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# C3 Adsorbed to a Polymer Surface Can Form an Initiating Alternative Pathway Convertase[1]

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**Contact between blood and a biomaterial surface induces an immediate complement-mediated inflammatory response. Under these conditions, the alternative pathway of complement is often initiated and amplified on the biomaterial surface. Adsorption of a protein such as C3 to a polymer surface induces conformational changes in the protein. Based on the expression on adsorbed C3 of conformational neoepitopes specific for bound C3 fragments, we have hypothesized that adsorbed C3 is able to bind factor B and form a functional C3,Bb convertase. Using a quartz crystal microbalance to monitor binding of proteins to a polymer surface, we have demonstrated that a functional C3-containing alternative pathway convertase can be formed, in particular, in the presence of properdin. These data indicate that adsorption of C3 induces conformational changes that turn C3 into a C3b-like molecule that is able to participate in the functioning of the alternative convertase, and they suggest a new mechanism for complement activation on a biomaterial surface.** ***The Journal of Immunology*****, 2002, 168: 5786–5791.**

The complement system is triggered by three separate pathways: the classical pathway, the lectin pathway, and the alternative pathway. The first two pathways form a common convertase, C4b,C2a, while the alternative pathway forms the alternative pathway convertase, C3b,Bb. Both convertases cleave C3 into C3a and C3b, providing more C3b that can participate in forming C3b,Bb complexes. By this mechanism a powerful amplification loop is formed that is stringently regulated by both soluble- and membrane-bound regulators (1).

Both the classical and lectin pathways are fast triggers of complement activation, and both are amplified by the alternative pathway, which can also trigger complement activation but needs a lag phase of 5–10 min to initiate the amplification loop (2, 3). Despite the occurrence of this lag phase, certain complement activators are distinct activators of the alternative pathway, including guinea pig (4, 5) and rabbit erythrocytes (6, 7) and certain biomaterial surfaces (8, 9).

Alternative pathway activation is a surface-oriented process. In the presence of regulators of complement activation, no efficient activation takes place in the fluid phase under nonpathological conditions. Introduction of microorganisms, biomaterials, or foreign bodies into the body allows contact between the blood plasma and a foreign surface. This interaction not only makes surface-bound nucleophilic groups available that can bind nascent C3b by attacking the thioester but also makes surfaces available to which C3 can be adsorbed through electrostatic and/or hydrophobic interactions.

According to the "tick-over theory," the alternative pathway is considered to be initiated by the generation of a fluid-phase convertase, iC3,Bb, which is formed from C3 with hydrolyzed thioester (iC3[3]; $C3H_2O$) and factor B (10, 11). The C3b-like iC3 is generated from native C3 by a spontaneous hydrolysis of the internal thioester in the protein, with a $t_{1/2}$ of 230 h (12). Through the activity of the iC3,Bb convertase, initiating C3b molecules are deposited on the target surface.

Circular dichroism studies in the far UV spectrum of both native C3 and C3b show ~40% $\beta$ structure and 60% random coil. When these forms of C3 are denatured in $3 \times 10^{-3}$ M SDS, the conformation is considerably changed. Both C3 and C3b exhibit a shift to 15% $\alpha$ helix and 25% $\beta$ structure, but with no apparent change in the random coil element, indicating a significant alteration in the backbone of the molecule (13). In parallel with this process, native C3 loses two-thirds of its exposed epitopes expressed only by native C3 (C3(N)), while one-third of the epitopes, stable epitopes expressed by both native and denatured C3 (C3(S)), are stable. In denatured C3, the C3(N) epitopes are interchanged with the neoepitopes expressed only by denatured C3 (C3(D)). C3(D) epitopes react exclusively with Abs raised against the isolated $\alpha$- and $\beta$-chains of the protein. C3(D) epitopes are distributed throughout the whole $\alpha$-chain but tend to be located in the vicinity of known protease cleavage sites, such as those of the convertase or factor I (14, 15).

An epitope similarity exists between physiologically bound C3b and soluble SDS-denatured C3. Bound C3b expresses C3(D), C3(S), and C3(N) epitopes, while the soluble, SDS-denatured form expresses C3(S) and C3(D) but no C3(N) epitopes. A comparison of the expressed C3(D) epitopes of C3b and denatured C3 using a radioimmunoassay has shown that the epitopes show nearly 70–90% similarity, suggesting that C3b undergoes a considerable conformational change upon activation and binding to a biological surface (16–18).

