Molecular Dissection of Interactions between Components of the Alternative Pathway of Complement and Decay Accelerating Factor (CD55)*

Received for publication, September 3, 2004, and in revised form, November 5, 2004
Published, JBC Papers in Press, November 9, 2004, DOI 10.1074/jbc.M410179200

Claire L. Harris**, Rachel J. M. Abbott†††, Richard A. Smith**, B. Paul Morgan‡, and Susan M. Lea‡
From the †Complement Biology Group, Department of Medical Biochemistry and Immunology, School of Medicine, Cardiff University, Heath Park, Cardiff, CF14 4XN, ‡Department of Biochemistry, Laboratory of Molecular Biophysics, Oxford University, South Parks Road, Oxford OX1 3QU, and **Adprotech Ltd., Chesterford Research Park, Little Chesterford, Essex, CB10 1XL, United Kingdom

The complement regulatory protein decay accelerating factor (DAF; CD55), inhibits the alternative complement pathway by accelerating decay of the convertase enzymes formed by C3b and factor B. We show, using surface plasmon resonance, that in the absence of Mg2++, DAF binds C3b, factor B, and the Bb subunit with low affinity (KD = 1.3 ± 0.5, 4.4 ± 10, and 20 ± 7 μM, respectively). In the presence of Mg2++, DAF bound Bb or the von Willebrand factor type A subunit of Bb with higher affinities (KD = 1.3 ± 0.5 and 2.2 ± 0.1 μM, respectively). Interaction with the proenzyme C3bBb was investigated by flowing factor B across a C3b-coated surface in the absence of factor D. The dissociation rate was dependent on the time of incubation, suggesting that a time-dependent conformational transition stabilized the C3b-factor B interaction. Activation by factor D (forming C3bBb) increased the complex half-life; however, the enzyme became susceptible to rapid decay by DAF, unlike the proenzyme, which was unaffected. A convertase assembled with cobra venom factor and Bb was decayed by DAF, albeit far less efficiently than C3bBb. DAF did not bind cobra venom factor, implying that Bb decay is accelerated, at least in part, through DAF binding of this subunit. It is likely that DAF binds the complex with higher affinity/avidity, promoting a conformational change in either or both subunits accelerating decay. Such analysis of component and regulator interactions will inform our understanding of inhibitory mechanisms and the ways in which regulatory proteins cooperate to control the complement cascade.

Complement (C) plays a central role in innate immune defense with the ability to rapidly opsonize or destroy microorganisms or infected cells. The alternative pathway (AP) provides an immediate, antibody-independent means to activate the C cascade, whereas the classic pathway relies in most cases on antibody to initiate activation of the first component, C1. A third pathway, the lectin pathway, is initiated by binding of mannann-binding lectin to sugar residues on bacterial cell walls. Other than the initiating factor (mannann-binding lectin and associated proteases), this pathway is identical to the classic pathway. The AP provides an immediate line of defense to potential infection, it “ticks over” continuously in plasma through low level activation of the central component, C3, probably via hydrolysis of the internal thioester bond (1). Tight regulation of this tick-over ensures that C is not activated to excess in plasma but provides enough active C3b to bind foreign surfaces and amplify the cascade swiftly when appropriate (2). The AP also amplifies the classic pathway by formation of further active convertases on C3b deposited during early amplification steps by the classic pathway convertase. The initiating stimulus of the AP is nucleophilic attack on the internal thioester bond in nascent C3b by an amine or hydroxy group present on a foreign or “activating” surface (3). This results in covalent binding of C3b and forms the nidus for C amplification. The C3 convertase is formed by binding of factor B (fB) to C3b in an interaction that requires a Mg2+ ion. The fB changes conformation such that the C3bB complex is capable of activating the zymogen factor D (fD), a serine protease present in plasma at about 2 μg/ml (4). FD cleaves fB into Ba (amino-terminal fragment) and Bb, the latter comprising an amino-terminal von Willebrand factor type A domain (vWFA) and a carboxyl-terminal serine protease (SP) domain. After release of Ba, Bb binds with higher affinity to C3b. It is likely that conformational changes in the vWFA domain transmit an allosteric signal to the SP domain, resulting in its activation and conferring it with the ability to cleave multiple molecules of C3 to nascent C3b, thereby amplifying the cascade (5, 6).

