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Complement Components C5 and C7: Recombinant Factor I Modules of C7 Bind to the C345C Domain of C5

Chuong-Thu Thai and Ronald T. Ogata

Studies reported over 30 years ago revealed that latent, nonactivated C5 binds specifically and reversibly to C6 and C7. These reversible reactions are distinct from the essentially nonreversible associations with activated C5b that occur during assembly of the membrane attack complex, but they likely involve some, perhaps many, of the same molecular contacts. We recently reported that these reversible reactions are mediated by the C345C (NTR) domain of C5. Early work by others localized the complementary binding sites to a tryptic fragment of C6 composed entirely of two adjacent factor I modules (FIMs), and to a larger fragment of C7 composed of its homologous FIMs as well as two adjoining short consensus repeat modules. In this work, we expressed the tandem FIMs from C7 in bacteria. The mobility on SDS-polyacrylamide gels, lack of free sulfhydryl groups, and atypical circular dichroism spectrum of the recombinant product rC7-FIMs were all consistent with a native structure. Using surface plasmon resonance, we found that rC7-FIMs binds specifically to both C5 and the rC5-C345C domain with K_D \approx 50 nM, and competes with C7 for binding to C5, as expected for an active domain. These results indicate that, like C6, the FIMs alone in C7 mediate reversible binding to C5. Based on available evidence, we suggest a model for an irreversible membrane attack complex assembly in which the C7 FIMs, but not those in C6, are bound to the C345C domain of C5 within the fully assembled complex. The Journal of Immunology, 2004, 173: 4547–4552.

The signature feature of complement is the ability to kill cells by puncturing their plasma membranes. This is conducted by a large heteropolymeric complex of proteins referred to as the membrane attack complex (MAC).

The formation begins when a protease attached to the target cell surface severs the α-chain of component C5 at a single site, yielding the small anaphylatoxin C5a and an activated C5b molecule. Activated C5b provides the nucleus for the sequential and essentially nonreversible addition of single copies of components C6, C7, and C8, and multiple copies of C9 to produce the MAC at the targeted site (reviewed in Refs. 1 and 2).

On the basis of ultracentrifugation studies, Müller-Eberhard and his colleagues (3, 4) reported over 30 years ago that even latent, nonactivated C5 binds to both C6 and C7. However, these interactions were reversible and therefore at least in part distinct from the nonreversible interactions that take place within the MAC. Nevertheless, they argued that there are only a limited number of such reversible interactions among the five MAC proteins, and suggested that they result from specific topological relationships that reflect the arrangement of individual components within the MAC itself (3, 4). More recently, DiScipio (5) examined the reversible interactions of C5 with C6 and C7 in a solid-phase assay in which C5 was linked to yeast cell walls through its carbohydrate groups. Using tryptic fragments, he localized the reversible binding site in C6 to a fragment composed only of its two tandem C-terminal factor I modules (FIMs; also called FIMAC). The equivalent tryptic fragment of C7 was not available, but DiScipio did localize the C5 binding site to a larger fragment composed of the corresponding C7 FIMs and two upstream short consensus repeats (SCRs; also called CCP domains). These observations demonstrated that FIMs mediate reversible binding of C6 to C5, and indicated that this is likely true for C7 as well, although they could not rule out participation of the C7 SCRs.

The significance of the reversible interactions is unclear, because a truncated C6 variant responsible for clinical subtotal deficiency has no FIMs, but still has bactericidal activity (6), and the common A form of mouse C6 also has no FIMs, but it too is active (7). DiScipio et al. (8) have constructed altered recombinant forms of C6 lacking the FIMs and found that the engineered C6des-FIMs protein is also active, albeit somewhat disabled, with ~65% of the alternative pathway activity and ~5% of the classical pathway activity of wild-type C6. They also found that the lower activity of C6des-FIMs is due primarily to a decreased ability to form a complex with C5b; formation of C5b,6 was ~14 times more efficient with wild-type C6 than C6des-FIMs, whereas the wild-type heterodimer was only about 2-fold more stable than C5b,6des-FIMs. These results demonstrated that the C6 FIMs enhance, but are not essential for lytic activity of the protein, or for formation of C5b,6.

