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The Third Component of Complement

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Preface

The third component of complement, C3, is one of the most versatile proteins and an important participant in immune surveillance and immune response pathways. Its multifunctionality is based on its ability to interact specifically with multiple serum complement proteins, cell surface receptors, and membrane-associated regulatory proteins. One of its most intriguing strategies of interaction with cell surfaces is the covalent binding of activated C3 through the internal thioester.

The field has expanded over the past 10 years and a wealth of information has accumulated. C3 from various species and many of the human C3 binding proteins have been cloned and expressed. Numerous cellular responses mediated by the different fragments of C3 have been described. The findings that C3 interacts in a ligand-receptor-like fashion with proteins of nonself origin such as the gC of herpes simplex virus, a 70-kDa protein from *Candida albicans*, proteins from Epstein-Barr virus, etc. has opened a new field of investigation. The papers assembled in this volume summarize the wealth of data on the various aspects of the C3 interactions; together they bring to the reader new information on the chemistry, molecular genetics, biology, and pathophysiology of C3 and C3-binding proteins. Emphasis is given to structural features as they relate to functions.

Spring 1989

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Table of Contents

Indexed in Current Contents

List of Contributors

You will find their addresses at the beginning of the respective contributions

Ahearn J. M.
Alsenz J.
Atkinson J. P.
Barnum S. R.
Becherer J. D.
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Dierich M. P.
Dodds A. W.
Fearon D. T.
Fey G.
Huemer H. P.
Hugli T. E.
Lambris J. D.
Law S. K. A.
Levine R. P.
Lublin D. M.
Muñoz-Cánoves P.
Nielsson B.
Perkins S. J.
Prodinger W. M.
Rosen H.
Sim R. B.
Tack B. F.
Vik D. P.
Volanakis J. E.

Participation of C3 and its Ligands in Complement Activation

J. E. VOLANAKIS

1 Introduction

The complement system comprises a group of proteins in the blood which upon activation generate fragments and protein-protein complexes expressing biological activities. The biochemistry, function, and genetics of the system have been recently reviewed (CAMPBELL et al. 1988; MÜLLER-EBERHARD 1988). Complement activation proceeds in a sequential cascase-like fashion that is similar to the activation of other humoral effector systems in the blood, such as the coagulation, the fibrinolytic, and the kinin-generating system. Complement-derived biologically active products mediate a variety of important functions including increased vascular permeability, chemotaxis of phagocytic cells, activation of inflammatory cells, opsonization of foreign particles and cells, and direct killing of foreign cells (reviewed in MÜLLER-EBERHARD and MIESCHER 1985). Thus, complement plays a major role in host defense against pathogens. Accumulating evidence suggests complement activation products also function as growth and/or differentiation factors for B cells and possibly have additional effects on other cells and tissues.

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Current Topics in Microbiology and Immunology, Vol. 153

The complement system consists of more than 30 distinct proteins (Table 1). In their native state these proteins are either serum soluble or associated with cell membranes. Functionally, complement proteins can be categorized as those participating in the activation sequences (the classical and the alternative pathway), those regulating the activation and the activities of the system, and those serving as receptors for active fragments. Certain complement proteins overlap these physicochemical and functional categories. The introduction of recombinant DNA methods in complement research in the early 1980s has contributed to the rapid acquisition of information regarding the primary structure, function, biosynthesis, and genetics of complement proteins and has paved the way for a structural definition of reactive sites and active centers.

2 Overview of Complement Activation

Despite the complexity implied by multiple interacting proteins, complement activation is characterized by relative simplicity and economy of design. The most important activities in terms of host defense are derived from two proteins, C3 and C5, which

Table 1. Proteins of the complement system

Prevalent form in native state	Functional group		
	Participating in activation sequence	Regulatory	Receptors
Serum soluble	C1q, C1r, C1s, D C4, C2, C3, B C5, C6, C7, C8, C9	C1INH C4bp, H, I, P C3a/C5a INA S protein	
Membrane associated		CR1, MCP DAF HRF	C1qR CR1, CR2, CR3, CR4, CR5 C3a/C4aR, C5aR

Established symbols have been used for most complement proteins. In addition, the following generally accepted abbreviations have been used: INH, inhibitor; C4p, C4b-binding protein INA, inactivator; R, receptor, e.g., CR1, complement receptor type 1; DAF, decay-accelerating factor; MCP, membrane cofactor protein; HRF, homologous restriction factor

are structurally homologous and probably represent gene duplication products (DE BRUIJN and FEY 1985; WETSEL et al. 1987). Additional biologically active fragments are derived from C4, another structural homolog of C3 (BELT et al. 1984) and perhaps also from C2 and factor B. Expression of activity requires cleavage of C3 and C5 by highly specific proteases, termed convertases (Fig. 1). There are two C3 and two C5 convertases. One of each is assembled during activation of complement by the classical or the alternative pathway. C3 convertases are bimolecular while C5 convertases

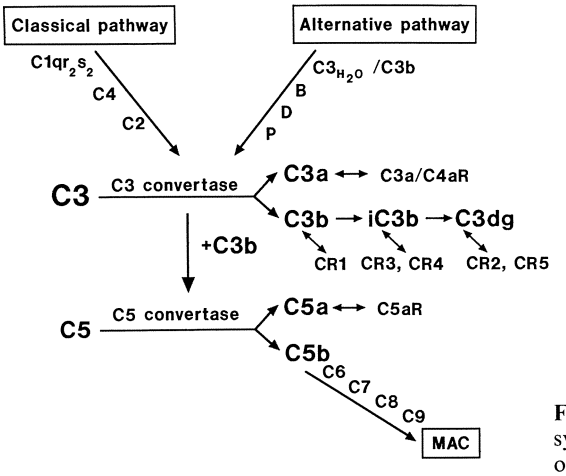

Fig. 1. Activation of the complement system. ↔, Ligand-receptor interactions

trimolecular protein complexes. The two activation pathways utilize different proteins to form these enzymes. In addition, the assembly of the convertases is initiated by different activating substances in the two pathways. However, the resulting enzymes have identical substrate and peptide bond specificity, giving rise to identical biological products.