The background tick-over of the AP and the potential for rapid amplification of all pathways on self cells means that strict control in both biological fluids and on the membranes is essential. Numerous C regulatory proteins (CReg) have evolved to meet the need to protect “self” from the potentially destructive effects of C (7). These proteins police the body, collaborating to control all pathways. The different CReg belong to various gene families, those encoded in the Regulators of Complement Activation (RCA) gene cluster on chromosome 1 comprise factor H, C4b binding protein, decay accelerating factor (DAF; CD55), membrane cofactor protein...
(CD46), and complement receptor 1 (CD35) (8). All contain a structural module termed the short consensus repeat (SCR), a domain comprising ~60 amino acids that confers C3b/C4b binding affinity to the proteins and contains the regulatory function of the molecule (9). The amino-terminal fragment of fB, Ba, also comprises three SCRs. The number of domains in CReg varies from four (in membrane cofactor protein and DAF) to 37 (complement receptor 1, “B” isoform). DAF is a glycosylphosphatidylinositol-anchored CReg that acts to accelerate decay of the naturally labile enzymes of the AP and CP, C3bBb and C4b2a (10, 11). Other than the SCRs, it comprises an elongated, heavily glycosylated “stalk” proximal to the membrane that projects the active site the correct distance from the membrane, allowing it to function efficiently (12, 13).

The C components, from C1 through C9, comprise a fascinating group of proteins whose activities are characterized by differing conformations and presentation of continuously changing faces or binding sites to other C components, receptors, or regulators. C3 and fB are no exception. C3 transformation starts after cleavage and release of C3α, continues after destruction of the internal thioester bond, and moves on by presentation of binding sites for components such as fB, Ba, and eventually C5 (14, 15). Along the way, binding sites for a multitude of regulators are formed; their sites may be distinct or overlapping and are often transient as the cascade progresses or C3b becomes inactivated (16). Cleavage and activation of C3b by fI results in the production of iC3b, C3c, and C3d; these inactivation products no longer bind complement regulatory proteins, or regulators. C3 and fB, and factor D were incubated with CVF, and factor B and C3b were separated into monomeric and dimeric forms by size exclusion chromatography using established methods (29). Factor D was purchased from Quidel Corporation (San Diego, MA). C3b was prepared from C3 using either of two methods. In the first, CVF was coupled to Sepharose CL-4B using the manufacturer’s protocol (Amersham Biosciences). A solid-phase convertase was formed by incubating CVF-Sepharose in normal human serum to form CVFB. The Sepharose was washed and incubated at 37°C with C3. C3b generated by cleavage was separated from C3a and other minor contaminants by anion exchange on a Source Q column (Amersham Biosciences). In the second method, C3, factor B, and factor D were incubated in complement fixation diluent (Oxoid Ltd., Basingstoke, UK) until total C3 cleavage had occurred. C3b, Bb, and Ba were purified by anion exchange chromatography. The C3b-containing fractions were pooled and concentrated, and monomeric C3b was separated from dimeric C3b by size exclusion on a Superose 6 column (Amersham Biosciences).

Recombinant human DAF comprising the four SCRs (soluble DAF (sDAF)) was isolated and refolded from Escherichia coli as described previously (30). The structure and function of sDAF has been studied previously, and the purified, refolded protein is known to consist of four correctly folded SCR domains and demonstrates the full range of complement-regulating and pathogen-binding activities associated with DAF purified from erythrocytes (13, 30, 31). Human DAF-Ig fusion protein comprising four SCR domains fused to human IgG1 Fc (DAF-Ig) was prepared as described previously (32). Recombinant vWF-A domain of human factor B was expressed in E. coli as a fusion protein with glutathione S-transferase using published methods (33) with the addition of a final gel filtration on a Superose 6 column (Amersham Biosciences) to yield protein at a purity of 98%, as assessed by SDS-PAGE electrophoresis (data not shown).

**RESULTS**

**Interaction of DAF with the AP convertase assembly**

DAF results of this study provide new insight into these associations and further guide our understanding of the complex mechanisms and protein interactions intrinsic to the C cascade.

**EXPERIMENTAL PROCEDURES**

**Preparation of Complement Components and Regulators—**C3, cobra venom factor (CVF), and factor B were prepared by classic column chromatography using established methods (29). Factor D was purchased from Quidel Corporation (San Diego, MA). C3b was prepared from C3 using either of two methods. In the first, CVF was coupled to Sepharose CL-4B using the manufacturer’s protocol (Amersham Biosciences). A solid-phase convertase was formed by incubating CVF-Sepharose in normal human serum to form CVFB. The Sepharose was washed and incubated at 37°C with C3. C3b generated by cleavage was separated from C3a and other minor contaminants by anion exchange on a Source Q column (Amersham Biosciences). In the second method, C3, factor B, and factor D were incubated in complement fixation diluent (Oxoid Ltd., Basingstoke, UK) until total C3 cleavage had occurred. C3b, Bb, and Ba were purified by anion exchange chromatography. The C3b-containing fractions were pooled and concentrated, and monomeric C3b was separated from dimeric C3b by size exclusion on a Superose 6 column (Amersham Biosciences).

