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Associative and Structural Properties of the Region of Complement Factor H Encompassing the Tyr402His Disease-related Polymorphism and its Interactions with Heparin

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Factor H (FH) is a major complement control protein in serum. The seventh short complement regulator (SCR-7) domain of the 20 in FH is associated with age-related macular degeneration through a Tyr402His polymorphism. The recombinant SCR-6/8 domains containing either His402 or Tyr402 and their complexes with a heparin decasaccharide were studied by analytical ultracentrifugation and X-ray scattering. The sedimentation coefficient is concentration dependent, giving a value of 2.0 S at zero concentration and a frictional ratio f/f_o of 1.2 for both allotypes. The His402 allotype showed a slightly greater self-association than the Tyr402 allotype, and small amounts of dimeric SCR-6/8 were found for both allotypes in 50 mM, 137 mM and 250 mM NaCl buffers. Sedimentation equilibrium data were interpreted in terms of a monomer–dimer equilibrium with a dissociation constant of 40 μM for the His402 form. The Guinier radius of gyration R_G of 3.1–3.3 nm and the R_G/R_O ratio of 2.0–2.1 showed that SCR-6/8 is relatively extended in solution. The distance distribution function *P(r)* showed a maximum dimension of 10 nm, which is less than the length expected for a linear domain arrangement. The constrained scattering and sedimentation modelling of FH SCR-6/8 showed that bent SCR arrangements fit the data better than linear arrangements. Previously identified heparin-binding residues were exposed on the outside curvature of this bent domain structure. Heparin caused the formation of a more linear structure, possibly by binding to residues in the linker. It was concluded that the His402 allotype may self-associate more readily than the Tyr402 allotype, SCR-6/8 is partly responsible for the folded-back structure of intact FH, and SCR-6/8 changes conformation upon heparin binding.

Keywords: factor H; X-ray scattering; homology modelling; ultracentrifugation

Introduction

In the innate immune defence system, C3 is the central complement component whose activation to C3b through the removal of the small anaphylatoxin C3a by cleavage initiates the alternative pathway in serum. Complement factor H (FH) regulates the alternative pathway to prevent host damage by C3b by acting as a cofactor for factor I in the breakdown of C3b to form iC3b.[1–3] It also accelerates the decay of the C3 convertase C3bBb, and competes with factor B for binding to C3b. FH

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Abbreviations used: FH, factor H; SCR, short complement regulator; AMD, age-related macular degeneration; aHUS, atypical haemolytic uraemic syndrome.

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consists entirely of 20 short complement regulator (SCR) domains, each of length about 61 residues. SCR domains constitute the most abundant domain type in the complement proteins, and are also known as short consensus repeat, Sushi or complement control protein domains.[4] The four N-terminal domains SCR-1 to SCR-4 bind to intact C3b, a second site in SCR-6 to SCR-10 binds to the C3c region of C3b, and a third site within SCR-16 and SCR-20 binds to the C3d region of C3b.[5,6] FH is thought to regulate surface-bound C3b activity by recognising charge (anionic) clusters on cell surfaces. Heparin has been used as a model for such interactions, sometimes being considered as representative of heparan sulphate. Heparin binding sites have been located in SCR-7 and SCR-20 and a third heparin binding site originally thought to be near SCR-13 has more recently been reported to be in SCR-9.[7–9] FH also interacts with proteins from pathogenic bacteria as part of immune evasion strategies.[2] Mutations and polymorphisms on FH are associated with atypical haemolytic uraemic syndrome (aHUS)[10] and age-related macular degeneration (AMD).[11–14]

