

THE MULTIFUNCTIONAL ROLE OF C3: STRUCTURAL ANALYSIS OF ITS INTERACTIONS WITH PHYSIOLOGICAL LIGANDS*

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Of the 20 distinct complement proteins recognized to date, C3 is probably the most versatile because of its pivotal role in both the classical and alternative pathways (for review, see Müller-Eberhard, 1975; Porter and Reid, 1979). Therefore, the structure and multiple functions of C3 have been the subject of intensive study.

C3 [molecular weight (mol. wt) 190,000], the most abundant complement protein in serum (1.2 mg/ml), consists of two polypeptide chains, α and β , linked by disulfide bonds and non-covalent forces. Cleavage of C3 by the classical or alternative pathway C3 convertases at an Arg-Ser peptide bond between positions 77 and 78 of the α chain generates C3a, a 9000 mol. wt fragment with anaphylatoxin activity, and C3b (Fig. 1). The C3b fragment can, for a brief period, bind covalently via a labile binding site (Müller-Eberhard *et al.*, 1966; Law *et al.*, 1979; Tack *et al.*, 1980) to cell surfaces, complex polysaccharides or immune aggregates. Unlike native C3, and C3b fragment expresses multiple binding sites, including sites for C5 (Vogt *et al.*, 1978), properdin (*P*) (Chapitis and Lepow, 1976; Schreiber *et al.*, 1975), factor H (Whaley and Ruddy, 1976; Weiler *et al.*, 1976), factor B (Fearon *et al.*, 1973; Brade *et al.*, 1973), factor I (Vogt *et al.*, 1977) and CR1, the C3b receptor (Nelson, 1953; Gigli and Nelson, 1968). Engagement of the binding sites on C3b has the following effects. Bound factor B becomes susceptible to cleavage and activation by factor D. Bound P stabilizes the resulting C3 convertase, bound C5 can be activated by C3b, Bb or C4b, 2a. Binding of factor H accelerates the decay-dissociation of the C3 convertase and modulates C3b for enzymatic degradation by factor I. Engagement of target cell-bound C3b with the CR1 on phagocytic cells causes cell adhesion, which may induce ingestion or extracellular killing.

Many of the activities exhibited by bound C3b are

regulated by factor I in the presence of H or CR1 (Harrison and Lachmann 1980; Ross *et al.*, 1982b; Lachmann *et al.*, 1982; Medicus *et al.*, 1983). The α chain of C3b is first cleaved at an Arg-Ser bond between residues 1298 and 1299 of the C3 sequence, generating a 62,500 *N*-terminal peptide and a 41,500 *C*-terminal peptide. A second cleavage near the *N*-terminus of a 41,500 peptide between amino acid residues 1281 and 1282 liberates a 2000 fragment. A third factor I mediated cleavage between amino acid residues 932 and 933 generates a 22,500 *N*-terminal peptide and a 39,000 C3dg peptide with concomitant dissociation of C3c (Ross *et al.*, 1982b; Lachmann *et al.*, 1982; Davis and Harrison, 1982, Davis *et al.*, 1984). Further degradation of C3dg with trypsin, elastase, or plasmin releases C3g (Ross *et al.*, 1982b; Davis *et al.*, 1984). In addition to the interactions with the complement component C5, factors B, H and I, and P the C3 molecule has the potential to bind to specific cell-surface receptors known as CR1, CR2, CR3 and C3aR (receptors for, respectively, C3b, C3d, iC3b and C3a) (Fig. 1). Cells endowed with C3a receptors include mast cells, smooth muscle cells, polymorphonuclear leukocytes and presumably certain subsets of T lymphocytes (Hugli, 1984; Morgan *et al.*, 1982). C3a causes the release of histamine, certain arachidonic metabolites and hydrolytic enzymes. Additionally, C3a leads to smooth muscle contraction and suppresses the specific and Fc fragment-induced polyclonal antibody responses *in vitro*. The CR1 found on erythrocytes, neutrophils, eosinophils, monocytes, macrophages, B and T lymphocytes, mast cells and glomerular podocytes, binds to the C3c domain of iC3, C3b, iC3b and also to C3c (for review see Dierich *et al.*, 1982; Fearon and Wong, 1983; Schreiber, 1984; Lambris and Tsokos, 1986). On phagocytic cells the major role of CR1 is to enhance phagocytosis of IgG-bearing particles (Ehlenburger and Nussenzweig, 1977). Recent studies have indicated a major role for erythrocyte CR1 in the clearing of immune complexes bearing C3b (Medof *et al.*, 1982). The CR2 (C3d receptor) resides on B and K lymphocytes (Lambris *et al.*, 1981; Dobson *et al.*, 1981; Perlmann *et al.*, 1981) and binds iC3b, C3dg

