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Structural insights into the central complement component C3

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Abstract

C3 is a central protein of the complement system, which is important to immune defense and provides a link between innate and adaptive immunity. Three pathways of complement activation converge at the activation of C3 yielding a diverse set of biological responses. This versatile and flexible molecule interacts with various proteins to fulfill its functions. Here we review recent insights gained from the crystal structure determinations of human, native C3 and its physiological down-regulation product C3c. The data provided, for the first time, a complete and detailed view of the composition and arrangement of the domains in C3. Comparison of C3 with C3c indicates marked flexibility of the molecule, particularly in the α-chain. We discuss the observed domain rearrangements, conformational changes and the location of various protein binding sites. These detailed, and structural, insights are important for developing models of the molecular mechanisms underlying the diverse biological activities of this large and complex molecule.

Keywords: Complement system; C3; α2-macroglobulin family; Innate immunity; Structure

1. Introduction

The mammalian complement system plays an important role in humoral immune defense against invading microorganisms [reviewed in (Carroll, 2004; Walport, 2001)]. The system may recognize and eliminate pathogens in the blood stream, elicit inflammatory responses and facilitate the adaptive immune response. Besides recognition and clearance of "non-self" material, the complement system is involved in clearance of "self" particles, such as apoptotic cells. Unduly complement activity, however, has been associated with a wide variety of inflammatory conditions and immune-complex diseases such as autoimmune diseases, sepsis, adult respiratory distress syndrome, hyperacute rejection of transplants, etc. In this sense, the complement system has often been referred to as a double-edged sword.

Complement activity is established by the interplay of 30–40 soluble plasma proteins and cell-surface proteins. Three routes of recognition lead to activation of the complement system: the classical (antibody-mediated) pathway, the lectin pathway and the alternative pathway. These pathways converge in the activation of the third complement component C3. C3 is activated proteolytically by enzyme complexes, the C3 convertases, yielding the anaphylatoxin C3a, which mediates inflammation (Bokisch et al., 1969), and the major fragment C3b. C3b can covalently attach to target surfaces via its reactive thioester (Law et al., 1979). Bound C3b provides a platform for the generation of more C3 convertases yielding amplification of the complement response (Muller-Eberhard and Gotze, 1972) and formation of C5 convertases initiating the terminal phase of complement activation, i.e. formation of the membrane-attack complex on target cells resulting in cell lysis. Furthermore, C3b may opsonize particles leading to phagocytosis and, thus, clearance of the tagged particles. Subsequent proteolysis of C3b results in the formation of fragment iC3b, and finally fragments C3dg and C3c (Harrison and Lachmann, 1980; Ross et al., 1982). iC3b and C3dg may stimulate B-cells providing a link between the innate and adaptive immune responses (Carroll, 2004). To fulfill all these roles, a diverse set of protein molecules interact with the various activated fragments of C3. This includes the protease of the (alternative pathway) convertases, complement regulators and cell-surface receptors on macrophages and B-cells (Lambris, 1988). The specificity of binding partners implies the existence of cryptic binding sites and, therefore, the importance of conformational differences between the C3 fragments.

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C3 and the homologous complement proteins C4 and C5 are members of the α2-macroglobulin (α2M) family of proteins. The proteins of this family have emerged over 700 million years ago (Sunyer et al., 1998). They are ubiquitous among metazoans and have a primary function in host defense (Blandin and Levashina, 2004). Other well known members of the family include the universal protease inhibitor α2-macroglobulin and the thioester-containing proteins (TEPs) from insects and nematodes (Budd et al., 2004). All these proteins are relatively large (1400–1800 amino-acid residues) with several marked functional features; a reactive thioester moiety enabling covalent attachment to target particles, a highly variable central segment likely involved in recognition; and, the propensity to undergo conformational changes yielding distinct protein-binding interactions (Budd et al., 2004; Chu and Pizzo, 1994; Lagueux et al., 2000). Structural insights into these large proteins, however, have remained scarce and fragmented.