It is interesting that the C3(D) epitopes of both purified C3 and C3b become exposed upon direct adsorption to a biomaterial surface, such as the plastic surface of a microtiter plate (19) or a

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[3] Abbreviations used in this paper: C3(D), neoepitopes expressed only by denatured C3; C3(N), epitopes expressed only by native C3; C3(S), stable epitopes expressed by both native and denatured C3; FPLC, fast performance liquid chromatography; iC3, C3 with hydrolyzed thioester; QCM-D, quartz crystal microbalance-dissipation.



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nitrocellulose membrane (20). The binding of anti-C3(D) Abs to C3 adsorbed on a plastic surface can be completely inhibited by soluble SDS-denatured C3 but not by native C3 (19). This finding raises the question of whether adsorbed C3 is autoactivated and is able to trigger alternative pathway activation by binding factor B, thereby generating a surface-bound initiating C3,Bb convertase. In previous studies it has been suggested that adsorbed C3 can mediate alternative pathway activation in serum that is in contact with a polystyrene surface (19, 21). In this work we show, by using a quartz microbalance technique (quartz crystal microbalance-dissipation (QCM-D)), that polystyrene-adsorbed purified C3 in the presence of factor B and properdin can form an initiating alternative pathway convertase that is able to cleave native C3.

## Materials and Methods

### *Purified proteins and Abs*

Fibrinogen was purchased from Chromogenix (Mölndal, Sweden). Factor D was purified from peritoneal fluid of patients with renal failure, as described by Catana and Schifferli (22). All other proteins were purified from human plasma. C3 and factor H were purified according to Hammer et al. (23), except that the factor H purification was preceded by a euglobulin precipitation (24). Factors B, I, and P were purified according to Lambris et al. (25), Fearon (26), and Medicus et al. (27), respectively. The proteins were drop-frozen in liquid nitrogen and then stored at −70°C. The proteins were thawed only once.

C3 was digested with trypsin (Sigma-Aldrich, St. Louis, MO) to give C3a and C3b (1% (w/w) trypsin for 5 min at room temperature) or C3c and C3d (2.5% (w/w) trypsin for 10 min at 37°C). The fragments were separated by gel filtration on a Sephadex G-100 column (Amersham Pharmacia Biotech, Uppsala, Sweden) equilibrated in PBS. Native C3 was partially converted to iC3 by incubating C3 in 0.05 M methylamine (pH 8) for 60 min at 37°C, followed by dialysis against PBS.

Three mouse mAbs against C3(D) epitopes were used in these studies: mAb 7D.406.4 (recognizing a 20-kDa fragment of C3c) (15), 7D.26.1 (recognizing C3d,g), and 7D.589.3 (recognizing a 40-kDa fragment of C3c) (21, 28). A mAb against activated protein C (DAKO, Glostrup, Denmark) was used as negative control.

### *PAGE and FPLC characterization of C3 and C3b preparations*

Preparations of C3 and C3b were analyzed by SDS-PAGE using 7.5% polyacrylamide gels (29), followed by Western blot analysis using HRP-conjugated rabbit Abs against human C3c (DAKO). The polypeptide chains of Coomassie-stained gels were quantified using NIH Image 1.54 (National Institutes of Health, Bethesda, MD) for Macintosh (Apple Computer, Cupertino, CA).

The relative amount of iC3 in the C3 preparations was quantified after separation of the two forms of C3 by fast performance liquid chromatography (FPLC) using a MonoS column (Amersham Pharmacia Biotech). The proteins were eluted using a linear NaCl gradient from 80 to 700 mM in 50 mM acetate buffer (pH 5.2), as described by Hack et al. (30). A C3 reference preparation containing iC3 was analyzed under identical conditions.

After washing of the plates with 10 mM sodium phosphate containing 0.15 M NaCl and 1 mM $Ni^{2+}$ (PBS-Ni), C3 and C3b adsorbed to microtiter plates were eluted with 85 mM Tris (pH 8) containing 15% (v/v) 2-ME, 8.5 mM EDTA, and 17% (w/v) SDS for 5 min at 100°C. The eluate was analyzed by SDS-PAGE, followed by Western blotting using anti-C3c.