The FIM or FIMAC domain is ~75 amino acid residues in length and contains four or five intradomain disulfide bonds. Its distribution is somewhat limited: C6 and C7 each have two tandem FIMs at their C termini, whereas complement factor I has a single N-terminal FIM (9). However, sequence comparisons suggest that the FIM is related to the follistatin domain (10) and may be a divergent composite of an N-terminal epidermal growth factor-like subdomain (also called FOLN in the SMART database; Ref. 11) and a C-terminal KAZAL-like subdomain (12, 13). However, this relationship is uncertain, because the disulfide bonding pattern determined by peptide analysis for the two FIMs in C6 (14) differs from the linkage pattern described for the follistatin domain by crystallography (12, 13). A fundamental difference is that, whereas

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2 Address correspondence and reprint requests to Dr. Ronald T. Ogata, Torrey Pines Institute for Molecular Studies, 3550 General Atomics Court, San Diego, CA 92121. E-mail address: rogata@tpims.org
3 Abbreviations used in this paper: MAC, membrane attack complex; FIM, factor I module; SCR, short consensus repeat; SPR, surface plasmon resonance; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); RU, response unit.
the disulfide bonding pattern of the follistatin domain reflects discrete epidermal growth factor-like and KAZAL-like subdomains, the pattern determined for the C6 FIM domains shows two disulfide bonds connecting these putative subdomains.

Using surface plasmon resonance (SPR), we recently found that the reversible interaction with C6 and C7 is mediated by a 150-residue-long domain at the C-terminal end of the C5 α-chain, distal to the proteolytic activation site at the N terminus (15). This domain, called C345C (and also NTR; Ref. 16), is present in both C5 and C5h. We found that recombinant C345C (rC5-C345C) binds to both C6 and C7, with a somewhat surprisingly strong preference for C7. Homologous C345C/NTR domains are also present in the corresponding C-terminal regions of the α- and γ-chains of the paralogous complement components C3 and C4, respectively, and in unrelated proteins including secreted frizzled-related protein, type I procollagen C-proteinase enhancer protein, and tissue inhibitors of metalloproteinases (16). This module functions as a metalloproteinase inhibitor in some proteins (17), but this does not appear to be the case with others (18) including the complement components.

The results of our studies with rC5-C345C (15), taken together with DiScipio’s results with the C6 and C7 (5), indicated that the reversible interaction of C5 with C6 is mediated by direct contact between the C345C domain of C5 and the FIMs in C6, and that binding to C7 likely involves a similar interaction with the corresponding C7 FIMs. In this report, we describe the production of the C7 FIMs dimer in a bacterial expression system and demonstrate that rC7-FIMs does bind to rC5-C345C as well as to native C5. We suggest a model for interactions within the fully assembled MAC in which the C7 FIMs play an essential role, whereas the C6 FIMs facilitate the initial step in MAC assembly formation of C5b,6—but do not participate in any significant interactions.

Materials and Methods
Reagents
Restriction enzymes were purchased from New England Biolabs (Beverly, MA), oligonucleotides from Sigma-Genosys (The Woodlands, TX), and pfu DNA polymerase from Stratagene (Cedar Creek, TX). Plasmid pET15b, the ORIGAMI strain of Escherichia coli, and protein extraction and purification reagents including BugBuster, benzamidine, protease inhibitor mixture, His-Bind purification kit, bionylated thrombin, and streptavidin agarose were purchased from Novagen (Milwaukee, WI). 5,5′-Dithio-bis(2-nitrobenzoic acid) (DTNB), L-cysteine hydrochloride, Tris, and Brilliant Blue R were from Sigma-Aldrich (St. Louis, MO), and isopyrophenyl β-thiogalactoside was from Fisher Biotech (Fair Lawn, NJ). All complement components, buffers, and reagents were purchased from Advanced Research Technologies (San Diego, CA).

Construction of expression plasmid
The DNA sequence encoding residues Asn671 to the C-terminal Gln631 of human C7 (19) were copied by PCR from the full-length C7 cDNA in the pBacPAK vector (a gift from Dr. R. DiScipio). Priming oligonucleotides were designed to include an Ndel restriction enzyme cleavage site immediately upstream of the N-terminal Asn residues, and a BamHI site in the 3′-untranslated region as well as a second TAA stop codon following the native TAG codon to ensure efficient translational termination. PCR products were digested with Ndel and BamHI, and ligated into NdeI/BamHI-digested PET15b to give PET/C7FIMs. Sequences of the final constructs were confirmed by automated DNA sequencing with ABI model 3100 instrument (Applied Biosystems, Foster City, CA) using the Big Dye Terminator, version 3.0, cycle sequencing reagents from the same company. This construct directs expression of the tandem C7 FIM domains with a 21-aminocarboxylydehyde-long N-terminal extension that includes a 6-residue-long His-tag followed by a thrombin cleavage site. Cleavage of the expressed recombinant product with thrombin removes the His-tag and yields the 153-residue-long rC7-FIMs, which includes the vector-derived 4-residue-long N-terminal extension, Gly-Ser-His-Met.

Protein expression and purification
Expression of rC7-FIMs in the ORIGAMI strain followed by affinity purification on a Ni2+-immobilized column, His-tag removal, and a second transit through the affinity column were conducted as described (15). The concentration of rC7-FIMs was determined from the calculated molar extinction coefficient at 280 nm of 12,600.

