaccharide or a mixed heparin tetradecasaccharide preparation. This shows that the shorter oligosaccharide binds with a comparable affinity to the longer one. A disaccharide did not bind (data not shown). The tetrasaccharide was therefore chosen for use in subsequent experiments.

In a series of ¹⁵N, ¹H HSQC spectra (Fig. 4C), only 18 out of 117 non-proline amino acid residues in fH⁻¹⁹–²⁰ exhibited a significant combined chemical shift difference (≥0.02 ppm) upon the incremental addition of up to 120 μM heparin tetrasaccharide (Fig. 4D). This implies that fH⁻¹⁹–²⁰ neither aggregates nor unfolds in the presence of this polyanionic ligand. It further suggests that the interaction of fH⁻¹⁹–²⁰ with the sulfated heparin tetrasaccharide is specific and localized and causes little if any changes in the structure of either module. A titration curve for residue Arg¹²³ (Fig. 4D) yielded a K_d of 9 μM. The perturbed residues map onto a single face of module 20, forming a swath that runs

**FIGURE 4. Heparin binding to fH⁻¹⁹–²⁰.** Gel mobility shift assays were performed on preincubated mixtures of protein (0–12 μg) and fixed amounts of 2-aminoacridone-labeled heparin tetradecasaccharide (A) or fully sulfated heparin tetrasaccharide (B). Protein binding is indirectly revealed by depletion of the band of free 2-aminoacridone-labeled oligosaccharide (light-pointing arrows). In panel A, this is also directly revealed by accumulation of a barely migrating, protein-heparin complex just below the well (left-pointing arrow). This is not visible in panel B as the complex with the smaller oligosaccharide is not anionic, so it does not enter the gel. C, HSQC spectra of fH⁻¹⁹–²⁰, free (black) and with 4, 10, 20, 43, or 120 μM heparin tetrasaccharide (red to blue). Perturbed cross-peaks are identified. The asterisk indicates the side chain. D, combined chemical shift differences as a function of residue number; (red, backbone; blue, side chain). Negative bars indicate non-Pro residues for which unambiguous assignments could not be obtained. The horizontal line indicates the significance threshold. Inset, combined chemical shift differences for a representative residue plotted against [ligand].
from the linker toward the C terminus, as viewed in Fig. 5A, roughly matching the distribution of electropositive charge (Fig. 3C). Among those residues thus implicated in binding to heparin (Fig. 5, A and B) are the triad of basic residues in the hypervariable loop (Arg1182, Lys1186, and Lys1188), the surrounding basic residues Lys1202, Arg1203, and Arg1215, and the C-terminal residues Lys1230 and Arg1231. No resonance assigned to a CCP 19 residue exhibited a significant change in chemical shift. The length of the affected region, ~24 Å, on CCP 20 is comparable with the length of 17 Å for a heparin tetrasaccharide, as determined in a fibroblast growth factor-heparin crystal structure (54). Thus, chemical shift perturbation studies identified a sulfated heparin tetrasaccharide-binding surface site of complementary charge and appropriate size on CCP 20.

In addition to those HSQC resonances with perturbed chemical shifts, four cross-peaks exhibited markedly reduced intensities following the titration. The 4 corresponding residues (Glu1145, Leu1164, His1165, Thr1193) form a cluster at the interface between the two CCPs (Fig. 3B), distal to the putative polyanion-binding site. Assuming, therefore, that these changes in intensity are not the consequence of direct contact with ligand, they likely arise from a change in intermodular dynamics. For example, motion of one module relative to the other on an intermediate time scale would produce such line-broadening effects. Taken together, these data indicate that a fully sulfated heparin tetrasaccharide binds to a discrete, preformed site on CCP 20 of fH but nonetheless elicits changes in the amplitude or the time scale of intermodular mobility.

Titration of a sample of 15N-labeled fH-19,20 with C3d resulted in incremental loss of signals in a series of HSQC spectra (data not shown), a consequence of line broadening arising from intermediate exchange between C3d-bound and free forms of fH-19,20. On the other hand, the addition of C3d to a sample of 15N-labeled single module 19 from fH yielded no significant spectral changes (data not shown), proving that module 20 is required for binding and consistent with previous studies implicating a binding site for C3d on CCP 20 (33, 44).