### *QCM-D analysis*

The QCM-D technique relies on the fact that a mass adsorbed onto the sensor surface of a shear-mode oscillating quartz crystal causes a proportional change in its resonance frequency ($f$). Changes in $f$ reflect the amount of mass deposited onto to the surface of the crystal. For thin, evenly distributed, and rigid films, an adsorption-induced $\Delta f$ is related to mass uptake ($\Delta m$) via the Sauerbrey relation (31): $\Delta f = -n\Delta m C^{-1}$, where $C$ (equivalent to 17.7 ng $cm^{-2}$ $Hz^{-1}$) is the mass sensitivity constant and $n$ is the overtone number. However, for proteins adsorbed from the aqueous phase, one must also be aware that water hydrodynamically coupled to the adlayer is included in the measured mass uptake (32). In addition, when the adsorbed material is nonrigid, additional energy dissipation (viscous loss) is also induced. The dissipation factor ($D$) reflects frictional (viscous) losses induced by deposited materials such as proteins adsorbed on the surface of the crystal. Hence, changes in the viscoelastic properties of adlayers (e.g., those induced by conformational changes) as well as differences between various protein-surface interactions can be monitored (33–35).

Analysis of adsorption kinetics by simultaneous measurement of both $f$ and $D$ was performed using a QCM-D instrument (Q-Sense, Gothenburg, Sweden), which is described in detail elsewhere (36). The volume of the chamber is 80 $\mu$l, and when the liquid in the chamber is exchanged 0.5 ml is added from a temperature loop; the excess volume is allowed to overflow. Sensor crystals (5 MHz), spin-coated with hydrophobic polystyrene, were used. Changes in $D$ and $f$ were measured on both the fundamental frequency ($n = 1$, i.e., $f \approx 5$ MHz) and the third ($n = 3$, i.e., $f \approx 15$ MHz) and fifth harmonic ($n = 5$, i.e., $f \approx 25$ MHz). Data from the measurements at the third harmonic are presented. All measurements were conducted at 25°C.

### *Assembly of alternative pathway convertases, as monitored by QCM-D*

Protein dilutions and QCM-D assessments were performed in PBS-Ni. $Ni^{2+}$ was used to stabilize the alternative pathway convertase (37). The experiments were performed at 25°C. A sensor crystal was coated with 200 $\mu$g/ml C3b or C3 for 50 min. Alternatively, the surface was precoated with 200 $\mu$g/ml fibrinogen for 50 min, followed by a 10-min incubation with PBS-Ni, before C3 and C3b (200 $\mu$g/ml) were added. Finally, all types of surfaces were washed with PBS-Ni for 10 min. After the coating procedure, the surface was incubated with factor B (38 $\mu$g/ml) for 10 min, followed by a 10-min incubation with factor D (10 $\mu$g/ml) before C3 (133 $\mu$g/ml) was added for 50 min. In some experiments properdin (15 $\mu$g/ml) was added together with factor B. The cycle was ended by a 10-min PBS-Ni rinse. Each of the described cycles of incubation with purified complement components was performed three times.

### *Enzyme immunoassay for the detection of C3a*

High-m.w. components were first precipitated by the addition of 20% (w/v) PEG 6000. The supernatant was collected after centrifugation at 3300 × $g$ for 30 min. Samples were diluted 1/10 in working buffer and analyzed as described previously (38); mAb 4SD17.3 was used as the capture Ab (20, 39). Bound C3a was detected with biotinylated rabbit anti-C3a, diluted 1/150, followed by HRP-conjugated streptavidin (Amersham, Slough, U.K.), diluted 1/500. Zymosan-activated serum, calibrated against a solution of purified C3a, was used as a standard; values are given as nanograms per milliliter. PBS containing 0.05% (v/v) Tween 20 and 0.02% (v/v) Antifoam (Amersham Pharmacia Biotech) was used as washing buffer. Washing buffer containing 1% BSA (w/v) served as the working buffer.