Circular dichroism
Circular dichroism measurements were conducted in 10 mM Tris-HCl/150 mM NaCl (pH 7.5) at a concentration of ~25 μM with a Jasco (Easton, MD) J-720 spectropolarimeter. Spectra were analyzed with the Jasco J-700 program for Windows Secondary Structure Estimation, version 1.10.00, Jasco.0.3, to assess secondary structure content.

DTNB assay for free sulfhydryls
Assays were conducted essentially as described (20). Briefly, protein solutions at 15–30 μM, and parallel standard solutions of cysteine hydrochloride were incubated at room temperature in 1.5 mM DTNB/50 mM NaPO4/2 mM EDTA (pH 7.2), and A412 was monitored for up to 3 h.

Surface plasmon resonance
SPR measurements were conducted on a Biacore 3000 instrument (Biacore, Uppsala, Sweden) with reagents, buffers, and data analysis software from the same company. Except as noted, ligands were immobilized by amine coupling to Biacore sensor chip CM5 activated with 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride and N-hydroxysuccinimide as recommended by Biacore. All experiments were conducted at 25°C in 0.01 M HEPES (pH 7.4), 0.15 M NaCl, and 0.005% surfactant P20, and sensor chips regenerated with 20 mM NaOH. The signal from a control blank sensor that had undergone activation and blocking without protein coupling was subtracted from all sensorgram plots. Binding affinities and kinetics were determined by data fitting using the BIAevaluation software from Biacore.

Results
Fig. 1 shows the schematic modular structures of the C5 α-chain, C6, and C7. In previous work (15), we showed that reversible binding of C5 to C6 and C7 (3) involves direct contact with the C345C domain of C5 (shaded in Fig. 1). Earlier work by DiScipio

![FIGURE 1. Schematic representation of the modular structures of the C5 α-chain, C6, and C7. Relative chain lengths and module sizes are approximately to scale. Most of the modules were defined as described in the SMART database (11). The α2-macroglobulin receptor binding domain-related (ARBR) domain is our own designation; this module is homologous to the receptor binding domain in α2-macroglobulin (24, 25). Sites labeled “T” in C6 and C7 are cleaved by trypsin in the native proteins. The C345C domain, FIMs, and SCR domains discussed in this report are shaded.](http://www.jimmunol.org/)

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used the ORIGAMI strain of expressed fragment. To fashion the appropriate disulfide forms of rC7-FIMs. Positions of molecular mass markers showing the electrophoretic migration of the reduced (2ME) and nonreduced (H11001) forms of rC7-FIMs. The upstream FIM contains four disulfide bonds, whereas the other has five, for a total of nine in the expressed fragment. To fashion the appropriate disulfide bonds, we used the ORIGAMI strain of E. coli, which has an oxidizing cytoplast that permits disulfide bond formation during protein synthesis (21). This method allowed us to isolate the soluble expressed product directly from bacterial cell lysates as we did previously with the C345C domains from C3 and C5 (15). 

rC7-FIMs has internal disulfide bonds and displays a nonstandard circular dichroism spectrum

Fig. 2 compares the electrophoretic migration of rC7-FIMs in the presence and absence of the reducing agent 2-ME. The reduced form shows the shift to lower mobility that is characteristic of a polypeptide with internal disulfide bonds, as observed earlier with rC5- and rC3-C345C (15). The minor contaminant seen in the reduced sample at M_r of ~15,000 may be a proteolytic fragment of rC7-FIMs; it was estimated to be ±5% by weight of the major product by visually comparing the intensities of stained bands among lanes loaded with different amounts of the final product. A DTNB assay measured 0.04 free sulfhydryl groups per molecule of rC7-FIMs in our purified sample (data not shown). Therefore, essentially all cysteines in the polypeptide are inaccessible to DTNB, consistent with the expected completely disulfide-bonded structure of the native FIMs (14). As shown in Fig. 2, the molecular mass of the reduced form estimated by electrophoretic mobility is substantially greater than the mass average of 16,936 calculated for the expected peptide. However, MALDI-TOF mass spectrometry (data not shown; conducted by the mass spectrometry laboratory of The Scripps Research Institute) measured a mass of 16,926, confirming the identity of the expressed product.