Here, we focus on recent insights that have been obtained through the structure determination of the full-length, native C3 molecule with its thioester moiety intact and the C3c molecule, which is the major breakdown product of the C3 molecule (Janssen et al., 2005). These structures provided for the first time insights into the intricate domain arrangement, the thioester protection mechanisms and flexibility of this type of proteins. With these structures available we can now develop detailed models for the underlying molecular mechanisms of C3 activation and regulation.

2. Structure determination of C3

The three-dimensional structure of C3, or of any of its homologues, has remained elusive for a long period of time. Typically for molecules of this size, the approach of structural biologists has been to determine structures of fragments or domains first. In the 1980s the first crystal structure determined of a fragment from a member of the α2M family was that of the anaphylatoxin C3a (Huber et al., 1980), followed 9 years later by the NMR solution structure of the homologous C5a (Zuiderweg et al., 1989). Since then only three other domain structures were determined by crystallography or NMR: (i) in 1998 the receptor-binding domain (RBD) of α2M (Jenner et al., 1998); (ii) the structure of C3d in 1998 (Nagar et al., 1998) and more recently (in 2002) that of the homologous C4d (van den Elsen et al., 2002); and finally (iii) most recently, in 2005, the C-terminal domain C345C of C5 (Bramham et al., 2005). These data provided structural insights for 40% of the protein molecule. However, the structure of the remaining 60% and the overall architecture remained elusive. This is exemplified by a conserved-domain search at http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi. This site presents two large single domain regions for C3: A2M_N (pfam01835) the α2M family N-terminal region corresponding to the β-chain of C3; and, A2M (pfam00207) the α2M family C-terminal region constituting most of the α-chain.

In our structural studies on C3 (Janssen et al., 2005) we first crystallized the proteolytic fragment C3c, which constitutes 72% of the complete C3. Analogous to limited proteolysis treatment, the naturally occurring breakdown of C3 into C3c in blood plasma yields a molecule forming the core structure of the molecule. Generally, such a core molecule exhibits less flexibility and is more amenable to crystallization. In addition, the molecule was further trimmed by enzymatic deglycosylation completely removing the glycan moiety on Asn-917. Crystals of this deglycosylated C3c typically diffracted to ∼3-Å resolution with the best crystal diffracting to 2.4-Å resolution. Crystal-structure determination was based on the classical heavy-atom derivative method. More expeditious methods were not applicable, because no significant structural models were available *a priori* for C3c to be used in molecular replacement; and, SeMet-MAD phasing was excluded because the protein was obtained from the natural, human source. In the end, the structure was determined by phasing from two heavy-atom derivatives and phase combination using 6-fold averaging over three crystals. The final electron-density map was of excellent quality. The final model of C3c consisted of 1109 residues forming 10 domains (coordinates of this model are available; entry 2A74 in the Protein Data Bank http://www.rcsb.org/pdb).

With the structure of C3c available, we were able to solve the structure of C3 more readily. To retain haemolytic activity C3 was precipitated by dialysis against 5 mM of MES buffer at a pH of 6.0 and the precipitate was stored at −80 °C. Shortly before the crystallization experiments precipitated C3 was resolubilized by dialysis against 10 mM of Tris buffer at a pH of 7.4. In this way, we obtained crystals of native, glycosylated human C3 with the thioester intact. Diffraction data was collected up to 3.3-Å resolution. The structure was solved by molecular replacement using the known structures of C3c and C3d (Nagar et al., 1998) followed by model building to complete the structure. The final model of C3 consisted of a total of 1611 residues forming 13 domains (entry 2A73 in the Protein Data Bank).

3. Domain organization

The structures of C3c and C3 display an intricate arrangement of 10 and 13 domains, respectively (Fig. 1). The core of the protein is formed by eight homologous domains, which we named macroglobulin (MG) domains referring to the related immunoglobulin fold and to the family of α2M proteins. The first five domains, MG1-5, are formed by residues of the β-chain. Most surprisingly, the next domain, MG6, is formed by residues from both the β- and α-chains, such that the two chains are intertwined. The last two MG domains, MG7-8, are provided by the α-chain. The other five domains are crafted onto this core of eight MG domains in two large insertions and one extension.