Recombinant human DAF comprising the four SCRs (soluble DAF (sDAF)) was isolated and refolded from Escherichia coli as described previously (30). The structure and function of sDAF has been studied previously, and the purified, refolded protein is known to consist of four correctly folded SCR domains and demonstrates the full range of complement-regulating and pathogen-binding activities associated with DAF purified from erythrocytes (13, 30, 31). Human DAF-Ig fusion protein comprising four SCR domains fused to human IgG1 Fc (DAF-Ig) was prepared as described previously (32). Recombinant vWF-A domain of human factor B was expressed in E. coli as a fusion protein with glutathione S-transferase using published methods (33) with the addition of a final gel filtration on a Superose 6 column (Amersham Biosciences) to yield protein at a purity of 98%, as assessed by SDS-PAGE electrophoresis (data not shown).

**Biosensor Analysis—**All analyses were carried out on a Biacore 3000 machine (Biacore International SA, Stevenage, UK), except for that shown in Fig. 4c, which was carried out on a Biacore 2000. Proteins were coupled to the sensor chip surface using an amine coupling chemistry as instructed by the manufacturers (N-hydroxysuccinimide/1-ethyl-3(3-dimethylaminopropyl)-carbodiimide hydrochloride coupling kit). For all kinetic analyses, a CM5 chip (carboxymethylated dextran surface) was used, data were collected at 25°C, the flow rate was maintained at 30 μl/min, and data from a reference cell were subtracted to control for bulk refractive index changes. The Rmax was kept low and the reaction rate high to eliminate mass transfer. However, this was controlled for where appropriate by varying flow rate and ensuring observed association rates did not vary. Samples were injected using the KINJECT command to ensure accurate association kinetic. Interactions were analyzed in HEPES-buffered saline (HBS; 10 mM HEPES pH 7.4, and 150 mM NaCl), 0.005% surfactant P20, and either 1 mM MgCl2 or 3 mM EDTA as stated in the text. Data were evaluated using Biacore evaluation software (Biacore International). Concentration of analytes was assessed using absorbance at 195 nm, molarities were calculated using the after extinction coefficients (molecular masses and coefficients obtained using Protein software, DNASTar: sDAF (1.3), C3b (1.03), factor B (1.43), Bb (1.29), and Ba (1.74)).
Interactions in the Alternative Pathway of Complement

2571

Interactions of fB with C3b

We sought to assemble the convertase on the sensor chip surface itself, the aim being to assess the affinity of DAF for the intact enzyme in addition to individual components. Low levels of C3b were immobilized to the chip surface and fB was flowed across in the presence of Mg$^{2+}$ (1 mM). It was immediately apparent that the interaction did not fit a simple 1:1 association model (Fig. 5). Furthermore, interaction of identical concentrations of fB with C3b for different lengths of time (1, 3, or 20 min) demonstrated that the dissociation became slower the longer the components interacted (Fig. 5b). This can be indicative of a conformational change that results in a tighter binding between components. Indeed, fitting the data from Fig. 5a to a conformational change model (BIAcore software) gave a good fit, although some heterogeneity was evident at high concentrations of fB (Fig. 5c).

Kinetic analysis after a 4-min association phase gave the following information: $k_{a1}, 1.36 \times 10^{4} \text{M}^{-1} \text{s}^{-1}$; $k_{d1}, 0.115 \text{s}^{-1}$; $k_{a2}, 5.53 \times 10^{-2} \text{M}^{-1} \text{s}^{-1}$; $k_{d2}, 1.78 \times 10^{-3} \text{s}^{-1}$ ($\chi^2, 4.2$). In this model, the off rate after the conformational change ($k_{d2}$) is much lower.

Formation of the AP Convertase and Interaction with DAF—

Either the active or inactive convertase was assembled on the chip surface by flowing fB over C3b in the presence or absence of fD. Either fB (46 µg/ml) or a mixture of fB (46 µg/ml) and fD (8 µg/ml) was flowed across the surface as indicated in Fig. 6a. Several differences in the two complexes were noted. First, cleavage of fB by fD altered the rate of decay of the active enzyme, prolonging its half-life. Second, the Mg$^{2+}$ ion in the active enzyme was “locked” in place, flowing 10 mM EDTA over C3bB(Mg$^{2+}$) dissociated fB from C3b, whereas it had no effect on decay of C3bBfB(Mg$^{2+}$).