Characteristic of the simplicity and economy of design of complement activation is the fact that C5 convertases are derivatives of C3 convertases (Fig. 1), and that C3 and C5 are activated by their respective convertases in similar fashion. In each case, a single peptide bond near the NH_2-terminus of the α-chain is cleaved to generate a small peptide, C3a or C5, and a large two-polypeptide chain fragment, C3b or C5b. Each of these four fragments expresses biological activities important to host defense. C3a, C5a, and C3b carry out their functions by interacting with specific receptors on the surface of effector cells (reviewed by FEARON and WONG 1983). Briefly, C3a, C5a, and also C4a, a homologous peptide generated during activation of C4, stimulate release of histamine from mast cells and blood basophils, contract smooth muscle, and increase vascular permeability. The structure and function of C3a is detailed in the article by HUGLI (this volume). C5a has additional functions evoking neutrophil responses, including chemotaxis, release of lysozomal enzymes, generation of oxygen radicals and increased adherence. C3b has multiple biologic activities that are mediated through interaction with a specific receptor, CR1, present on most blood cells.

The biologic consequences of C3b-CR1 interactions include clearance of immune complexes and phagocytosis and are discussed in the article by FEARON and AHEARN in this volume. C3b in complex with factor H, CR1, or membrane cofactor protein (MCP) is cleaved by factor I, a serine proteinase, to iC3b (DAVIS and HARRISON 1982), which is recognized by specific receptors, CR3 and CR4 (p150/95), present on leukocytes, resulting in enhanced phagocytosis. The structure and function of CR3 and CR4 are described in the article by ROSEN and LAW (this volume). Further cleavage of the α′-chain of iC3b by I in the presence of CR1 (ROSS et al. 1982; MEDOF et al. 1982) generates C3c and C3dg. The latter fragment is recognized by specific

receptors, CR2, on B lymphocytes and also by distinct receptors, CR5, on neutrophils. CR2 is discussed in the article by FEARON and AHEARN (this volume). The biologic activity of C5b does not depend on interaction with specific receptors. Instead, C5b initiates the assembly of a large protein-protein complex, termed membrane attack complex (MAC; MÜLLER-EBERHARD 1988), by interacting sequentially with C6, C7, C8, and C9. The resulting complex interacts directly with the lipid bilayer of membranes through hydrophobic domains of the participating proteins and eventually forms a transmembrane channel which leads to cell death.

The potential pathogenic potential of complement activation is controlled efficiently by several regulatory proteins (reviewed by VOLANAKIS 1988) that act at points of enzymatic amplification of the activation sequence and also at the level of effector molecules. They effectively control the extent of activation of the complement cascade and also protect the cells of the host from the pathogenic potential of complement activation products.

3 Participation of C3 in Complement Activation

From the above brief overview it is evident that C3 is the pivotal protein of the complement system (BARNUM 1989). It is the most abundant complement protein in blood (130 mg/dl) composed of two disulfide-linked polypeptide chains, α (115 kDa) and β (75 kDa). Clearly, cleavage of the Arg-77–Ser-78 bond near the NH_2-terminus of the α-chain of C3 is the key event in complement activation not only in terms of the multiple biologic activities expressed by C3a, C3b, and the further proteolytic degradation fragments of C3b, but also in terms of the multiple functions of C3b in the activation sequence per se. As shown in Fig. 1, C3b participates in the formation of the C3 and C5 convertases of the alternative pathway and of the C5 convertase in the classical pathway. Molecular interactions leading to the formation of these enzymes and the structure of participating proteins is discussed below.

4 C3 Convertase of the Alternative Pathway

4.1 The Noncatalytic Subunit $C3_{H2O}$/C3b

Formation of the alternative pathway C3 convertase is intimately related to certain unique structural and functional features of C3. The structure of C3 is discussed in the article by BECHERER et al. (this volume) and the biosynthesis and genetics of C3 in the article by BARNUM et al. (this volume). Structural features relevant to the assembly of C3 convertase are considered here briefly. The complete primary structure of human C3 has been deduced from the nucleotide sequence of overlapping cDNA clones (DE BRUIJN and FEY 1985). The molecule is encoded as a single 1663 amino acid long polypeptide chain, which includes a 22-residue signal peptide and four Arg residues linking the COOH-terminus of the β-chain to the NH_2-terminus of the α-chain. The Arg linker is removed during posttranslational modification leading to the two-polypeptide chain structure of the mature protein.