The first insert is located in MG6 and is formed by residues 578-745. This segment includes the linker region (LNK), the tetra-arginine pro-C3 processing site, the anaphylatoxin (ANA) domain and a linker (α'NT) that connects the ANA domain back to MG6. A remarkable feature of this insert is its geometric arrangement. The insert loops completely through the central hole formed by the MG1-6 domains. Because the processing site is included in this segment, the mature protein appears as two chains that are intertwined in domain MG6. In the structure of C3, the C-terminus of the β-chain and N-terminus of the α-chain

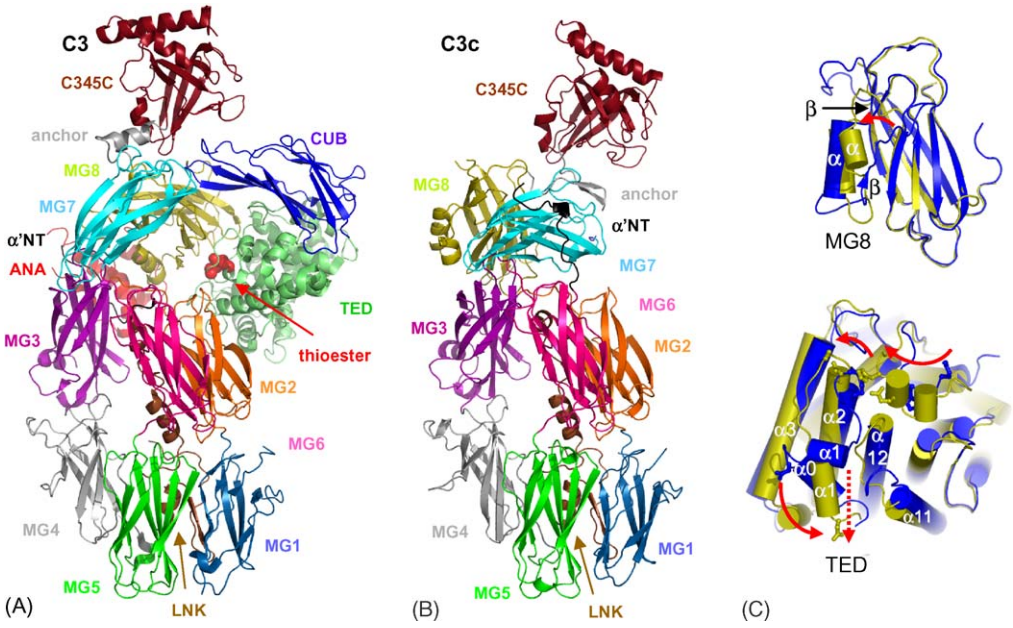

Fig. 1. Structures of human complement component C3 and C3c. (A) Ribbon representation of C3. Each of its 13 domains is shown in a different colour with domain names as indicated. Additional features indicated are the intact thioester displayed as red spheres, the anchor region shown in grey, and the α'NT in black. (B) Ribbon representation of C3c, its 10 domains are coloured according to A. (C) Superposed MG8 of C3 (blue) and MG8 of C3c (olive) with the secondary structure elements of the β-α-β/β-α-α transition indicated and superposed TED domain of C3 (blue) and C3d (olive) (Nagar et al., 1998) with residues of the thioester and acyl-imidazole intermediate shown in stick representation. Conformational changes are indicated by red arrows.

are far apart; there is a gap of 53 Å between the last β residue (Pro-643) and first α residue (Val-651) present in the model. In pro-C3 the two ends of the chains are connected through the tetra-arginine (residues 646-649). Thus, in pro-C3 the LNK-ANA arrangement must be drastically different.

Residues 913-1334 form a segment that may be considered an insert between domains MG7 and MG8. These residues form the CUB domain and the TED domain that carries the reactive thioester. The TED domain itself (residues 963-1268) is inserted in loop β5-β6 of the CUB domain. Consequently, CUB is an intertwined domain consisting of two distinct parts of the polypeptide chain (residues 912-962 and 1269-1330). The cleavage fragments C3d, C3g and C3f are part of this CUB-TED insert.