In view of these experimental limitations, a theoretical approach to identification of the C3d-binding site was adopted. Those residues that are most likely to form the C3d-binding site of CCP 20 were inferred by Hellwage et al. (44), on the basis of a sequence comparison with module 5 of fH-related protein 3 that also binds to C3d and a panel of CCP modules that do not bind to C3d. The fH-binding residues in C3d were determined previously using a peptide-mapping approach (55). Adopting these residues in C3d and in fH-19,20 as anchor points and starting from randomly placed structures of fH-19,20 and C3d (43), the docking program HADDOCK (42) produced highly consistent structural models of the
complex in which ~1100 Å² of protein surface area is buried (Fig. 5A). This inferred C3d-binding site lies on the opposing face of CCP 20 to the experimentally delineated glycosaminoglycan (GAG)-binding site.

**DISCUSSION**

The C-terminal 125 amino acid residues of fH comprise its 19th and 20th CCPs and represent a region that is a hotspot for aHUS-linked mutations. CCP 20 is a structural outlier among CCPs. The unusual three-dimensional structure of CCP 20 reflects its divergent sequence (56) (Fig. 6) and is consistent with its unique possession of both heparin and C3d-binding sites. Interestingly, the other putative polyanion recognition modules of fH, CCP 7 and CCP 13, do not show high sequences similarity to CCP 20 and are unlikely to bear much structural resemblance.

A range of flexibilities among CCPs has been noted previously (56, 57). The intermodular orientation of fH~19–20 is relatively well defined (Fig. 1A and D), consistent with the exceptionally short linking sequence between CCP 19 and CCP 20 of just 3 bulky residues. Furthermore, the intermodular interface is stabilized by van der Waals interactions of two loops of CCP 20, a loop of CCP 19 and the linker region (Fig. 3B). Nonetheless, a limited degree of intermodular motion, on time scales not manifested in heteronuclear NOEs or line broadening, cannot be ruled out.

The C-terminal CCP pair of fH not only contains most of the fH mutations that are associated with aHUS but is also a locus for binding of polyanionic carbohydrates, endothelial cells, and C3d/C3b. Chemical shift perturbation studies using sulfated heparin tetrasaccharide revealed a discrete, ~24 Å long swath of side chains on one face of CCP 20 involved in heparin binding. The partially exposed Trp (Trp1183) plays a central role in the binding site since it not only helps to hold in place Arg1182 and Lys1186 but also contributes a surface for potential van der Waals interactions. The electrostatic face of fH~19–20 extends across the intermodular linker from CCP 20 into CCP 19 (Fig. 3C); however, the binding of heparin tetrasaccharide is restricted to CCP 20. It is possible, however, that a longer oligosaccharide would bind across both modules. The binding site on CCP 20 includes 3 residues (Arg1203, Lys1230, and Arg1231) previously suggested to be part of the heparin-binding site based on sequence comparisons (44). The polyanion-binding site identified in the present study also overlaps with the heparin-binding site containing Arg1182, Lys1186, and Lys1188 postulated on the basis of a modeled structure (58) and with a site proposed on the basis of homology with CCP 4 of a viral complement regulator, which included Arg1182, Lys1202, Arg1203, Lys1230, and Arg1231 (52).

Interestingly, the C-terminal domains of factor H-related proteins 3 and 4, although highly similar in sequence and therefore in structure to CCP 20 (Fig. 6), do not bind heparin (44). It is clear from the current studies that critical heparin-binding site residues are replaced in these proteins, Trp1183 with Gly, Lys1186 with Asp, Arg1203 with Leu, and Arg1231 with Gln, whereas residues equivalent to the C-terminal Lys1230 and Arg1231 are absent. The published mutagenesis data also support the NMR-derived polyanion-binding site as shown. A triple fH~15–20 mutant (R1203E,R1206E,R1210E) did not bind to heparin or endothelial cells (33) in agreement with the central position of Arg1203 in the polyanion-binding site; a double fH~15–20 mutant (R1230E,R1231E) was able to bind to a commercial heparin column but did not bind to endothelial cells (33); and a R1215G mutant version of factor H bound to neither heparin nor endothelial cells.