An additional important posttranslational modification takes place before secretion of C3 (IIJIMA et al. 1984). It involves the formation of a thioester bond between Cys-1010 and Gln-1013 of the pro-C3 polypeptide chain (TACK et al. 1980; THOMAS et al. 1982). The mechanism leading to the formation of the thioester bond is unknown, however it has been shown that in C4, which has a similar thioester bond, this process precedes proteolytic processing of the single polypeptide chain into the three chains of mature C4, but that it follows core N glycosylation (KARP 1983). VAN LEUVEN (1982) has suggested that the thioester bond forms during protein folding by the action of a transglutaminase-like active site in the polypeptide chain. Further folding of the polypeptide chain results in burying of the thioester bond in the hydrophobic interior of the molecule where it is protected from water and thus, relatively stable. The chemistry and function of protein thioester bonds are discussed in the article by LEVINE and DODDS (this volume). Under physiologic conditions the thioester bond in native C3 undergoes hydrolysis at slow rates with a half-life of 230 h giving rise to $C3_{H2O}$ (PANGBURN and MÜLLER-EBERHARD 1980), which displays a free sulfhydryl at Cys-1010 and a Glu at residue 1013 (numbering of pro-C3). Hydrolysis of the thioester results in conformational changes (ISENMAN et al. 1981) accompanied by loss of certain functional activities and acquisition of others. Among the latter is the ability of $C3_{H2O}$ to form Mg^{2+}-dependent complexes with complement factor B.

Formation of the $C3_{H2O}B(Mg^{2+})$ complex is considered to represent the first step towards formation of the so-called "initiation" C3 convertase of the alternative pathway (PANGBURN et al. 1981). In a second step, the serine proteinase, factor D catalyzes the cleavage of the Arg-228–Lys-229 peptide bond in B resulting in the release of the NH_2-terminal, 30 kDa, Ba fragment of B and the formation of the $\overline{C3_{H2O}Bb}$ complex which expresses C3 convertase activity. This series of reactions initiated with the hydrolysis of the thioester bond in native C3 and concluding with the formation of the initiation C3 convertase and cleavage of C3 into C3a and C3b is considered to occur in the blood continuously at slow rates (NICOL and LACHMAN 1973). Thus, a constant supply of small amounts of freshly generated C3b is available at all times. Both the $\overline{C3_{H2O}Bb}$ C3 convertase and the products of its catalytic action, C3a and C3b, are quickly inactivated by the control proteins, C3a/C5a INA and factors H and I.

Cleavage of C3 by a C3 convertase results in a pronounced conformational change in C3b (ISENMAN and COOPER 1981) associated with an extremely labile (metastable) thioester bond apparently resulting from its exposure on the surface of the molecule (LAW et al. 1980; SIM et al. 1981; TACK et al. 1980). The metastable thioester bond of C3b has a short half-life estimated at 60 μs and is highly reactive towards nucleophiles. The reactive carbonyl serves as acyl donor resulting in the formation of ester or amide bonds on reaction with hydroxyl or amino groups, respectively. Thus, C3b becomes covalently attached to the surface of neighboring cells or proteins displaying reactive nucleophiles. Alternatively, the metastable thioester bond reacts with H_2O to give fluid-phase C3b, which like $C3_{H2O}$ can form an unstable fluid-phase C3 convertase and thus contribute to the continuous physiologic low-level cleavage of C3. The fate of surface-bound C3b seems to be entirely dependent on the nature of the surface. C3b bound to the surface of a nonactivator of the alternative pathway, e.g., host's red cells, reacts preferentially with factor H which acts as a cofactor for the enzyme, factor I.

Cleavage of the α-chain of C3b by I results in iC3b which is subsequently cleaved further to C3c, released in the fluid phase, and C3dg, which remains bound to the surface. Additional control proteins CR1, decay-accelerating factor (DAF), and MCP on the membrane of host cells also interact with bound C3b preventing its interaction with B, dissociating C3b-bound B, and in the case of CR1 and MCP acting as cofactors for I. The structure and function of factor H is discussed in the article by VIK et al. (this volume); DAF and MCP are discussed in the article by LUBLIN and ATKINSON (this volume). In contrast, C3b bound to the surface of an activator of the alternative pathway has a higher avidity for factor B than for H (FEARON and AUSTEN 1977a, b) and thus can form a C3 convertase. Activators include various polysaccharides, lipopolysaccharides, bacteria, viruses, fungi, parasites, tumor cells, and certain immunoglobulins in complex with antigen (reviewed in (MÜLLER-EBERHARD and SCHREIBER 1980). The exact chemical features allowing discrimination between activators and nonactivators and their mode of action are not completely understood. Experiments by FEARON (1978) demonstrated that removal of sialic acid from the surface of sheep erythrocytes resulted in decreased binding of H to erythrocyte-bound C3b, thus transforming the cells from nonactivators to activators of the alternative pathway. Similar effects of sialic acid have been described for other cell surfaces (KAZATCHKINE et al. 1979; OKADA et al. 1982; EDWARDS et al. 1982). Subsequent experiments (reviewed in PANGBURN and MÜLLER-EBERHARD 1984) indicated sialic acid is probably not the only chemical moiety determining the properties of a surface with respect to alternative pathway activation.

The C3 convertase formed on the surface on an activator of the alternative pathway is termed "amplification" convertase because it forms part of a positive feedback loop, forming additional C3 convertase. Its assembly proceeds through steps identical to those described for the initiation C3 convertase. Factor B binds to C3b in the presence of Mg^{2+}; it is then cleaved by factor D giving rise to the $\overline{C3bBb}$ complex. An additional protein, properdin, plays an important role in the upregulation of the amplification C3 convertase. Properdin binds to the surface-bound $\overline{C3Bb}$ complex resulting in its stabilization (FEARON and AUSTEN 1975; MEDICUS et al. 1976). Binding of properdin may also protect the amplification C3 convertase from the action of the regulatory proteins, factors H and I. However, eventually the amplification C3 convertase comes under control with the release of Bb in an inactive from and the degradation of C3b to iC3b.