## Results

### *Characterization of the C3 and C3b preparations by native PAGE, SDS-PAGE, and FPLC*

The purity of the C3 and C3b preparations was assessed by column chromatography and SDS-PAGE. SDS-PAGE analysis demonstrated that the C3 preparation was devoid of C3b (Fig. 1*A*). No

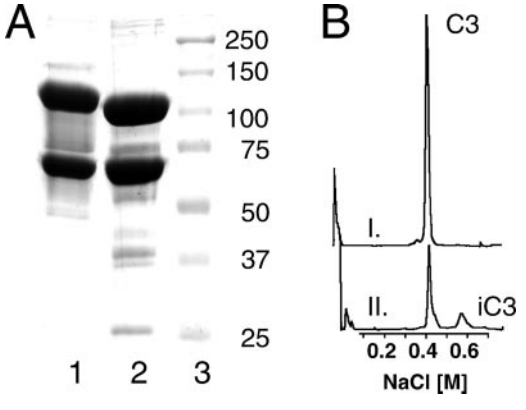


**FIGURE 1.** Characterization of the C3 and C3b preparations. *A*, C3 (*lane 1*) and C3b (*lane 2*) analyzed by SDS-PAGE and Western blotting using a polyclonal anti-C3c Ab. *Lane 3* contains molecular mass standards. *B*, Chromatograms of native C3 (I) and methylamine-treated C3 (II) eluted from a MonoS column using a gradient of NaCl from 80 to 700 mM in 50 mM acetate buffer (pH 5.2). The first peak is C3 at 420 mM, and the second peak is iC3 at 590 mM. The C3 preparation shows no sign of iC3.

iC3 was found in the C3 preparation after separation by FPLC on a MonoS column. Reference C3 and iC3 eluted at 420 and 590 mM NaCl, respectively (Fig. 1*B*).

*Adsorption of C3 and C3b to polystyrene*

When 200 $\mu$g/ml C3 or C3b was allowed to adsorb to the polystyrene surface of the QCM-D sensor for 50 min, a frequency shift of −150 Hz was observed with C3 and a −220-Hz shift was seen with C3b (Fig. 2*A*).

To characterize the binding property of the polystyrene-bound C3, C3 was incubated in polystyrene wells of microtiter plates already coated with either C3 or C3b, then eluted with a mixture of SDS and 2-ME. The eluted proteins were analyzed by SDS-PAGE/Western blotting using a HRP-conjugated polyclonal Ab against C3c (Fig. 2*B*). When compared with purified native C3, there was an enrichment of high-m.w. C3-related polypeptides in the eluate (Fig. 2*B*, *lanes 3* and *4*). This band was not visible on the parallel Coomassie-stained gels and was estimated to account for less than 1% of the polypeptides. No such high-m.w. polypeptides were seen in soluble native C3 or in eluates of wells adsorbed with C3 alone in the absence of further C3 incubation (data not shown).

We then analyzed the binding of anti-C3(D) Abs to immobilized C3 or C3b using the QCM-D technique (Fig. 3). For these experiments a hydrophobic polystyrene surface was first coated with C3 or C3b, blocked with PBS containing 0.1% Tween 20, and rinsed by incubating for 10 min with PBS-Ni. Control mAb (anti-protein C) was then added to the QCM-D chamber, followed sequentially by three mAbs specific for particle-bound C3 fragments. Each 20-min incubation with mAb was separated by a 10-min PBS-Ni incubation. Incubation with the control mAb caused no frequency shift. In contrast, the specific mAbs caused a frequency shift of −20 to −51 Hz on both the C3- and C3b-coated surfaces (Fig. 3). Based on similar water content and molecular mass (160 and 185 kDa for IgG and C3, respectively), this corresponds to a partial binding of each mAb to between 15 and 40% of the C3 molecules.

*Monitoring the generation of surface-bound convertases*

Fig. 4 presents a typical cycle of convertase generation, as monitored by QCM-D. The polystyrene surface, already coated with C3b, was first incubated in buffer for 10 min, and a slight frequency increase was detected as a result of a loss in mass. Factor B was then allowed to bind to the C3b for 10 min, causing a frequency drop. The C3b,B complex was then activated by factor D and, as a result of the loss of the Ba fragment of factor B, the frequency increased. Following the addition of C3 there was a significant frequency decrease, because C3 was activated to C3a and C3b, and C3b was bound to the surface.