At the C-terminal end the C345C domain is connected to MG8 via a short anchor region. This C-terminal extension is common to the complement proteins C3, C4 and C5, and not to other members of the α2M family. The anchor region links the C345C domain to both the MG8 domain, through the polypeptide chain, and to the MG7 domain, through a disulphide bridge. As such, the position and orientation of C345C depends on both MG7 and MG8 by a riding motion.

Overall, the structures of C3 and C3c have revealed an intricate arrangement and composition of 13 and 10 domains, respectively (Fig. 1). The data shows that the previously listed A2M_N and A2M regions consist of the eight MG domains forming the core and the LNK, CUB and TED domains. Neither sequence alignments nor intron/exon boundaries indicated the observed domains. The fact that intron/exon boundaries do not overlap with domain boundaries is also observed for other ancient multidomain proteins unique to metazoans. This probably results from a continual insertion and removal of introns (Patthy, 1999). This notion of ancientness is further supported by the lack of sequence identity among the eight MG domains, which do not show a single conserved amino-acid residue.

4. Structural rigidity and flexibility

Comparison of the structures of C3 and C3c suggests that domains MG1-6 form a structurally stable platform, that we called the β-ring, onto which the flexible domains of the α-chain are crafted. Even though large movements of domains occur (with maximum displacements up to 50 Å for residues of MG8) most domains themselves remain relatively unchanged. Three significant exceptions are the MG8 domain, the small anchor region that links C345C to MG7 and MG8; and, the TED domain when compared to the structure of C3d. Another striking structural change involves the linker between the ANA and MG6 domain, referred to as α'NT, that will be discussed in Section 5.1.

4.1. Flexible motif in MG8

MG8 of C3 plays a crucial role in the double protection mechanism of the highly reactive thioester. First, it contributes a large part of the hydrophobic/aromatic pocket that protects the thioester against nucleophiles; and second, it prevents the formation of the free thiolate and acyl-imidazole intermediate necessary for the high-reactivity with hydroxyl nucleophiles (Law and Dodds, 1997). MG8 drastically changes position from C3 to C3c. In addition, the domain itself undergoes a conformational change (as shown in Fig. 1C): residues 1388-1420 change

conformation from a β-α-β to a β-α-α motif in the conversion of C3 to C3c. This structurally variable segment is not part of the MG core fold (it forms a segment in between strands βC and βC' of the MG fold). The β-α-β motif is also observed in the structure of the isolated RBD of α2M. In α2M this segment is important for the binding to the α2M receptor (Jenner et al., 1998). In C3 this segment is involved in protecting the thioester, as it bridges contacts between TED (through Arg-1405, Ser-1408, Lys-1409 and Glu-1411) and the ANA domain (through Gln-1398, Leu-1399, Val-1403, Asp-1404 and Tyr-1406) stabilizing the overall structure of C3. In addition, residues 1402-1435 were found to be important for properdin binding (Daoudaki et al., 1988), which stabilizes the C3bBb, C3-convertase complex. In contrast to C3 where the segment is largely buried, the segment is exposed in C3c. We hypothesize that C3c resembles C3b in this respect. The change from hidden to exposed and the conformational change from β-α-β to a β-α-α may be a critical feature for properdin binding to the C3bBb complex.

4.2. The anchor region is structurally variable

The anchor region (residues 1475-1495) connects the C-terminal C345C domain to the body of the molecule via the peptide chain to MG8 and through a disulphide bond (Cys-851 to Cys-1491) to MG7. The anchor region has an additional internal disulphide bond from Cys-1484 to Cys-1489. Surprisingly this region undergoes a drastic conformational change in the conversion of C3 to C3c from an α-helix to a β-hairpin, respectively, apparently not restricted by the internal disulphide bond. Possibly the conformational change is induced by the large movement of MG7 in the conversion of C3 to C3c onto which the anchor seems to "ride". The conformational change of the anchor might be important for factor B binding (Kolln et al., 2004) in the formation of the C3 convertase.