The broadening of some resonances upon titration with heparin tetrasaccharide can be explained by a change in flexibility between CCPs 19 and 20 of fH. It is interesting that binding of a heparin decasaccharide to CCP 4 of viral complement regulator stabilizes a 29.5° rotation of CCP 4 relative to CCP 3 (52), an adjustment that could modulate complement regulatory function. A similar mechanistic link between glycosaminoglycan binding and biological function could exist for fH~19–20. The NMR-derived distance restraints do not define absolutely intermodular angles in fH~19,20 (Fig. 1D). Thus, heparin binding to fH could modify intermodular motion by selectively stabilizing a subset of conformations, potentially modulating the fH-C3b interaction.

Previous work had identified residues in fH and C3d likely to interact, and these were employed together with the two three-dimensional structures in a docking procedure to show that a highly plausible C3d-binding site lies on the opposing face to the GAG-interaction site (Fig. 5A). This arrangement allows for the interesting possibility of a tertiary complex formed by C3d (or C3b), factor H, and a GAG. Previous *in vitro* competition studies between C3d and commercially available glycosaminoglycan preparations yielded paradoxical results (33) that neither support nor invalidate this model as shown. A panel of polyanionic molecules inhibited C3d binding to a fH~15–20 construct; on the other hand, C3d actually enhances heparin binding to fH~15–20 (33). In other systems, glycosaminoglycans are known to enhance protein-protein interactions by “gluing” together the binding partners (59). However, polyanions apparently weaken rather than strengthen *in vitro* interactions between CCP 20 and C3d (33). Alternatively, a GAG molecule might promote fH dimerization, or it might bind simultaneously...
to CCP 20 and to CCP 7 within the same molecule of fH (Fig. 5C). This latter possibility is feasible given the bent-back structure of fH inferred from solution studies (60) and given the long intermodular linkers (up to 8 residues) in the CCP 12–14 region of fH that allow neighboring modules to fold back against one another. This would bring CCP 7, and therefore CCPs 1–4, close to C3 (Fig. 5C) and so enhance their effectiveness in terms of competing for factor B binding as well as decay acceleration and/or cofactor activity.

Of the several dozen fH mutations so far identified in aHUS patients, more than half occur in CCPs 19 and 20 (18). Many of the remaining missense mutations involve structurally critical residues in CCPs 14 to 18. The new structural data allow assessment of the likely consequences of the five aHUS-associated mutations in CCP 19 and the larger cluster of mutations in CCP 20 (Fig. 6). In CCP 19, C1163W will disrupt structure, whereas V1134G and W1157R will destabilize its hydrophobic core. The mutation Y1142D would change the flexibility and orientation between CCPs 19 and 20. In the case of D1119G, the greater local flexibility imparted by substitution with a Gly could perturb the structure or dynamics of the CCP 19 hypervariable loop that immediately follows. Both hypervariable loops (of CCP 19 and CCP 20) occupy the same face of fH—19–20, and the CCP 20 hypervariable loop contains several residues implicated in heparin binding. Therefore D1119G would indirectly interfere with a putative CCP 19 extension of the observed CCP 20 polyanion-binding site, which was not evident in studies with the tetrasaccharide but is important for interactions with longer ligands.

In CCP 20, the majority of disease-associated mutations will perturb surface properties, whereas a few are likely to destabilize the structure (V1197A, F1199S, P1226S, G1194D) or cause cross-linking (R1210C) (16). Substitutions of partially solvent-exposed Leu1189 for Phe or Arg (V1197A, F1199S, P1226S, G1194D) or cause cross-linking (R1210C) and/or cofactor activity.

The new structural data overwhelmingly support a disease model that hinges on an impaired ability of fH to properly recognize polyanionic self-markers, allowing alternative pathway activation to proceed unchecked on surfaces that are not enclosed by a membrane carrying other regulators. In the microvasculature of the glomerulae in which the basal lamina is exposed to complement proteins by large gaps (fenestrata) between endothelial cells, this is a potentially pathogenic phenomenon. The data do not exclude the contribution to the disease process of a degraded affinity for C3d/C3b in some of the mutants. That a single nucleotide polymorphism corresponding to an amino acid substitution in another polyanion-binding region of fH (CCP 7, Fig. 5C) predisposes to a second rare kidney disease, as well as to age-related macular degeneration (7), is suggestive of a similar underlying patho-physiological mechanism. Given the cooperative manner in which they work to suppress complement activation, additional sequence variants elsewhere among the RCA proteins might also be needed before complement-mediated damage manifests as clinical symptoms.

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