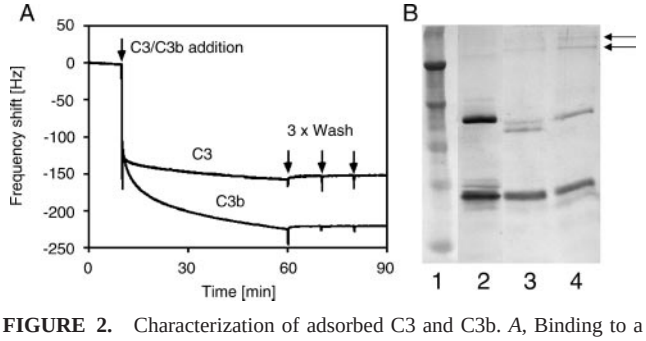


**FIGURE 2.** Characterization of adsorbed C3 and C3b. *A*, Binding to a polystyrene surface of 200 $\mu$g/ml C3 or C3b in PBS containing 1 mM $Ni^{2+}$ at 25°C, as monitored by QCM-D. Protein was added at 10 min, and buffer exchange was performed at 60, 70, and 80 min. The binding was detected by the QCM-D technique. *B*, Polystyrene wells coated with 200 $\mu$g/ml C3 or C3b were incubated with 133 $\mu$g/ml C3 for 50 min. After a 10-min wash the adsorbed proteins were eluted with 0.1 M Tris (pH 8) with 20% (w/v) SDS, 5% (v/v) 2-ME, and 10 mM EDTA. The eluted proteins were analyzed by SDS-PAGE, followed by Western blotting using polyclonal anti-C3c. Shown are the molecular mass standards (*lane 1*), native C3 (*lane 2*), eluate from the C3b-coated wells (*lane 3*), and the eluate from the C3-coated wells (*lane 4*).

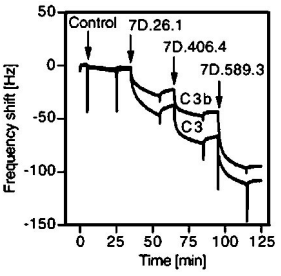


**FIGURE 3.** Binding of mAbs (25 $\mu$g/ml) to C3 or C3b adsorbed to a polystyrene surface, as monitored by the QCM-D technique. mAbs used were anti-protein C (control) and the anti-C3(D) mAbs 7D.26.1 (C3d,g), 7D.406.4 (20-kDa fragment of C3c), and 7D.589.3 (40-kDa fragment of C3c). The Abs were incubated with the C3- or C3b-adsorbed surface for 20 min each, with each incubation separated by a 10-min buffer incubation.

*Generation of surface-bound C3b,Bb and C3,Bb*

After coating of the polystyrene surface with either C3b or C3, factor B, factor D, and C3 were sequentially added (Fig. 5, *A* and *B*). For the C3b-coated surface, the frequency shifts obtained for the three repeated cycles after C3 incubation in the absence of properdin were −31, −37, and −40 Hz, respectively. A control cycle performed without addition of factor D resulted in no frequency shift (data not shown). Similarly, when the surface was precoated with fibrinogen, no frequency shift was observed (Fig. 5*A*).

Similar experiments on adsorbed C3 in the absence of properdin (Fig. 5*B*, −P) gave frequency shifts of −7 Hz in each cycle, except in a cycle without factor D, when the shift was only −1 Hz (data not shown). As was observed for C3b, there was no frequency shift when the surface was pretreated with fibrinogen (Fig. 5*A*).

*Generation of C3b,Bb,P and C3,Bb,P complexes*

Convertase complexes were then generated in the presence of properdin (Fig. 5, *A* and *B*, +P), which was added together with factor B. For adsorbed C3, the frequency shift during the C3 incubations was decreased from −7 Hz in all cycles to −7, −10, and −17 Hz, respectively. For C3b, the corresponding frequency shifts were raised from −31, −37, and −40 Hz, respectively, to −55, −79, and −82 Hz.

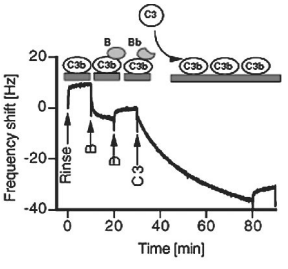


**FIGURE 4.** A typical cycle in the assembly of an alternative pathway convertase, as monitored by QCM-D. The convertase was generated by the sequential addition of factor B, factor D, and C3 to adsorbed C3b.