4.2 The Catalytic Subunit Factor B

Complement factor B is a 90-kDa single-polypeptide chain glycoprotein, structurally and functionally homologous to complement component C2. The genes for both proteins are located within the major histocompatibility complex locus on the short arm of human chromosome 6 (CARROLL et al. 1984; WHITEHEAD et al. 1985) and are less than 500 bp apart (CAMPBELL and BENTLEY 1985). The 5′ end of the C2 gene lies approximately 600 kb from the 5′ end of the gene encoding the HLA-B antigen and the 3′ end of the factor B gene approximately 30 kb from the 5′ end of the C4A gene (DUNHAM et al. 1987; CARROLL et al. 1987). The genes encoding tumor necrosis factors α and β were mapped between the C2 and the HLA-B genes and a novel gene, termed RD, between the factor B and the C4A genes (LÉVI-STRAUSS et al. 1988).

The complete primary structure of factor B has been determined from cDNA and protein sequencing (MOLE et al. 1984). The polypeptide chain consists of 739 amino acid residues with a calculated M_r of 83000. It exhibits 39% residue identity with C2 (BENTLEY 1986; HORIUCHI et al. 1989) which, along with the close proximity of the corresponding genes, indicates a gene duplication event at a distant evolutionary time. The polypeptide chain of B contains four sites for potential N-linked glycosylation and the mature protein 8.6% carbohydrate (TOMANA et al. 1985). Transmission electron micrographs have revealed a three-domain globular structure for B (SMITH et al. 1984a; UEDA et al. 1987). The gene segment coding for each of the three domains appear to have been derived from three unrelated gene superfamilies (Fig. 2). Thus, factor B represents an example of a "mosaic" protein (DOOLITTLE 1985) which is also true for several other complement proteins, including C1r, C1s C2, C7, C8α, C8β, C9, and factor I (reviewed in CAMPBELL et al. 1988).

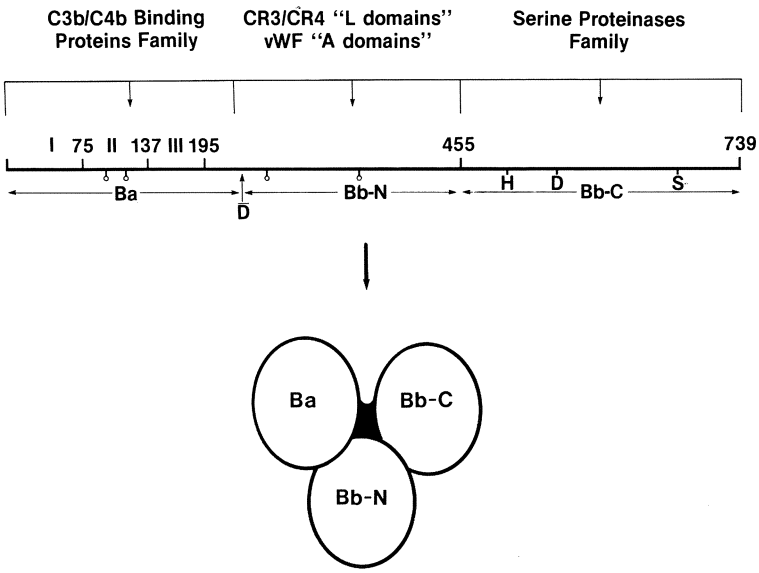

Fig. 2. Diagrammatic representation of the mature polypeptide chain of factor B. *Top*, the probable derivation of the gene segments encoding each of the three domains from three unrelated gene superfamilies; *thick line*, polypeptide chain: ⇣, Possible N-glycosylation site; *H*, *D*, *S*, active site residues His, Asp, and Ser, respectively; I, II, III, consensus repeat units. *Bottom*, a schematic representation of the electron microscopic appearance of the molecule

The NH_2-terminal domain of factor B, termed Ba, contains three direct consensus repeats, each approximately 60 amino acid residues long. Each repeat is encoded by a separate exon (CAMPBELL et al. 1984). Interest in the structure and function of these repeats stemmed from their presence in variable numbers in C2, CR1, CR2, H, MCP, DAF, and C4p, all of which bind fragments of C3 and/or C4. Members of this newly

described gene superfamily (reviewed in REID et al. 1986) also include proteins not known to bind C3 and/or C4 fragments such as β_2-glycoprotein I, clotting factor XIII, and the interleukin 2 receptor. Each of these proteins contains a different number of consensus repeats from two in C1r (JOURNET and TOSSI 1986) to 30 in CR1 (KLICKSTEIN et al. 1987). The consensus repeat units are contiguous, and in the complement regulatory proteins they represent the major structural element. The most characteristic shared structural feature among repeats is the presence of four invariable disulfide-linked half-cystine residues. It has been determined that in β_2-glycoprotein I (LOZIER et al. 1984) and in C4bp (JANATOVA et al. 1989) the first and third half cystines and the second and fourth are disulfide linked giving rise to a compact triple-loop structure. It seems likely that the same bonding pattern is present in the consensus repeats of all other proteins in the superfamily, including the three repeats of factor B.