C3b was also bound to the surface using the AP, which resulted in coupling of nascent C3b via its thioester to hydroxy groups present on the dextran-coated surface. We used a modification of a described previously technique in which repetitive cycles of fB/fD with C3 resulted in AP amplification on the chip surface (36). To increase efficiency of activation we deposited the first nidus of C3b on the chip surface using a fluid phase convertase, CVFBB (Fig. 6b). Each subsequent “cycle” illustrated in Fig. 6b comprised incubation with fB and fD to form active convertase followed by incubation with native C3. After C3b deposition, buffer was flowed across the surface for 16 h to allow dissociation of noncovalently bound C3b and of any active convertase. To study the interaction of DAF with the convertase, fB and fD were flowed over the surface. The amount of active/inactive convertase on the chip surface was varied by titrating the amount of fD in the incubation (Fig. 6c). In the presence of fD, the surface reached an equilibrium (Fig. 6c, E), unlike the proenzyme, which displayed complex kinetics during formation (Figs. 5 and 6c, A). When soluble DAF was flowed across the active convertase, decay of the Bb subunit was virtually instantaneous (Fig. 6c, B–E). Decay of the enzyme was so efficient that it was impossible to measure binding and an affinity of DAF for the intact convertase. This contrasted with flow of DAF across the inactive enzyme in which rapid and total decay was not apparent (Fig. 6b, A). Uncleaved fB was dissociated from the surface at the end of the incubation using EDTA as shown in Fig. 6. Alignment of individual sens-
grams at either the fB/fD injection or the DAF injection demonstrates the variation in decay and identical binding of DAF to C3b on the chip surface (Fig. 6d).

**Formation of CVFBb and Interaction with DAF**—We have demonstrated that DAF interacts with both subunits of the active enzyme and that decay is rapid and efficient. The convertase enzymes formed from CVF (C3b-like molecule found in cobra venom) and fB are very stable and are assumed to be resistant to decay by human regulators. To analyze the interaction with DAF, CVF was immobilized on the sensor chip surface and DAF was flowed across; no interaction between CVF and DAF was evident even at high concentrations (47 μM) (Fig. 7a). To assess whether DAF could decay the CVF-containing convertase, fB and fD were flowed sequentially across the CVF surface and a C3b-coated surface (Fig. 7b). In the absence of fD, the CVFB complex dissociated rapidly and the surface could be regenerated with EDTA. Inclusion of fD resulted in an EDTA-stable surface that efficiently cleaved C3 to C3b (data not shown). Formation of the active enzyme was less efficient than with human C3b, but enzyme that did form was very stable (Fig. 7b). When DAF was flowed across the surface at concentrations (15 μM) much higher than those bringing about immediate decay of C3bBb, it was apparent that it could indeed accelerate decay of CVFBb. However, this was far less efficient than with the native, human enzyme. The high concentration of DAF used in this experiment is apparent in Fig. 7b, where binding to the C3b-coated surface can be seen during the DAF inject (indicated in the figure).

**DISCUSSION**

We have used SPR technology to analyze the interaction of DAF with individual components of the AP convertase and also with the intact (pro)enzyme and to visualize assembly and decay of the convertase in real time. We demonstrate that DAF interacts with the individual components C3b and fB with low affinity. The equilibrium dissociation constant (KD) for the interaction of DAF with fB is 44 μM, demonstrating that this is a significantly weaker interaction than that of DAF with C3b monomer amine-coupled (n = 5)

| Protein interacting with DAF                        | kₐ | kₐ | KD  | χ²  |
|-----------------------------------------------------|----|----|-----|-----|
| C3b monomer amine-coupled (n = 5)                   | ND | ND | 14 ± 1 | 1.2 ± 0.7 |
| C3b dimer amine-coupled (n = 3)                     | ND | ND | 13 ± 1 | 2.5 ± 1.3 |
| Thioester-coupled C3b (n = 4)                       | ND | ND | 7 ± 1  | 6.1 ± 6.4 |
| fB (no Mg²⁺) (n = 4)                                | ND | ND | 44 ± 10| 1.2 ± 0.6 |
| fB (1 mM Mg²⁺) (n = 4)                              | ND | ND | 74 ± 30| 2.3 ± 3.2 |
| Bb (no Mg²⁺) (n = 4)                                | ND | ND | 20 ± 7 | 2.3 ± 0.8 |
| Bb (1 mM Mg²⁺) (n = 2)                              | 824, 1420 | 1.4, 1.2 | 1.7, 0.8 mean 1.3 | 3.7, 1.8 |
| vWF-A (1 mM Mg²⁺) (n = 6)                           | 830 ± 30 | 1.8 ± 0.03 | 2.2 ± 0.1 | 0.4 ± 0.3 |

*ND, not determined.*

**TABLE I Parameters measured that define DAF-ligand interactions**

Values given are mean ± S.D. of multiple experiments (n) other than Bb (1 mM Mg²⁺), where individual data sets are illustrated. Concentration ranges of analytes are as indicated in figures.
C3b where the interaction with amine-coupled C3b is characterized by a $K_D$ of $14 \pm 1 \mu M$ and with thioester-coupled C3b with a $K_D$ of $7 \pm 1 \mu M$ (Table I and Figs. 2 and 3). The small differences between the $K_D$ values for DAF interactions with differently coupled C3b probably reflect the more optimal presentation of binding sites in the natively coupled C3b compared with the amine coupled protein, in which steric hindrance is more likely to occur.