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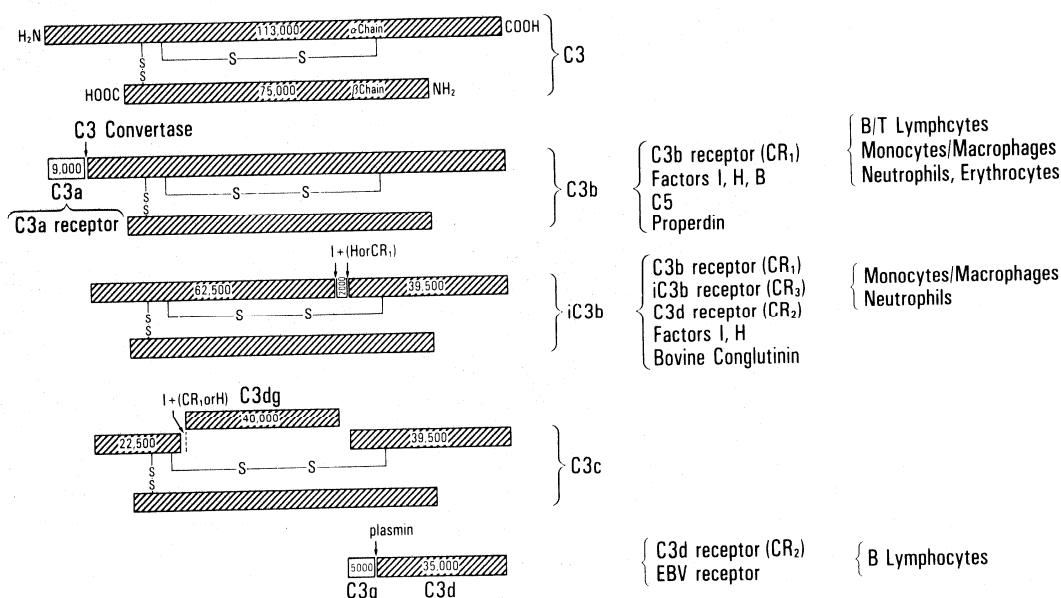


Fig. 1. Degradation of C3 and reactivity of its fragments with other complement proteins and cellular complement receptors.

and C3d. The major function of this receptor is the regulation of lymphocyte proliferation and growth (Lambris *et al.*, 1982; Meuth *et al.*, 1983; Melchers *et al.*, 1985). The CR3 (iC3b receptor) found on phagocytic cells and K lymphocytes binds to iC3b (Ross and Lambris, 1982a). CR3 on neutrophils and macrophages plays a major role in mediating respiratory burst and phagocytosis (Newman and Johnson, 1979; Newman *et al.*, 1980).

Localization of the individual binding sites on the C3b molecule has been approached recently by using monoclonal antibodies (MoAb) to C3b that compete with the binding of P, factor H, factor B and C5 (Burger *et al.*, 1982; Tamerius *et al.*, 1982). Although each of these antibodies selectively inhibited the binding of the different ligands to C3b, it was not possible to decide whether a given antigenic determinant was part of a specific functional site or located in its proximity. In addition, allosteric effects could not be ruled out.