UEDA et al. (1987) demonstrated that Fab fragments of a monoclonal antibody recognizing an epitope on the Ba domain inhibited in a dose-dependent fashion binding of intact B to red cell-bound C3b. In addition, PRYZDIAL and ISENMAN (1987) showed that Ba could inhibit the formation of the $\overline{C3bBb}$ convertase. They also demonstrated a specific, metal-ion independent interaction between Ba and C3b by using a cross-linking reagent. Taken together, these data indicate that Ba contains a binding site for C3b. Similar data have indicated the corresponding NH_2-terminal domain of C2, C2b, contains a similar binding site for C4b (NAGASAWA and STROUD 1977, KERR 1989; OGLESBY et al. 1988). The topology and chemical nature of these sites are unknown; however, it seems reasonable to assume they are contained within one of the consensus repeats. Several considerations indicate that the presence of this binding site on Ba cannot fully account for the initial binding of B to C3b or $C3_{H2O}$. First, Mg^{2+} is required for binding of B to C3b, and the Mg^{2+} binding site has been localized on Bb (FISHELSON et al. 1983). Second, neither Ba nor Bb in isolated form have substantial affinity for C3b. Third, Bb remains bound to C3b after cleavage of B by D. Electron micrographs of the C3 convertase, $\overline{CoVFBb}$, formed with the cobra analog of C3b, CoVF, have clearly shown Bb attached to CoVF through one of its two globular domains (SMITH et al. 1982). It thus seems likely that initial binding of B to C3b/$C3_{H2O}$ depends on two low-affinity binding sites, one on Ba and the other on one of the other two domains of B. Mg^{2+} apparently acts as an allosteric effector of the latter site.

The middle domain of factor B (residues 229–454; Fig. 2, Bb-N) exhibits amino acid sequence homology to a 187 amino acid residue long region near the NH_2 terminus of the α-chain of CR3 (Mac-1; CORBI et al. 1988) and to a homologous region on the α-chain of CR4 (p150/95; CORBI et al. 1987). Both of these leukocyte adhesion glycoproteins have binding affinity for iC3b, and interestingly they both require the presence of divalent cations for binding iC3b (ROSS et al. 1983; MICKLEM and SIM 1985). CR3 and CR4 are structurally homologous to extracellular matrix receptors such as the vitronectin receptor, the fibronectin receptor, and glycoprotein IIb and are therefore considered to be members of a gene superfamily of cell-cell and cell-matrix receptors termed integrins (HYNES 1987). However, no other integrin has on its α-chain a region similar to those found in CR3 and CR4, which led to its designation as L domain to indicate its presence on leukocyte integrins only. A domain termed "A domain," homologous to the middle domain

of factor B, is present in three imperfect tandem repeats in the polypeptide chain of von Willebrand factor (vWF) (SADLER et al. 1986). vWF is a glycoprotein playing an essential role in hemostasis. The region of vWF containing the A domains is believed to contain binding sites for collagen and platelet glycoprotein Ib (GIRMA et al. 1986).

The middle domain of C2 (residues 218–444) is homologous to that of factor B, but its homology to the L domains of CR3 and CR4 and to the A domains of vWF is not as strong as that of factor B. The middle domain of factor B and of C2 have been proposed (SMITH et al. 1982; PRYZDIAL and ISENMAN 1987) to contain binding sites for C3b and C4b, respectively, but direct supporting evidence is missing. However, studies on the oxidation of C2 by I_2, which results in stabilization of the $\overline{C4b2a}$, C3 convertase of the classical pathway (POLLEY and MÜLLER-EBERHARD 1967), demonstrated that the effect of I_2 is due to oxidation of the single free thiol of Cys-241 within this domain (PARKES et al. 1983). The relative stability of the C4b2aoxy convertase is apparently the result of a higher affinity binding of C2aoxy to C4b as compared to C2a as indicated from a slower rate of dissociation from the complex. Conversely, reduction of the free thiol abolishes the hemolytic activity of C2. It is of further interest that guinea pig C2, which forms a more stable convertase than human C2 (KERR and GAGNON 1982), has an Ala residue substituting for Cys-241 of human C2. Taken together these data are consistent with the suggestion (SMITH et al. 1982) that the middle domain of B contains a binding site for C3b/$C3_{H2O}$.

The carboxyl-terminal domain of factor B (Fig. 2, Bb-C) is homologous to serine proteinases, including highly conserved segments around the active site residues and the substrate binding site. Both intact B and fragment Bb express esterolytic activity, and the active site has been mapped with peptide thioester substrates homologous to the sequences at the cleavage/activation site of C3 and C5 (KAM et al. 1987). Bb was shown to be about ten fold more reactive towards these synthetic substrates than B and approximately 1000-fold less reactive than trypsin. This low catalytic efficiency is consistent with the mode of action of factor B in the activation sequence of the alternative pathway. Proteolytic activity against C3 is expressed by Bb only in the context of a complex with $C3_{H2O}$ or C3b. Comparison to other serine proteinases indicates the main distinctive structural feature of the carboxyl-terminal domain of factor B is the absence of a free NH_2-terminal residue and of the highly conserved amino terminal region that is characteristic of all members of this large family of homologous enzymes. In all other eukaryotic serine proteinases the positively charged α-NH_2-terminal residue is generated during activation from their zymogen form and plays an essential role in the molecular rearrangement that results in the enzymatically active conformation of the catalytic center (STROUD et al. 1975). In the apparent absence of a free NH_2-terminus, assumption of the catalytically active form of Bb must be achieved through a different mechanism probably involving C3b. A 33-kDa fragment obtained from a partial digest of B with porcine elastase and consisting essentially of the COOH-terminal domain of the protein was shown to exhibit serine esterase as well as hemolytic activity (LAMBRIS and MÜLLER-EBERHARD 1984). Interestingly, this fragment also displayed binding affinity for C3b.