DAF did not interact with the Ba subunit of fB but did interact with the Bb subunit. The affinities of DAF for fB and Bb in EDTA were comparable ($44 \pm 10$ and $20 \pm 7 \mu M$, respectively). However, the affinity of DAF for the Bb subunit was increased $>10$-fold to $1.3 \mu M$ ($K_D$) in the presence of Mg$^{2+}$; a similar affinity was obtained for the isolated vWFA domain (2.2 $\mu M$), suggesting that this domain mediates the contact with DAF (Fig. 4). Indeed, a previous report defined two surface patches on opposing surfaces of the vWFA domain that may be involved in the decay of C3bBb by DAF; one of these may mediate binding to C3b, whereas the other may be involved in binding DAF (26). It is interesting that DAF has a higher affinity for Mg$^{2+}$-bound Bb. It is known that the Mg$^{2+}$ coordination site of fB is in the active site cleft of the vWFA domain, a site that is involved in the binding to C3b, and that the structure of this domain and of Bb is more stable in the presence of the cation. A conformational dependence of the vWFA domain on the presence of a metal ion has been demonstrated by various spectroscopic techniques (6); in particular, the $\alpha$-helix A7 is conformationally mobile between the metal-free and metal-bound forms of the vWFA domain. Our data show that DAF may bind distinct conformational states of this molecule with different affinities. Although we have demonstrated a direct interaction of DAF with the isolated Bb fragment, the physiologically relevant complex is Bb in association with C3b. Bb has little or no affinity for C3b after dissociation, indicating that fluid phase Bb differs in conformation from C3b-bound Bb (18). It is known that the vWFA domain in Bb, in common with similar domains in other molecules such as CR3 or von...
Willebrand factor, exists in high and low affinity conformations (37). After cleavage of fB by fD, Bb binds to C3b with higher affinity. The vWFA domain probably transmits an allosteric signal resulting in proteolytic activity in the SP domain of Bb (6). We cannot determine whether the affinity of DAF that we demonstrate here is for a low or high affinity conformation of vWFA domain.

The initial binding of fB to surface bound C3b is complex and clearly does not follow a simple 1:1 Langmuir interaction (Fig. 5). The data fit a model in which a conformational change occurs, resulting in a higher affinity interaction with a slower dissociation rate (kd). This model can only be applied when the change is slow and occurs over the period of the association phase; extremely rapid changes are more likely to fit a simple 1:1 interaction. It is known that fB changes conformation upon binding C3b allowing it to induce a proteolytically active conformation of fD (4). However, inclusion of fD in the incubation resulted in a very different binding profile (Fig. 6), the binding rapidly reached a plateau and an equilibrium was obtained. It is interesting that the resulting complex was resistant to EDTA (Fig. 6a); this is in agreement with previous work, which has shown that a transition in Bb and tight binding of the vWFA active site cleft to C3b protects the Mg ion from chelation by EDTA (19, 38). The binding profile of fB in the presence of fD was more typical of a simple interaction, implying that if conformational changes have occurred they were rapid and an “end-point” was swiftly reached. Others have previously reported that fB can bind C3b (and CVF) and form an active enzyme in the absence of fD (39–41). It is possible that the conformational change evident in the absence of fD in Fig. 5 represents a slow transition to an active conformation in which the SP domain of fB can cleave C3b; fD-mediated cleavage of fB to Bb may act to expedite this transition.

We have shown that DAF binds individual components of the AP convertase with low affinity, the crucial question is how does DAF bind the intact convertase? DAF must “recycle” to protect self cells from C attack. It is clear that if DAF bound to C3b with the same affinity as to the convertase, it would rapidly become saturated with C3b at a site of C activation and would not be available for decay of the active convertase. In 1986, Pangburn examined the ability of DAF and other CReg to decay the zymosan-bound convertases in the presence of fluid phase competitors such as C3b, Bb, C3bB, and C3bBb (42). Apparent association constants (appKa) of DAF with C3b and
the active enzyme. To monitor the decay of the convertase

DAF for C3bB or C3bBb was

bibilized on the chip surface via amine coupling. Interaction with factor B

"conformational change" model; dashed lines are modeled data,

association curve.

evaluation software and sensorgrams overlaid at the start of the disso-

association curve

lengths of time (1, 3, and 20 min), data were normalized using BIAcore

association for 4 min.