Recently six binding sites involving the C3 molecule were studied: (a) the binding sites on C3 for CR2 (Lambris *et al.*, 1985b), factor H (Lambris *et al.*, 1985a), P (Lambris *et al.*, 1984c) and conglutinin (Hirani *et al.*, 1985a); (b) the binding site on H for C3b (Alsenz *et al.*, 1985a,c) the binding site on B for C3b (Lambris and Müller-Eberhard, 1984b).

THE CR2 BINDING SITE ON C3

The C3d fragment of C3 in addition to its metastable membrane binding site contains a site that binds to CR2 and an antigenic determinant recognized by MoAb 130 (Tamerius *et al.*, 1982). Both the CR2 binding site and the MoAb 130 antigenic site are expressed when C3b is cleaved to iC3b and sub-

sequently to C3dg and C3d. MoAb 130 inhibits the binding of C3d to CR2. In order to localize the CR2 binding site and the MoAb 130 antigenic site, C3d was cleaved with CNBr. Of two major resulting fragments, one was unreactive but the other reacted with both CR2 and MoAb 130. The latter had a mol. wt of 8600 and extended from amino acid residue 1199 and to residue 1274 of the C3 sequence. Based on the secondary structure and hydrophilicity predictions (Chou and Fasman, 1974; Kyte and Doolittle, 1982) several overlapping peptides were selected for synthesis. The peptides covered the sequence between amino acid residues 1209–1236 (KFLTTAKDKNRWEDPGKOLYNVEATSYA). Differential binding of these synthetic peptides to CR2 and MoAb 130 as well as comparison of the amino acid sequences of human C3 and murine C3 (de Bruijn and Fey, 1985; Wetsel *et al.*, 1984), located the CR2 binding site to residues 1227–1232 and the MoAb 130 neoantigenic site to 1217–1232 of the C3 sequence (Lambris *et al.*, 1985b,c; Fig. 2). Preliminary experiments with these synthetic peptides have shown that they mimic C3d (Lambris *et al.*, 1982) or C3dK (Meuth *et al.*, 1983) in inhibiting lymphocyte proliferation induced by mitogens or by the mixed lymphocyte reaction (Lambris and Tsokos; unpublished observations).

THE FACTOR H BINDING SITE IN C3

Localization of the factor H binding site within the C3 molecule was approached by using anti-human H anti-idiotypic antibodies (aaH), which have been shown to bind to C3b and to inhibit the binding of H to C3b (Lambris and Ross, 1982). According to an ELISA, in which the C3 fragments were fixed to

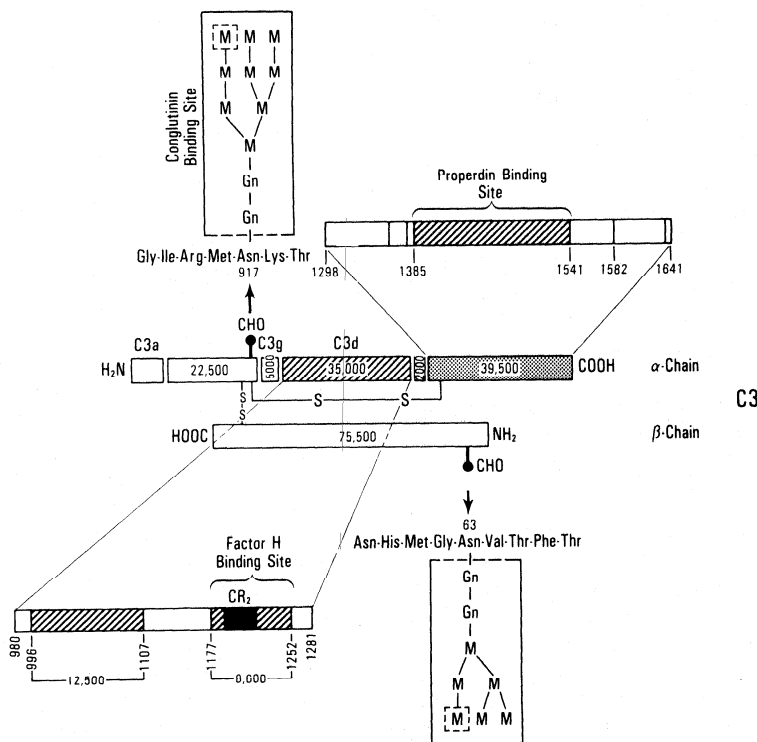


Fig. 2. A schematic representation of the C3 molecule. All assignments are based on direct structural analyses (de Bruijn and Fey, 1985; Lambris *et al.*, 1984a; 1985a,c; Hirani *et al.*, 1985a,b).