4.3 Factor D

Complement factor D is the enzyme that catalyzes the cleavage of C3b-bound B, thus completing the assembly of the C3 convertase of the alternative pathway, $\overline{C3bBb}$. Factor D is a 24-kDa single-polypeptide chain serine proteinase. The serine proteinase nature of the enzyme was initially demonstrated by its irreversible inhibition by diisopropyl fluorophosphate (FEARON et al. 1974) and subsequently confirmed by amino acid sequence homologies with other members of the serine proteinase family of enzymes (VOLANAKIS et al. 1980; DAVIS 1980). The almost complete primary structure of factor D has been deduced from amino acid sequencing (JOHNSON et al. 1984; NIEMANN et al. 1984). The polypeptide chain consists of 222 amino acid residues with a calculated M_r of 23750. The primary structure of D exhibits about 40% identity with the B chain of human plasmin and an average of 35% identity with the pancreatic enzymes kallikrein, trypsin, chymotrypsin, and elastase. A much stronger homology (62%) is observed between factor D and a mouse protein, termed adipsin, synthesized by adipocytes and secreted in the blood (COOK et al. 1985, 1987). Recent studies (ROSEN et al. 1989) have indicated that adipsin has factor D-like functional properties i.e., it can cleave factor B into Ba and Bb only in the presence of C3b, and it can substitute for factor D in hemolytic assays. It thus seems likely that adipsin is mouse factor D.

Factor D isolated from serum of normal individuals or from urine of patients with Fanconi's syndrome (VOLANAKIS and MACON 1987) lacks the amino-terminal activation peptide that characterizes other serine proteinase zymogens. In addition, it exhibits esterolytic activity against peptide thioesters, homologous to the sequence of the factor B activation/cleavage site (KAM et al. 1987). However, the catalytic efficiency of D is 10^3–10^4 times lower than that of $\overline{C1s}$ and trypsin. The low esterolytic activity of purified factor D is compatible with the apparent absence of a zymogen for the enzyme in blood (LESAVRE and MÜLLER-EBERHARD 1978). A similar absence of a structural zymogen was noted in biosynthetic studies using U937 and HepG2 cells which secreted only active D (BARNUM and VOLANAKIS 1985a, b). A partial cDNA clone for factor D was isolated from a U937 library (MOLE and ANDERSON 1987). The nucleotide sequence of the insert has not been reported. However, it was reported to contain 17 amino acid residues at the NH_2-terminus not present in the sequence of D isolated from serum. It is not clear whether this sequence represents a leader or an activation peptide. However, it is interesting to note that an activation peptide coded for in the adipsin mRNA is apparently cleaved off before secretion of the enzyme by adipocytes or by mammalian cells transfected with adispin cDNA (ROSEN et al. 1989; B. SPIEGELMAN, personal communication).

In the absence of a structural zymogen, other mechanisms are apparently contributing to the regulation of D activity in blood, including the extremely restricted specificity of the enzyme and its rapid catabolic rate. Studies in our laboratory (VOLANAKIS et al. 1985; SANDERS et al. 1986) have demonstrated that factor D is filtered through the glomerular membrane and catabolized in the proximal renal tubules, resulting in a fractional catabolic rate of 59.6% per hour (PASCUAL et al. 1988). This rapid catabolic rate maintains very low plasma levels of the enzyme (1.8 ± 0.4 μg/ml; BARNUM et al. 1984) and thus may contribute to the regulation of its activity. In fact, factor D is the limiting enzyme in the activation sequence of the

alternative pathway (LESAVRE and MÜLLER-EBERHARD 1978). The mode of action of D may also contribute significantly to the regulation of its enzymatic activity. Active D cleaves its single known substrate, factor B, only in the context of the Mg^{2+}-dependent $C3_{H2O}$/C3bB complex. Active site mapping of D with peptide thioesters (KAM et al. 1987) and active site inhibitors (C. M. KAM et al. unpublished data) revealed some interesting functional features. As mentioned above, D was found to express esterolytic activity against Arg thioesters, but its catalytic efficiency was three to four orders of magnitude lower than that of $\overline{C1s}$ and trypsin. One of the most interesting findings was that extension of the substrate to include a P_3 or P_4 residue corresponding to the cleavage site of B resulted in loss of esterolytic activity. In contrast to its low reactivity with peptide thioester substrates, D reacted with active site inhibitors at rates comparable to those measured for trypsin. For example, APMSF inhibited D with a $K_{obs}/[I]$ of $110 M^{-1} s^{-1}$ as compared to $150 M^{-1} s^{-1}$ measured for trypsin. On the basis of these results, it has been proposed that the active center of D as it exists in serum exhibits a zymogen-like conformation, characterized by an obstructed binding site, and that the active conformation is induced by the substrate, $C3_{H2O}$/C3bB.