b

This selective decay may be related to the >10-fold higher affinity of DAF for the active convertase, C3bBb, was visualized in real time. It was immediately apparent that dissociation of Bb from C3b was virtually instantaneous, and it was not possible to measure an affinity of DAF for the active convertase directly. In support of the ELISA-based assay it was evident that DAF decayed the active enzyme and had little effect on the inactive convertase. By titrating the amount of active convertase on the chip surface it was possible to see this differential decay (Fig. 6c). In the absence of any fD, a small amount of DAF-mediated decay was evident (Fig. 6c, A), we do not know what this represents, but it is possible that a portion of C3bBb has undergone a subtle transition such that it is in an “active” conformation, and the affinity for DAF is enhanced as discussed above rather than this being the observation of decay of the inactive convertase. When DAF was flowed across the C3b-coated surface in the absence of any fB or fD, an increase in resonance units was evident (Fig. 6b, alignment at DAF injection). This represented binding of DAF to C3b and decreased again rapidly after the DAF injection was completed; the off rate for this interaction is extremely rapid, as illustrated in Fig. 2. After an identical injection of DAF over the C3bB- or C3bBb-coated surface, the same decrease in resonance units was evident at the end of the inject and dissociation was rapid (Fig. 6d). The dissociation of DAF from the surface was evident above the background decay of fB from C3b, which remained unaltered. These preliminary data imply that in our assay system, the affinity of DAF for C3b was unaffected by the presence or absence of proteolytically inactive fB. We are currently conducting further investigation of the kinetics of this interaction. Attempts to stabilize the C3bBb surface using chemical cross-linking to measure the affinity of DAF for the active enzyme were not successful. Although the release of Bb from the surface was prevented, an interaction with DAF could not be visualized. Either the cross-

linking reagent destroyed the interaction sites with the convertase, or DAF did indeed decay the components, but they remained loosely tethered to each other via the cross-linker without any physical protein/protein interaction. There are various reasons why DAF might decay the active but not the inactive convertase. First, the SCR-containing subunit of fB, Ba, might directly compete for the same binding site on C3b. Second, the presence of Ba might stabilize the C3bB complex; a direct interaction of Ba with C3b has been demonstrated (45). Several points of contact will increase the avidity of the interaction. Third, as suggested above, Bb might adopt a conformation in the absence of Ba that binds DAF with a higher affinity. Finally, it is also possible that the Ba domain partially blocks the binding site on Bb for DAF; it has been shown previously that Ba contacts both the vWFA and SP domains in inactive fB (5).

It is interesting that DAF accelerates decay of the CVFBb convertase, albeit with much lower efficiency than decay of C3bBb (Fig. 7b). DAF had no affinity for the isolated C3b-like component of the enzyme, CVF (Fig. 7a). In the study described above, Pangburn (42) demonstrated that the affinity of DAF for CVFBb was identical to that of DAF for Bb. Although we show here that the accelerated decay of CVFBb mediated by DAF was very inefficient, it was the only way we could regenerate the surface of the chip. Neither EDTA nor another powerful CReg, soluble recombinant complement receptor 1, could regenerate the surface back to uncomplexed CVF. The inefficiency of this decay implies that either the conformational change in Bb resulting in its release is slowed by the presence of CVF or that DAF works by also inducing a transition in C3b (not seen with CVF) that accelerates decay of the enzyme.
FIG. 6. Assembly and decay of the AP C3 convertase in real time.  

(a), C3b was amine-coupled to the chip surface. fB was flowed across in HBS-Mg²⁺ as indicated; incubation in 10 mM EDTA decayed the complex. In contrast, C3bBb was not decayed by 10 mM EDTA. b, C3b was deposited on to the acceptor surface using a method similar to that already described previously (36). The AP was amplified by repeated cycling (in HBS-1 mM Ni²⁺) at 30 °C of C3 followed by fB and fD. Convertases and non-covalently bound C3b was allowed to decay for 16 h before using the surface. c, different amounts of active convertase were assembled on the chip surface by varying the concentration of factor D in the incubation. As the quantity of active convertase increased, the ability of sDAF to decay the components was greater. FB, fD, sDAF, and EDTA were injected at the indicated time-points. Decay mediated by (0.8 μM) sDAF is indicated by a double-headed arrow. FB was injected at 450 μg/ml and fD at 0, 2.5, 5, 10, and 20 ng/ml (lettered A–E, respectively). d, sensorgrams from c are aligned at the start of the fB/fD injection to allow for comparison of convertase assembly or at the end of the DAF inject to allow comparison of DAF-mediated decay.
Current concepts regarding AP convertase assembly and decay are as follows. Ba mediates initial binding of fB to C3b, and the avidity of binding may be enhanced by sites on the Bb subunit which interact with C3b. If sites in Bb interact with C3b, the conformation differs from that of the dissociated subunit, Bb, which has much decreased affinity for C3b. After binding of fB, a conformational change occurs such that the complex activates the zymogen fD, which in turn cleaves fB into Bb and Ba. Ba is released from the complex (although this interaction is reversible). Release of Ba results in a higher affinity complex between C3b and Bb, the Mg$^{2+}$ ion in the active site cleft of the vWFA domain is locked into the enzyme through association with C3b, the complex becomes more stable with a longer half-life, and the SP domain in Bb is activated such that it can proteolytically cleave C3. However, this complex is subject to decay by DAF. We show that DAF binds individual components of the convertase in the following order of affinity: Bb $>$ C3b $>$ fB. It is also possible that DAF binds to C3bBb complex in vivo by binding of properdin to the C3bBb complex (46). We are currently examining the effect of properdin on DAF binding to the AP convertase and to individual components. Although the exact mechanisms of assembly and decay of the AP convertase are still unclear, we have come a long way in deciphering the ways in which C is activated and regulated. Data presented here and similar analyses of other C regulators will provide a more informed picture of their...
cooperation in inhibiting the complement activation pathways and the mechanisms responsible for release from the convertase and recycling of the regulatory proteins.