4.3. Conformational changes in the TED domain

The TED domain forms a double, α6-α6, helical bundle. The helices alternate outside-in with the even helices forming the central 6 helical bundle and the odd numbered helices forming a shell around the inner bundle. Structures of isolated C3d (Nagar et al., 1998; Zanotti et al., 2000), C4d (van den Elsen et al., 2002) and C3d in complex with CCP domains 1 and 2 of CR2 (Szakonyi et al., 2001) have been solved previously. These structures showed little structural differences among each other. In contrast, large structural changes are apparent between the TED domain of C3 and the C3d structure, which appear correlated with the switch from the thioester to the free thiolate and acyl-imidazole intermediate (Fig. 1C). In C3 the intact thioester sits on top of helix α2, which adopts a strained 3_{10} helical conformation. In C3d the thioester residues move upward (by 3/4 of helical turn) and the helix straightens into a typical α-helix conformation. This up, and sideways, movement of helix α2 creates space for loop 1103-1115, which is now free to move in. This loop, which was largely disordered in C3, adopts a well-defined conformation in C3d, where it forms two short helical turns (1105-1108 and 1110-1112). The straightening of the helix α2 and the upwards movement of the thioester are possibly correlated with changes in the N-terminus of TED. This N-terminus displays two small helices, α0 and α1 that are packed in between helix α3, loop α11-α12 and helix α2. This region (residues 965-981) is extended in C3d with helix α0 expelled. Potentially, these changes may be induced by a pulling force on the N-terminus in the conformational change from C3 to C3b.

The remainder of the domain is conformationally stable. By and large, the structural changes are limited to helices α0, α1, α2 and α3 that rearrange as discussed. Most of the interaction faces, therefore, do not undergo large changes in themselves and appear structurally stable. In contrast large changes in accessibility are expected for the interfaces of TED in the conversion of C3 to C3b, i.e. TED is nestled in C3, whereas a more exposed position is expected for TED in C3b.

5. Location of binding sites

C3 and its derived products are versatile molecules that are able to interact with over 30 different ligands from self and non-self (Lambris, 1988; Lambris et al., 1998); and, new ligands are still being found (Helmy et al., 2006). The affinity for many of these ligands changes in going from C3 to C3b, iC3b and finally to C3c and C3d with most ligands binding to the activated forms, C3b and iC3b. Numerous studies have provided insights into the binding sites of these ligands. However, up till now the structural data was lacking to provide a comprehensive view of these binding sites. Here, we discuss the proposed binding sites with regard to the structures of C3 and C3c (see also (Janssen and Gros, 2006)) (Fig. 2). Unfortunately, no structural data are yet available for C3b and iC3b. Therefore, potential conformational changes must be considered that will affect the arrangement of the putative binding sites.

5.1. Convertase formation

The complement amplification step is initiated by the formation of the C3 convertase through the binding of factor B to C3b and subsequent proteolytic activation of factor B. By binding to C3b, factor B becomes sensitive to factor D which cleaves it into Ba, which dissociates from the convertase, and Bb, which stays attached. This yields the active (and short-lived) convertase complex of the alternative pathway C3bBb that will amplify the complement response by cleaving additional C3 into C3a and C3b. So far four separate sites on C3b have been determined that may either represent a binding site or residues that are indirectly involved in the binding of factor B. These putative binding sites are; (i) residues 727-745 (Fishelson, 1991), which form the α'N-terminus (α'NT), especially acidic residues Asp-730, Glu-731, Glu-736 and Glu-737 (Taniguchi-Sidle and Isenman, 1994); (ii) residues 933-942 (O'Keefe et al., 1988), which form strand β4 and its flanking loops in the CUB domain of C3; (iii) residues 200-220 (Inal and Schifferli, 2002), which form strand βG of MG2, the connecting MG2-MG3 loop and βA of MG3; and (iv) the C-terminal C345C domain (formed by residues 1496-1641) (Kolln et al., 2005; Kolln et al., 2004). Together these data indicate four structurally distinct regions located in the top part,