microtiter plates, possibly altering their conformation, both aaH and factor H bound to C3b, C3d or the α chain of C3 but not to C3c or the β chain of C3. Binding studies with CNBr-generated C3d fragments (Lambris *et al.*, 1985b) showed that both factor H and aaH bound to the 8600 mol. wt fragment (amino acid residues 1199–1274). Synthetic peptides covering the amino acid sequence 1209–1236 showed no reactivity with either factor H or aaH, suggesting that the factor H binding site, or part of it, is located either within the segment between residues 1199–1208 or 1237–1274 of the C3 sequence (Lambris *et al.*, 1985a) (Fig. 2). These data suggest that the factor H site is masked in native C3d and is expressed upon its fixation to microtiter plates.

THE PROPERDIN BINDING SITE IN C3

The (P) binding site within the C3 molecule was localized by testing the binding of P to solid phase C3 fragments. Briefly, ELISA plates were coated with C3 fragments, then P was added, and its binding was monitored by goat anti-P and peroxidase-labeled rabbit anti-goat Ig antibodies. The results of direct binding showed that P binds to C3c as well as to C3b, confirming earlier results which showed that P binds to C3c but not to C3d (Chapitis and Lepow, 1976). Preincubation of solid phase bound with anti-C3c antibodies completely abolished the binding of P to C3b, whereas preincubation with anti-C3d antibodies

had no effect. The P binding site was further localized by analyzing the binding of P to the isolated α or β chains of C3 or to α -chain fragments (62,500, 22,500 and 39,500). P bound to the α chain and to its 39,500 C-terminal fragment but not to the β chain or the other α -chain fragments. Thus, the binding site in C3 for P was placed in the 39,500 fragment (Lambris *et al.*, 1984b). Cleavage of the 39,500 fragment by CNBr generated several distinct fragments, one of which expressed the ability to bind P. Limited amino acid sequencing placed it between amino acids 1385 and 1541 of the C3 sequence (Fig. 2; unpublished observations).

THE CONGLUTININ BINDING SITE IN C3

Conglutinin is a bovine plasma protein of unknown physiologic function, with the ability to bind to human C3 (Lachmann, 1976). This interaction involves the carbohydrate entity of the C3 molecule. The carbohydrate portions of C3 are N-linked high-mannose oligosaccharides (Hirani *et al.*, 1985b) which are linked to asparagine residues 67 (β chain) and 917 (α chain) (Hirani *et al.*, 1985a; de Bruijn and Fey, 1985). Testing the binding of C3 fragments to solid-phase conglutinin, revealed that C3b, iC3b, C3c, the α chain and the 22,500 α -chain fragment bound to conglutinin but not C3d, the β chain or the 39,500 α -chain fragment. The binding was inhibited by EDTA, N-acetylglucosamine, mannose and abol-

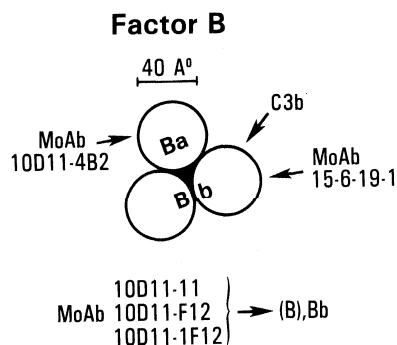


Fig. 3. A schematic representation of factor B. The three domain structure of factor B is based on electron-microscopic data (Smith *et al.*, 1982). All other assignments are based on direct structural analysis (Lambris and Müller-Eberhard, 1984b; La Motte *et al.*, 1981).

ished by deglycosylation of C3. Based on these results, it is concluded that conglutinin binds to the carbohydrate entity linked to the asparagine residues at position 917 of C3 (Hirani *et al.*, 1985a, b; Fig. 2).