4.4 Properdin

Properdin is the final protein participating in the assembly of the amplification C3 convertase of the alternative pathway. It is necessary for the formation of a stable C3 convertase and therefore for efficient activation of the pathway. Properdin was first described by PILLEMER and his associates (1954) as an important component of host defenses against pathogens in a series of experiments demonstrating the existence of an alternative pathway for complement activation. Human properdin is a glycoprotein consisting of cyclic oligomers of a polypeptide chain of approximate M_r of 50000 (MINTA and LEPOW 1974; MEDICUS et al. 1980; DISCIPIO 1982). A partial amino acid sequence of human properdin has been published (REID and GAGNON 1981) and a cDNA clone coding for mouse properdin has been isolated and sequenced (GOUNDIS and REID 1988). The deduced amino acid sequence of mouse properdin indicated a 441-residue-long polypeptide chain with two sites for potential N glycosylation. The most interesting structural feature of properdin is the presence of six tandem consensus repeats, each approximately 60 amino acid residues in length, occupying the middle of the polypeptide chain. Alignment of the repeats to maximize homologies indicates 35 of the average 60 residues in each repeat are conserved, including six Cys, six Pro, and three Trp residues. Consensus repeats homologous to those found in properdin are also present in the adhesive glycoprotein thrombospondin (LAWLER and HYNES 1986) and in the complement proteins C6 (D. N. CHAKRAVARTI, personal communication), C7 (DISCIPIO et al. 1988), C8α (RAO et al. 1987), C8β (HOWARD et al. 1987), and C9 (DISCIPIO et al. 1984). Interestingly, the three thrombospondin consensus repeats are within a 70-kDa chymotryptic fragment known to contain binding sites for matrix proteins, including type V collagen, laminin, and fibronectin (MUMBY et al. 1984). It is of further interest that the properdin consensus repeat exhibits structural homology to conserved regions near the COOH-terminus of the polypeptide chains of circumsporozoite proteins from *Plasmodium falciparum, P. knowlesi,* and *P. vivax* (GOUNDIS and REID 1988).

An electron microscopic study of human properdin (SMITH et al. 1984b) revealed the protein to be polydisperse, consisting of cyclic dimers, trimers, tetramers, and higher oligomers. Trimers represented the most abundant form accounting for 45% of the observed oligomers followed by dimers (30%) and tetramers (10%). This distribution is in reasonably good agreement with that determined for properdin oligomers in human serum (PANGBURN 1989). Properdin protomers appeared as long, flexible rods exhibiting a reproducible sharp bend near the middle. No isolated monomers were detected, and no redistribution of individual oligomers was observed, indicating a high avidity of association between binding sites (SMITH et al. 1984b).

As mentioned above, the function of properdin relates to its ability to bind to the $\overline{C3bBb}$, C3 convertase resulting in a decreased rate of dissociation of Bb and thus stabilization of the enzyme (FEARON and AUSTEN 1975; MEDICUS et al. 1976). The primary binding specificity of properdin is for the C3b subunit of the convertase. Complexes of properdin and C3 in the fluid phase have been demonstrated (CHAPITIS and LEPOW 1976), and binding of properdin to red cell-bound C3b (EC3b) can be shown. An affinity constant of $2.9 \times 10^7\ M^{-1}$ with a 1:1 stoichoimetry was determined for the binding of properdin to zymosan-bound C3b (DISCIPIO 1981). The binding site for properdin has been mapped within a 34 amino acid residue segment of the α-chain of C3 (DAOUDAKI et al. 1988). The affinity of properdin for zymosan-bound C3b was found to increase by approximately four fold in the presence of factor B (DISCIPIO 1981). Similarly, the affinity of properdin is higher for EC3bBb than for EC3b (FARRIES et al. 1988). Thus, it seems likely that Bb also has binding sites for properdin, although complexes between B or Bb and properdin have not been demonstrated.

4.5 Assembly of the Alternative Pathway C3 Convertase

A hypothetical model for the assembly of the alternative pathway C3 convertase, based on the information reviewed above, is shown in Fig. 3. Similar models have been proposed previously (PRYZDIAL and ISENMAN 1987; OGLESBY et al. 1988). Factor B is depicted as a three-domain structure based on its electron microscopic appearance (SMITH et al. 1984a; UEDA et al. 1987). Initial binding of B to activator-bound C3b depends on two low-affinity binding sites, one on the NH_2-terminal and the other on the middle domain of B. Mg^{2+} apparently acts as an allosteric effector of the latter binding site (FISHELSON et al. 1983). The precise topology of the corresponding sites on C3 has not been determined. Studies by BURGER et al. (1982) demonstrated that monoclonal antibodies reacting with the α-chain of C3 and the C3c fragment inhibited the binding of B but not that of H to surface-bound C3b. Anti-C3d monoclonals had a reverse effect, i.e., they inhibited binding of H but not of B. However, C3c has no binding affinity for B. More recently, O'KEEFE et al. (1988) obtained a C3 fragment, termed C3o, by digesting C3 with a protease derived from cobra venom. C3o is similar to C3c except for an additional ten amino acids (residues 955–964, pro-C3 numbering) at the carboxyl terminus of the NH_2-terminal fragment of the α′-chain of C3c. C3o was able to interact with B in the presence of Mg^{2+} in a manner similar to C3b, i.e., C3o-bound B could be cleaved by D into Ba and Bb. It thus seems likely that the region 955-Glu-Gly-Val-Gln-Lys-Glu-Asp-Ile-Pro-Pro-

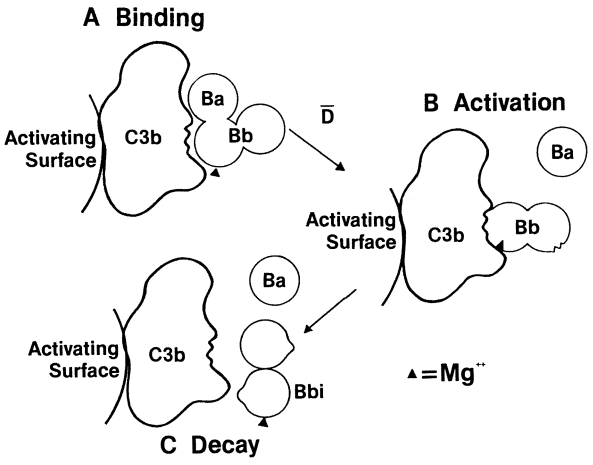

Fig. 3. Hypothetical model for the assembly (A), activation (B), and decay (C) of the C3 convertase of the alternative pathway of complement activation

964 of the α-chain of C3 represents one of the presumed two-factor B binding sites. A thermodynamic study of the binding of factor B to fluid-phase C3b or $C3_{H2O}$ (PRYZDIAL and ISENMAN 1988) indicated that, under physiologic conditions, hybrophobic interactions are dominant in C3bB or $C3_{H2O}B$ complex formation. Ionic forces are also likely to contribute to the interactions since low ionic strength enhances complex formation (DISCIPIO 1981). The association constant for binding of B to C3b was determined to be $2.5 \times 10^6\ M^{-1}$, whereas a five fold lower affinity was measured for the $C3_{H2O}B$ interaction (PRYZDIAL and ISENMAN 1988).