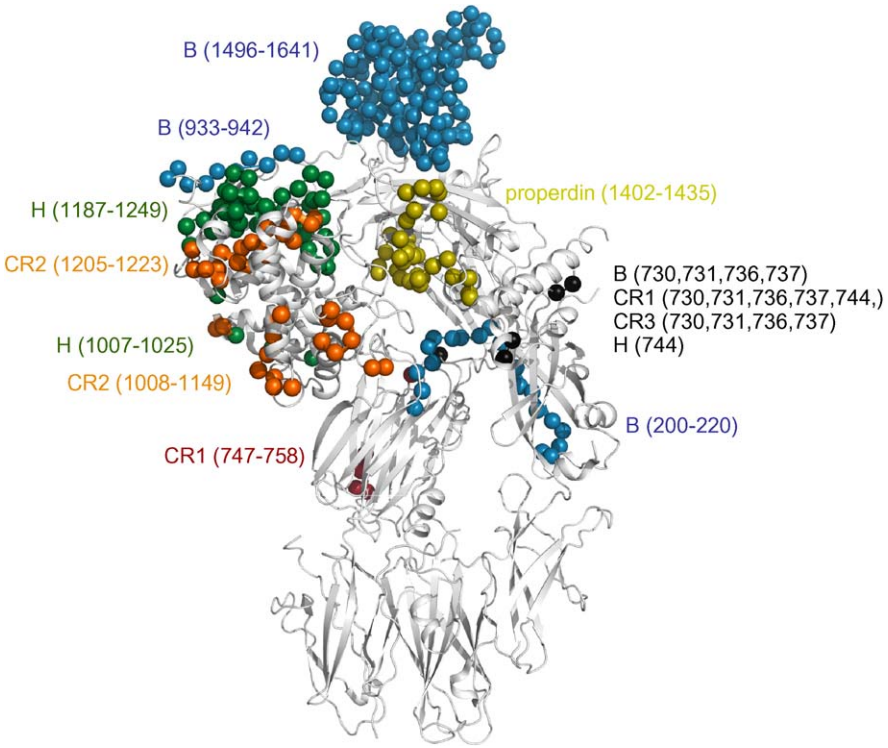

Fig. 2. Putative binding sites mapped on the structure of C3. Proposed binding sites (as discussed in Section 5) are indicated by residue numbering and spheres, coloured according to ligand; factor B (blue), factor H (green), CR1 (red), CR2 (orange), properdin (olive) and the α'NT containing binding sites for factor B, CR1, CR3 and factor H (black).

mostly within the α-chain, of the C3 molecule (Fig. 2). Structural changes are expected in the conversion of C3 to C3b (Isenman et al., 1981). Nevertheless the C345C domain is exposed in both C3 and C3c, which suggests that it is also exposed in C3b and thus accessible for factor B binding. The segment formed by residues 933-942 (O'Keefe et al., 1988) on CUB is exposed in C3 and proteolytically removed by factor I in C3c (Lachmann et al., 1982). In the structure of C3 it is located close to the C345C domain and possibly even after conformational changes factor B can bind simultaneously to both regions. In contrast, residues 200-220 are inaccessible to proteins in both C3 and C3c, even though these residues are exposed to the solvent. A conformational change would be required for factor B binding to this segment. Such a change is not observed in this region when comparing C3 and C3c. Strikingly, the α'NT region with the four acidic residues required for factor B binding (Taniguchi-Sidle and Isenman, 1994) is buried in C3 by the ANA domain and surface exposed in C3c. The α'NT region undergoes a drastic conformational change from C3 to C3c. By sliding through the ring formed by the β-chain, it moves from the ANA side to the opposite, MG6 side of the molecule. However, at this stage we do not know in which conversion step this large structural rearrangement of the α'NT occurs and, thus, on which side the α'NT resides when factor B binds to C3b.