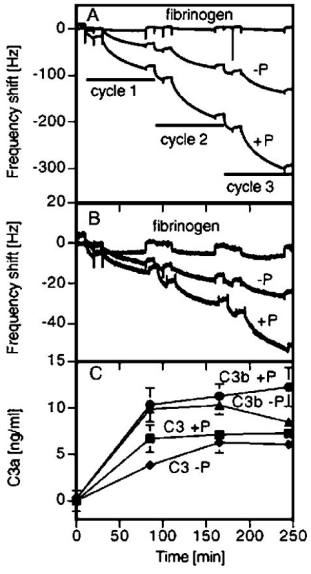


**FIGURE 5.** Generation of alternative pathway convertases on adsorbed C3b and C3 in the presence or absence of properdin, as monitored by QCM-D. Polystyrene surfaces were coated with 200 $\mu$l/ml C3b (*A*) or C3 (*B*), either directly or after precoating with fibrinogen. For each experiment the alternative convertase was assembled in three identical cycles; factor B (10 min), factor D (10 min), C3 (50 min), and buffer (10 min) were sequentially incubated. Each bar represents one cycle. The experiments were performed with (+P) or without (−P) properdin, which was added together with factor B. The experiment on fibrinogen was performed without properdin only. All steps was conducted in PBS containing 1 mM $Ni^{2+}$. *C*, Generation of C3a in the supernatants from the experiments in *A* and *B*.

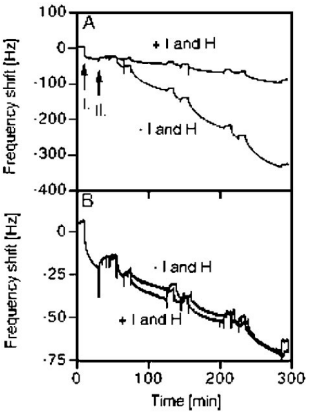


**FIGURE 6.** Generation of alternative pathway convertases on adsorbed C3b and C3 treated with factors I and H. Polystyrene surfaces were coated with either 200 $\mu$l/ml C3b (*A*) or 200 $\mu$l/ml C3 (*B*), and the adsorbed proteins were incubated simultaneously with factors I and H. Surfaces not exposed to factors I and H were used as controls. The addition of factors I and H (I.) and the start of the wash (II.) are indicated. After these initial steps, factor B (10 min), factor D (10 min), C3 (50 min), and buffer (10 min) were sequentially incubated in three cycles. The binding was monitored by QCM-D.

*Generation of C3a by the surface-bound convertase complexes*

Supernatants obtained after the C3 incubations shown in Fig. 5, *A* and *B*, were then analyzed for the presence of C3a (Fig. 5*C*). A significant generation of C3a could be detected after incubation with either immobilized C3b or C3, but the addition of properdin (Fig. 5*C*, +P) markedly increased the level of C3a that was generated in the supernatant.

*Regulation by factor I and factor H*

In the final set of experiments, adsorbed C3 and C3b were treated with factor I and factor H before the convertases were assembled in the presence of properdin (Fig. 6). In the case of adsorbed C3 (Fig. 6*B*), this treatment did not affect the ability to form new convertase complexes. However, the ability of C3b to form convertase complexes was greatly reduced (Fig. 6*A*). The frequency shifts during the C3 incubations were only −10, −17, and −23 Hz, as compared with −55, −79, and −82 Hz in the absence of treatment with factors I and H.

## Discussion

Surface plasmon resonance has previously been used to study the assembly of the alternative pathway convertase (2, 40). In the present study we have used a new method, the QCM-D, to demonstrate that C3 bound by adsorption to a biomaterial surface can form an initiating alternative convertase. In this system, polystyrene-adsorbed C3 and C3b were each incubated sequentially with factor B, followed by factor D and C3. Subsequent analysis indicated that both adsorbed C3 and C3b generated convertases (C3,Bb and C3b,Bb) that cleaved C3, as evidenced by the binding of C3 fragments to the polystyrene surface in the QCM-D chamber and by generation of fluid-phase C3a. The addition of properdin to both incubation mixtures amplified the reaction significantly, indicating that C3,Bb,P and C3b,Bb,P were formed. Although C3b,Bb and C3b,Bb,P were more efficient than the corresponding C3-containing complexes, these experiments clearly showed that surface-bound C3-containing convertases are formed on the polystyrene surface.