REFERENCES

1. Pangburn, M. K., Schreiber, R. D., and Muller-Eberhard, H. J. (1981) J. Exp. Med. 154, 856–867
2. Nicol, P. A., and Lachmann, P. J. (1973) Immunology 24, 259–275
3. Law, S. K., and Levine, R. P. (1977) Proc. Natl. Acad. Sci. U.S.A. 74, 2701–2705
4. Volanakis, J. E., and Narayana, S. V. (1996) Protein Sci. 5, 553–564
5. Hinshelwood, J., and Perkins, S. J. (2000) J. Mol. Biol. 301, 1267–1285
6. Hinshelwood, J., and Perkins, S. J. (2000) J. Mol. Biol. 298, 135–147
7. Morgan, B. P., and Harris, C. L. (1999) Complement Regulatory Proteins, Academic Press, London
8. Holoers, V. M., Cole, J. L., Lublin, D. M., Seya, T., and Atkinson, J. P. (1985) Immunol. Today 6, 188–192
9. Reid, K. B., and Day, A. J. (1989) J. Mol. Biol. 216, 2164–2171
10. Harrison, R. A. (1996) in Weir’s Handbook of Experimental Immunology, Volume II. Cell Surface and Messenger Molecules of the Immune System (Herzenberg, L. A., Herzenberg, L. A., and Blackwell, C., eds) pp. 75.11–75.50, Blackwell Science, Cambridge, MA
11. Harrison, R. A. (1996) in Weir’s Handbook of Experimental Immunology, Volume II. Cell Surface and Messenger Molecules of the Immune System (Herzenberg, L. A., Herzenberg, L. A., and Blackwell, C., eds) pp. 75.11–75.50, Blackwell Science, Cambridge, MA
12. Coyne, K. E., Hall, S. E., Thompson, S., Arce, M. A., Fujita, T., Anstee, D. J., Rosse, W., and Lublin, D. M. (1992) J. Immunol. 149, 2906–2913
13. Lukacik, P., Roversi, P., White, J., Esser, D., Smith, G. P., Billington, J., Williams, P. A., Rudd, P. M., Wormald, M. R., Harvey, D. J., Crispin, M. D., Radcliffe, C. M., Dwek, R., Evans, D. J., Morgan, B. P., Smith, R. A., and Lea, S. M. (2004) J. Biol. Chem. 279, 2753–2757
14. Isenman, D. E., Kells, D. I., Cooper, N. R., Muller-Eberhard, H. J., and Pangburn, M. K. (1991) J. Biol. Chem. 266, 10673–10676
15. Isenman, D. E., Kells, D. I., Cooper, N. R., Muller-Eberhard, H. J., and Pangburn, M. K. (1981) Biochemistry 20, 4458–4467
16. Lambris, J. D., and Muller-Eberhard, H. J. (1986) Mol. Immunol. 23, 1237–1242
17. Harrison, R. A., and Lachmann, P. J. (1989) Mol. Immunol. 25, 9–29
18. Fishelson, Z., and Pangburn, M. K. (1996) J. Mol. Biol. 258, 1519–1525
19. Tuckwell, D. S., Xu, Y., Newham, P., Humphries, M. J., and Volanakis, J. E. (1990) Biochemistry 29, 6605–6613
20. Fishelson, Z., Pangburn, M. K., and Muller-Eberhard, H. J. (1997) Immunology 101, 1279–1284
21. Fishelson, Z. (1997) J. Biol. Chem. 272, 2216–2221
22. Kuttner-Kondo, L. A., Mitchell, L., Hourcade, D. E., and Medof, M. E. (2001) J. Immunol. 167, 2164–2171
23. Williams, P., Chaudhry, Y., Goodfellow, I. G., Billington, J., Powell, R., Spiller, O. B., Evans, D. J., and Lea, S. (2005) J. Biol. Chem. 276, 30691–30696
24. Uhrinova, S., Lin, F., Ball, G., Bremek, K., Uhrin, D., Medof, M. E., and Barlow, P. N. (2000) Proc. Natl. Acad. Sci. U.S.A. 100, 4718–4723
25. Lea, S. (2002) Biochem. Soc. Trans. 30, 1014–1019
26. Hourcade, D. E., Mitchell, L., Kuttner-Kondo, L. A., Atkinson, J. P., and Medof, M. E. (2002) J. Biol. Chem. 277, 1107–1112. Epub 2001 Nov 10