The labile complexes C3bB and C3bBb are stabilized over 10-fold by properdin binding (Fearon and Austen, 1975; Hourcade, 2006). Properdin binding increases the affinity of factor B for C3b and prevents the cleavage of C3b to iC3b by factor I. Residues 1402-1435 form a binding site for properdin (Daoudaki et al., 1988) (Fig. 2). These residues are part of MG8 forming the conformationally flexible β-α-β/β-α-α motif (residues 1388-1420), as discussed above. Not only does this segment alter its conformation, it also changes from hidden to exposed in the conversion of C3 to C3c. Possibly this site is also exposed in the C3bB and C3bBb complexes. Furthermore, the properdin C3bBb interaction has been proposed to be mainly ionic strength dependent (DiScipio, 1981). This is in good agreement with the mainly positively charged properdin (Sun et al., 2004) and mainly negatively charged C3 and C3c (Janssen et al., 2005).

5.2. Decay acceleration activity

Regulation of complement is crucial to prevent severe damage to host tissues. Inhibition of convertase activity, and thus inhibition of complement amplification, is achieved by various regulators that accelerate the dissociation of the convertases, called "decay-acceleration activity" (DAA) (Kirkitadze and Barlow, 2001) and that can discriminate between self and non-self. These regulators belong to a family of homologous proteins called "regulators of complement activation" (RCA). Factor H, decay-accelerating factor (DAF, CD55) and complement receptor 1 (CR1, CD35) are three important members of this family. Various studies (Becherer et al., 1992; Fishelson, 1991; Lambris et al., 1996; Oran and Isenman, 1999; Taniguchi-Sidle and Isenman, 1994) have shown that residues 727-767 of C3b form an important interaction site for both factor H and CR1. Mutational studies have further pinpointed crucial residues in this region. For factor H these are acidic residues Glu-744 and

Glu-747 and for CR1 these are acidic residues Asp-730, Glu-731, Glu-736, Glu-737, Glu-747, Glu-754 and Glu-755 located on α'NT (729-745) and on MG6 (535-577 and 746-806) (Fig. 2). This indicates that both factor H and CR1 have a primary binding site on MG6. The fore-mentioned residues are the same or are close to residues important for factor B interaction. This is consistent with the observation that factor H, CR1 and factor B compete for C3b or C3(H_2O) binding (Weiler et al., 1976). Factor H has at least one additional binding site situated on the TED domain (Lambris et al., 1988). Very recently two different models of a complex between C3d and the C-terminal two CCP domains (CCP19-20) of factor H were proposed (Herbert et al., 2006; Jokiranta et al., 2006). One model suggests that residues Asp-1007, Glu-1008, Glu-1010, Arg-1020, Glu-1025 and Lys-1262 of TED/C3d are involved in factor H binding (Jokiranta et al., 2006), whereas the other proposes a binding site somewhere between residues 1187-1249 (Herbert et al., 2006) (Fig. 2). Residues proposed in both cases are for a large part solvent exposed in C3, however some parts of the 1187-1249 region are hidden, i.e. beginning of helix α10 (by MG8), a side of α11 (by CUB), loop α11-α12 (by CUB) and the N-terminal part of α12 (by MG8), which could explain the specificity of factor H for C3b and not C3. However, factor H is an extended molecule consisting of 20 CCP domains, steric hindrance of this or other interaction sites determining specificity cannot be excluded at this stage. Thus, the exact mode of interactions involved in DAA remains unclear, but a general model of steric hindrance disrupting the C3bBb interactions appears plausible.

5.3. Cofactor activity

A second mechanism of complement regulation is the processing of C3b into iC3b by the protease factor I with the aid of additional cofactors. Processing into iC3b induces structural rearrangements and changes the binding properties, e.g. factor B and properdin can no longer bind. The cofactors which display the "cofactor activity" (CA) are factor H, CR1 and membrane-cofactor protein (MCP) which also belongs to the RCA family of proteins (Medicus et al., 1983; Pangburn et al., 1977; Ross et al., 1982; Seya et al., 1986). This cofactor-induced processing of C3b occurs in three cleavage steps. First factor I cleaves between residues Arg-1281 and Ser-1282 in loop β6-β7 of the CUB^f part of CUB which generates $iC3b_1$. A second cleavage occurs between Arg-1298 and Ser-1299, in strand β8 of CUB^f generating C3f (2 kDa) and $iC3b_2$ (Davis and Harrison, 1982; Harrison and Lachmann, 1980). Third, factor I cleaves between Arg-932 and Ser-933 in loop β3'-β4 of the CUB^g part, resulting in the formation of C3dg (40 kDa) and C3c (135 kDa) (Lachmann et al., 1982). This indicates that factor I might bind in various ways to the CUB domain assisted by one of the cofactors which might be involved in the unraveling of the CUB domain enabling factor I to cleave.