C3b/$C3_{H2O}$-bound B interacts with D inducing the active conformation of the enzyme which in turn catalyzes the cleavage of the Arg-228–Lys-229 bond in B. Cleavage of B induces a transient conformational change in Bb resulting in higher binding affinity for C3b, sequestration of Mg^{2+} (FISHELSON et al. 1983), and expression of proteolytic activity for C3 (Fig. 3). The transient conformation can be stabilized by properdin. Decay of the C3 convertase is due to dissociation of Bb which is accelerated by binding of H, CR1, DAF, and MCP to C3b. The conformation of dissociated Bb in Fig. 3 is depicted as different from that in the native molecule based on the electron microscopic appearance of Bb which is characterized by projections and angular surfaces (UEDA et al. 1987). In addition, the finding of 99% loss of C3-cleaving activity of dissociated, as compared to C3b-bound, Bb (FISHELSON and MÜLLER-EBERHARD 1984) indicates a diffent conformation of the active center.

5 C5 Convertase of the Alternative Pathway

Cleavage of C3 by the amplification C3 convertase formed on the surface of an alternative pathway activator results in the deposition of many C3b molecules in the vicinity of the convertase and eventually leads to the formation of a $\overline{C3bBbC3b}$

complex which expresses C5 convertase activity (DAHA et al. 1976; MEDICUS et al. 1976). It was originally thought that the second C3b molecule in the trimolecular complex was bound covalently to the surface of the activator in the immediate vicinity of the C3 convertase. The second C3b molecule was shown to provide a binding site for C5 which allowed its cleavage by Bb into C5a and C5b (VOGT et al. 1978; ISENMAN et al. 1980). The C3b-C5 interaction was found to exhibit a stoichiometry of 1:1 and an association constant of $4.8 \times 10^5\ M^{-1}$ for fluid-phase C3b (ISENMAN et al. 1980) and $5.7 \times 10^6\ M^{-1}$ for bound C3b (DISCIPIO 1981). Recently, KINOSHITA et al. (1988) demonstrated the second C3b molecule in the $\overline{C3bBbC3b}$ complex is covalently attached through an ester bond to the α'-chain of the first, surface-bound C3b molecule. Thus, the alternative pathway C5 convertase can be described as a trimolecular complex in which the catalytic subunit, Bb is bound noncovalently to a covalently linked C3b dimer. The association constant for binding of C5 to the C3b dimer is $1.5\text{–}2.4 \times 10^8\ M^{-1}$, significantly higher than that for monomeric C3b. This finding suggests (KINOSHITA et al. 1988) that C5 binds to both C3b molecules in the complex through two relatively low affinity sites resulting in an increased avidity for the C3b-C3b complex and, thus, selective binding of C5 to the convertase.

6 C5 Convertase of the Classical Pathway

In the classical pathway the C5 convertase forms by a mechanism similar to that in the alternative pathway, i.e., C3b binds to the C3 convertase resulting in a switch of substrate specificity from C3 to C5. The classical pathway C3 convertase is a bimolecular complex, $\overline{C4bC2a}$, assembled on the surface of activators through steps similar to those described for the alternative pathway (reviewed in REID and PORTER 1981; MÜLLER-EBERHARD 1988). The main difference between the two pathways is that in the classical pathway the activating enzyme, $\overline{C1s}$, generates both the noncatalytic and the catalytic subunits of the convertase, C4b and C2a, respectively. Further, $\overline{C1s}$ circulates in the blood in enzymatically inactive zymogen form, C1s, as part of Ca^{2+}-dependent complex $C1qr_2s_2$ of three proteins. Activation of C1s by $\overline{C1r}$ follows binding of the recognition protein C1q to a classical pathway activator and the ensuing autocatalytic activation of C1r (LOOS 1982; COLOMB et al. 1984; SCHUMAKER 1987). $\overline{C1s}$ cleaves C4, a protein structurally homologous to C3, into C4a and C4b resulting in the covalent binding of C4b to the activator through a transacylation reaction involving the reactive carbonyl group of the internal thioester of C4 and hydroxyl or amino groups, depending on the C4 isotype, on the surface of the activator (LAW et al. 1984; ISENMAN and YOUNG 1984). C2, a homolog of factor B, binds to surface-bound C4b in a Mg^{2+}-dependent reaction and is cleaved by $\overline{C1s}$ into C2b, released in the fluid-phase and the two-domain C2a which remains bound to C4b, representing the catalytic domain of both the C3 and the C5 convertase. Similarly to Bb, C2a expresses proteolytic activity for C3 only in the context of a complex with C4b. Cleavage of C3 by the $\overline{C4b2a}$ C3 convertase results in deposition of C3b molecules on the surface of the activator. These C3b molecules can initiate the assembly of the amplification C3 convertase depending on the chemical nature of the activating surface. Formation of C5 convertase requires the covalent binding of