27. Fishelson, Z., Seya, T., Harrison, R. A., and Atkinson, J. P. (1999) Immunol. Lett. 7, 30–41
28. Lambris, J. D., Luo, Z., Ogleshy, T. J., Atkinson, J. P., Hack, C. E., and Becherer, J. D. (1996) J. Immunol. 156, 4821–4832
29. Harrison, R. A. (1996) in Weir’s Handbook of Experimental Immunology, Volume II. Cell Surface and Messenger Molecules of the Immune System (Herzenberg, L. A., Herzenberg, L. A., and Blackwell, C., eds) pp. 75.11–75.50, Blackwell Science, Cambridge, MA
30. White, J., Lukacik, P., Esser, D., Steward, M., Giddings, N., Bright, J., Morgan, B. P., Lea, S. M., Smith, G. P., and Smith, R. A. G. (2004) Protein Sci. 13, 2406–2415
31. Anderson, K. L., Billington, J., Pettigrew, D., Cola, E., Roversi, P., Simpson, P., Chen, H. A., Urvi, P., du Merle, L., Barlow, P., Medof, E., Smith, R. A. G., Nowicki, B., Le Bouguenec, Lea, S. M., and Matthews, S. (2004) Mol. Cell 15, 647–657
32. Harris, C. L., Lublin, D. M., and Morgan, B. P. (2002) J. Immunol. Methods 268, 245–256
33. Williams, S. C., Hinshelwood, J., Perkins, S. J., and Sim, R. B. (1999) Biochem. J. 342, 625–632
34. Lea, S. M., Powell, R. M., McKee, T., Evans, D. J., Brown, D., Stuart, D. I., and van der Merwe, P. A. (1996) J. Biol. Chem. 271, 30443–30447
35. Lin, H. H., Stacey, M., Saxby, C. J., Kostt, V., Chaudhury, Y., Evans, D., Gordon, S., Mc Knight, A. J., Handford, P., and Lea, S. (2001) J. Biol. Chem. 276, 24160–24169
36. Koikiranta, T. S., Westin, J., Nilsson, U. R., Nilsson, B., Hellwage, J., Lofas, S., Gordon, D. L., Ekdahl, K. N., and Meri, S. (2001) Int. Immunopharmacol. 1, 495–506
37. McIvor, H. R., and Liddington, R. C. (1997) J. Clin. Invest. 100, 777–881
38. Fishelson, Z., Pangburn, M. K., and Muller-Eberhard, H. J. (1983) J. Biol. Chem. 258, 7411–7415
39. Vogt, W., Dames, W., Schmidt, G., and Dienminger, L. (1977) Immunochemistry 14, 201–205
40. Cooper, N. R. (1973) J. Exp. Med. 138, 451–460
41. Vogel, C. W., and Muller-Eberhard, H. J. (1982) J. Biol. Chem. 257, 8292–8299
42. Pangburn, M. K. (1986) J. Immunol. 136, 2216–2221
43. Fujita, T., Inoue, T., Ogawa, K., Iida, K., and Tamura, N. (1987) J. Exp. Med. 166, 1221–1228
44. Hourcade, D. E., Mitchell, L. M., and Medof, M. E. (1999) Immunopharmacology 42, 167–173
45. Prydz, E. L., and Isenman, D. E. (1987) J. Biol. Chem. 262, 1519–1525
46. Medical, R. G., Gotze, O., Muller-Eberhard, H. J. (1976) J. Exp. Med. 144, 1076–1093
Molecular Dissection of Interactions between Components of the Alternative Pathway of Complement and Decay Accelerating Factor (CD55)
Claire L. Harris, Rachel J. M. Abbott, Richard A. Smith, B. Paul Morgan and Susan M. Lea

J. Biol. Chem. 2005, 280:2569-2578.
doi: 10.1074/jbc.M410179200 originally published online November 9, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M410179200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 42 references, 20 of which can be accessed free at http://www.jbc.org/content/280/4/2569.full.html#ref-list-1