5.4. C3b fragments signaling

Two fragments generated by the processing of C3b, i.e. iC3b and finally C3dg, have an important role in the initiation of adaptive immune responses (Carroll, 2004). In addition, iC3b acts as an opsonin resulting in phagocytosis of tagged particles by leukocytes. The recognition of iC3b is mediated by the integrin, complement receptor 3 (CR3, $\alpha_M\beta_2$, CD11b/CD18, Mac-1) on leukocytes (Kamata et al., 1995; McGuire and Bajt, 1995; Ueda et al., 1994) and by the very recently discovered complement receptor of the immunoglobulin superfamily (CRIg) on Kupffer cells (Helmy et al., 2006). As for factor B, factor H and CR1 the α'NT has also been shown to be important for the CR3 interaction (Taniguchi-Sidle and Isenman, 1994), implicating that this region is also exposed in iC3b (Fig. 2). As CR3 does not bind to C3b it is possible that there are differences in or near the α'NT between C3b and iC3b which determines the specificity. Another possibility is that these ligands bind multiple regions which are conformationally different between C3b and iC3b. This is supported by various studies which show that multiple regions of CR3 contribute to iC3b interaction (Li and Zhang, 2003; Xiong and Zhang, 2001).

Binding of complement receptor 2 (CR2, CD21), consisting of 15 or 16 CCP domains, with surface bound iC3b or C3dg and the B-cell antigen receptor complex results in the stimulation of B-cells. This provides a link between the innate and the adaptive immune system (Carroll, 2004). Multiple studies have been performed on the interaction of CR2 with iC3b and C3dg (Clemenza and Isenman, 2000; Diefenbach and Isenman, 1995; Esparza et al., 1991; Kalli et al., 1991; Lambris et al., 1985; Lowell et al., 1989; Molina et al., 1995; Morikis and Lambris, 2004; Sarrias et al., 2001) (Fig. 2). A few years ago, the structure of a complex between C3d and two domains of CR2 (CCP1-2) was solved (Szakonyi et al., 2001). However this complex is controversial. In this complex the two CCP modules of CR2 adopt a V-shaped arrangement that would not be likely for the physiologically glycosylated CR2, which adopts a more extended conformation (Prota et al., 2002). Moreover, this structure of the complex showed interactions only between CCP2 of CR2 and C3d and no direct interactions between CCP1 of CR2 and C3d. Two recent studies by the same authors showed that CCP1 of CR2 probably contacts C3d directly (Gilbert et al., 2005; Hannan et al., 2005). Although significant structural differences are apparent between TED of C3 and C3d, the CR2 CCP2-binding site is very similar in the two structures. This indicates that conformational changes do not play a role for this subsite. Furthermore, this site is completely exposed in native C3, which would suggest that CCP1, and not CCP2, of CR2 discriminates between the conformational states of C3 versus iC3b and C3dg. Possibly its binding site is either inaccessible in C3 or is different in conformation.

6. Concluding remarks

The structures of C3 and C3c provide a great wealth of information. They show in detail the composition of the molecules and the location of the many interaction sites that are known for C3 and its activated fragments. However, a number of important questions remain, such as what is the arrangement of domains and, thus, the interaction sites in the activated molecules C3b and iC3b. Furthermore, the mechanisms of convertase formation and regulation are still poorly understood. The structures of C3

and C3c, however, provide a new starting point for additional structural and mutagenesis studies to elucidate the molecular mechanisms underlying the diverse functions of C3 and its derivatives.

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