Structural studies of FH are hindered by its size, glycosylation, inter-SCR flexibility and multiple ligand binding sites. Constrained solution scattering modelling and electron microscopy suggested that FH exhibited folded-back structures and may exist in monomeric and dimeric forms.[15–17] To date, NMR and crystal structures for FH have been reported for SCR-5, SCR-15/16 and SCR-19/20.[4,18–21] The homology modelling of all 20 SCR domains has been described and updated, based on 27 NMR and crystal structures for SCR proteins.[17,22] The consensus results of these structural studies showed that a typical SCR domain contains six to eight β-strands b1–b8, of which b2–b7 are shown in Figure 1.[23] The aHUS and AMD mutation sites suggest that the SCR domains are associated with functional binding roles, while the inter-SCR linkers are relatively unimportant in this context.[23] The orientation between two adjacent SCR domains is varied and not easily predicted.[24] An explanation is required for why FH exhibits a folded-back structure while this is not observed for related proteins such as complement receptor type 2 (CR2) with 15/16 SCR domains.[25]

X-ray scattering and ultracentrifugation in combination with constrained modelling leads to medium resolution molecular structures.[26,27] The solution structure of small fragments of FH can be studied by this approach. The SCR-6/8 fragment is of interest, not only because it possesses a heparin site, but because a common Tyr402His polymorphism in SCR-7 (corresponding to residue 384 in mature FH) is associated with AMD.[11–14] Individuals who carry a single copy of the His402 allele in the polymorphism have a two- to fourfold increased risk of AMD. Individuals who carry two copies of the His402 allele have a five- to sevenfold increased risk. Molecular structures for both allotypes of the SCR-6/8 domains, including their complex with heparin, may elucidate the increased AMD risk caused by the Tyr402His substitution. Accordingly we have performed ultracentrifugation, scattering and constrained modelling studies on the SCR-6/8 fragment. The His402 allotype may show a slightly greater propensity to self-associate than the Tyr402 allotype. Unlike most of the SCR solution structures we have studied[25] SCR-6/8 shows a bent domain arrangement, and the Tyr402His polymorphism site is on an exposed region between β-strands b2 and b3 in this structure (Figure 1). Tyr402His is close to the heparin binding site in SCR-7, and the two forms of SCR-6/8 exhibit differential binding to heparin.[28,29] We investigated the SCR-6/8 complex with a heparin decasaccharide (dp10) to determine if dp10 leads to a conformational change in SCR-6/8. The implications of these findings for AMD are discussed.

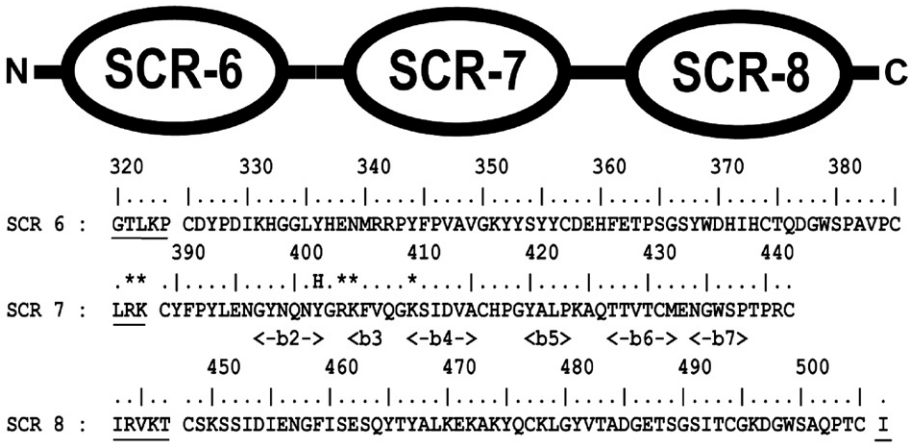

Figure 1. Cartoon of the FH SCR-6/8 domains and its sequence. The position of the Tyr402His polymorphism is highlighted by an H symbol. Note that Tyr402 is numbered as Tyr384 in earlier FH publications if the signal peptide is not included. The inter-SCR linker sequences are underlined together with the N-terminal G residue from the expression vector. Five residues identified as putative heparin binding sites are denoted by asterisks (*). The approximate locations of the six β-strands of the SCR secondary structure are denoted by b2 to b7 underneath the SCR-7 sequence.