Many polymers are known to activate the alternative pathway, and previous reports that a monoclonal anti-properdin Ab is able to block alternative pathway activation in an in vitro model of cardiopulmonary bypass (41) are in accordance with the properdin dependence of the C3,Bb convertase that we have observed. We have also shown in the present study that precoating of the polystyrene with fibrinogen could completely block the adsorption of C3 and therefore the formation of the convertase complexes.

An interesting difference was observed between the properties of adsorbed C3 and C3b, in that C3b was shown to be susceptible to cleavage by factors I and H, but adsorbed C3 was not influenced by this treatment. Therefore, it appears that the adsorbed C3 is not regulated by these factors. Such a situation would allow an initial convertase to be formed without being immediately down-regulated by the regulator factor I. The reason for the factor I resistance is not clear. It has been shown that substantial amounts of C3b must be bound to a host cell surface to achieve interaction with factor H (42). However, the first layer of C3 is due to adsorption of the protein to the polystyrene surface, which makes other mechanisms possible, such as an extreme conformation of the bound C3 that has no affinity for factor I and/or H, or concealment of factor I and/or H binding sites facing the solid phase.

In these experiments several measures were taken to rule out the possibility of forming fluid-phase iC3,Bb or C3b,Bb convertases, which could activate and deposit C3b on the surface. First, the C3 preparations were characterized by FPLC and SDS-PAGE before use and were found to contain no visible iC3 or $\alpha'$-chain of C3b. Second, after adsorption of C3 or C3b, and after each C3 cycle, we washed the incubation chamber for 10 min. The volume of each protein solution was more than six times the volume of the incubation chamber, so that the chamber was extensively washed with respect to the previous protein. Finally, the concentrations of C3 and factor B used to assemble the convertases were adjusted to be

approximately one-eighth of those in serum; further dilution would prevent assembly of the convertase complexes, because the alternative pathway is extremely dependent on a high protein concentration (43, 44).

To characterize the "contact-activated" C3 in our system, we reacted anti-C3(D) with the adsorbed C3. All the tested anti-C3(D) mAbs bound to adsorbed C3 and gave a frequency shift of ~−30 Hz in the QCM-D analysis. This result means that the mAbs bound to at least 20% of the bound C3 molecules, indicating that a large portion of the C3 molecules were conformationally changed. It is also likely that these molecules have a disrupted thioester, because expression of C3(D) epitopes coincides with breakage of the thioester (45). Some of the C3(D) epitopes were probably also expressed by dimeric C3 complexes (C3 covalently bound to C3), because SDS-PAGE/Western blotting under reducing conditions showed that a minor fraction of the C3 that was eluted from the surface appeared as high-molecular mass bands on the gel. As an indicator of the function of the bound C3, the binding of factor B (molecular mass, 93 kDa) in the first cycle was assessed and shown to approach −7 Hz at saturation (Fig. 6). These data indicate that ~10% of the factor B molecules were complexed with adsorbed C3. The small portion of dimers on the surface is not able to mediate this factor B binding. Taken together, our data suggest that adsorption of C3 to polystyrene causes a conformational change of the molecule that most likely results in a disrupted thioester bond (13). This form of "contact activation" of the protein serves as an alternate means by which the alternative pathway may be initiated independently of the "tick-over" of the thioester in fluid-phase C3. It is possible that surface contact is responsible for a significant part of the iC3 generation, as indicated by exposure of C3(D) epitopes on C3 adsorbed to a number of surfaces, indicating that the protein is conformationally changed; similarly, iC3 is formed on the interface between gas and fluid, as assessed by an anti-C3a Ab that detects only conformationally changed C3 (38, 46). This contact with different interfaces is impossible to avoid in vitro. Therefore, it is likely that "contact activation" of C3 is an initiator of the alternative pathway not only in vitro but also in vivo and ex vivo during the extracorporeal circulation involved in hemodialysis, cardiopulmonary bypass, and plasmapheresis.

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