A C3 BINDING SITE ON FACTOR B

Limited degradation of native factor B with porcine elastase yielded five fragments with respective mol. wts of 36,000, 35,000, 33,000, 31,000 and 25,000. Binding studies showed that the 33,000 fragment expressed metal-dependent affinity for C3b. The 33,000 fragment was found to be part of the Bb fragment of factor B as evidence by its reactivity with MoAb 15-6-19-1 (LaMotte *et al.*, 1981). The purified 33,000 fragment had the following activities: (a) esterolytic activity for the synthetic substrate *N*^a-acetyl-glycyl-L-lysine methyl ester; (b) residual hemolytic activity of factor B; and (c) inhibition of alternative pathway activation by rabbit erythrocytes. The latter resulted from a 33,000 fragment's high affinity for C3b and its lower hemolytic activity as compared to factor B. Limited sequence analysis placed the 33,000 fragment between residues 211 and 505 of the Bb sequence (Lambris and Müller-Eberhard, 1984b). These results suggest that the C3b binding site of Bb observed in this study is located inside or near the catalytic domain (Fig. 3). The

possibility remains, however, that this site is not identical to the physiological binding site of factor B.

THE BINDING SITE IN FACTOR H FOR C3B

Treatment of human factor H with trypsin produced a 38,000 and a 142,000 fragment linked by disulfide bonds (Hong *et al.*, 1982; Alsenz *et al.*, 1985a). Both fragments were purified either by SDS-preparative gel electrophoresis or gel filtration on Sephadex G200 following reduction with β -mercaptoethanol. The binding of C3b to factor H or its fragments was tested by ELISA. After incubation with serial dilutions of C3b, C3b binding to H was assayed with a goat anti-C3b and rabbit anti-goat Ig labeled with peroxidase. Using this assay system we found that C3b bound to intact factor H, trypsinized factor H and the 38,000 polypeptide but not to the 142,000 polypeptide (Alsenz *et al.*, 1985a). To substantiate that the 38,000 peptide contained the binding site for C3b, six different MoAbs to factor H (MAH 1, 2, 3, 4, OX23, 24) (Schulz *et al.*, 1984; Sim *et al.*, 1983) were used. By direct ELISA or Western blot analysis, all six bound to the 38,000 polypeptide but none to the 142,000 polypeptide. Monoclonal antibodies MAH 1, 2, 3 and OX 24 but not MAH4 or OX 23 were able to block binding of factor H to C3b. Amino terminal amino acid sequence analysis of the 38,000 factor H fragment placed it at the *N*-terminal position of factor H. These results suggest that the binding site of factor H for C3b resides in the *N*-terminal segment of factor H (Alsenz *et al.*, 1985b; Fig. 4).

From the data summarized in this paper, it may be concluded that the chemical or enzymatic degradation of a complement protein may be useful in determining the structure of sites involved in the interactions with physiological ligands.

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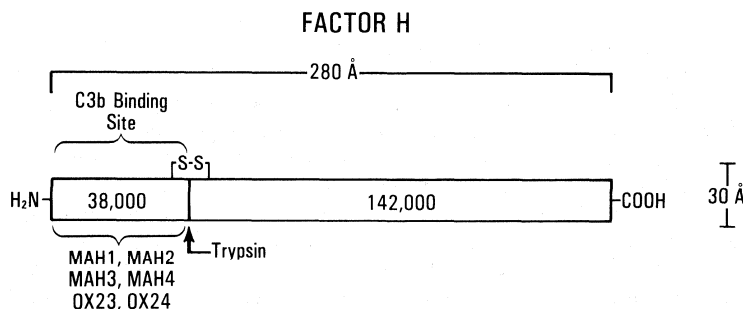


Fig. 4. A schematic representation of factor H. The size of H is based on electromicroscopic data (Smith *et al.*, 1983). For other assignments, see Alsenz *et al.*, 1985a,b).

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