Fig. 3 shows the circular dichroism spectrum of rC7-FIMs. Although this spectrum differs somewhat from that reported for the tryptic fragment containing the corresponding C6 FIM dimer (22), both spectra exhibit similar nonstandard features that preclude estimation of secondary structure by standard algorithms. A preliminary 1H nuclear magnetic resonance analysis of rC7-FIMs showed little or no evidence of oligomers at concentrations of ≥200 μM (P. Barlow and J. Bramham, unpublished observation), and hence it is very unlikely that the recombinant product aggregates at the much lower concentrations used in the studies described here.

rC7-FIMs binds to C5 and rC5-C345C, but not to C3

Fig. 4 shows SPR sensorgrams tracking the binding of C7-FIMs, both free in solution and immobilized on the SPR surface, to C3, C5, and their recombinant C345C domains. Fig. 4A shows the results of pulsing a 1 μM solution of rC7-FIMs over separate SPR flow cells bearing immobilized C3, C5, or rC5-C345C. Fig. 4B shows the results of pulsing solutions of rC3-C345C and rC5-C345C, each at 1 μM, and C5 at 0.25 μM over a surface bearing 1000 response units (RU) of rC7-FIMs. Both free in solution and immobilized on the SPR surface, rC7-FIMs shows unambiguous binding to C5 and rC5-C345C, but not to C3 or rC3-C345C. This is consistent with the specific binding of C5 and rC5-C345C to the intact C7 protein that we reported earlier (15).

Fig. 5 shows the SPR responses for a surface bearing 1000 RU of immobilized rC7-FIMs pulsed with varying concentrations of C5 (panel labeled C5) and rC5-C345C (panel labeled CC). Similar experiments (not shown) were conducted, in which solutions of rC7-FIMs at multiple concentrations were pulsed over surfaces bearing immobilized C5 or rC5-C345C. Data from several independent measurements with rC7-FIMs, C7, rC5-C345C, and C5 in both orientations were used to derive the equilibrium and kinetic constants listed in Table I.

![Figure 2](http://www.jimmunol.org/)

**FIGURE 2.** 13% SDS-polyacrylamide gel stained with Brilliant Blue showing the electrophoretic migration of the reduced (+2ME) and nonreduced (−2ME) forms of rC7-FIMs. Positions of molecular mass markers are shown at the left.

![Figure 3](http://www.jimmunol.org/)

**FIGURE 3.** CD spectrum of rC7-FIMs. Our standard secondary structure simulation program was unable to estimate the secondary structure content of this module because of the nonstandard features of this spectrum.

![Figure 4](http://www.jimmunol.org/)

**FIGURE 4.** SPR sensorgrams showing that rC7-FIMs binds specifically to C5 and rC5-C345C. **A.** Shown are the responses of immobilized C5 (dotted line), rC5-C345C (solid line), and C3 (dashed line) to pulses of 1 μM rC7-FIMs. In this experiment, 5000 RU of C5, 200 RU of rC5-C345C, and 5000 RU of C3 were immobilized on the SPR surface. **B.** Shown are the responses of 1000 RU of immobilized rC7-FIMs to 0.25 μM C5 (dotted line), 1 μM rC5-C345C (solid line), and 1 μM rC3-C345C (dashed line).
With two exceptions, the equilibrium binding data in Table I show a striking consistency. They indicate that either in solution or immobilized on the SPR surface, C5 and rC5-C345C bind to C7 with essentially the same affinity, \( K_d = 40-70 \) and 50–100 nM, respectively; and that both bind to immobilized C7 with a similar but slightly higher affinity (\( K_d = 30 \) nM). The near identity of the results with C7 and rC7-FIMs demonstrate that glycosylation of the first FIM in C7 (19) is not necessary for binding to C5, because rC7-FIMs is not glycosylated. The two exceptions to the general consensus were the affinities measured with C7 in solution and C5 or rC5-C345C immobilized on the SPR surface. In those cases, the measured dissociation constants were 300- and 10-fold lower (higher affinity) than in the inverse orientation, with the differences due entirely to much slower dissociation rates. Because of the otherwise general agreement of the measurements, we infer that the latter results are incorrect, perhaps because of a non-specific affinity of C7 for the carboxymethyl dextran matrix on the SPR chip while it is bound to its immobilized target. However, this assumption is based only on strength of numbers, and we cannot exclude the possibility that the predominance of lower affinities is itself caused by artifacts in the method. With this reservation, we conclude that C345C and the C7 FIMs bind to each other, both as independent structures and within their respective full-length proteins, with a dissociation constant of \( \sim 50 \) nM.

### Table I. Equilibrium and kinetics constants from SPR for binding of C5 and rC5-C345C to C7 and rC7-FIMs

<table>
<thead>
<tr>
<th>Immobilized(^a)</th>
<th>Solution</th>
<th>( K_d ) (nM)</th>
<th>( k_d ) (M(^{-1})s(^{-1}))</th>
<th>( k_{on} ) (s(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>C5(^b)</td>
<td>C7</td>
<td>0.1</td>
<td>( 4 \times 10^4 )</td>
<td>( 0.04 \times 10^4 )</td>
</tr>
<tr>
<td>C5</td>
<td>rC7-FIMs</td>
<td>70</td>
<td>( 3 \times 10^4 )</td>
<td>( 2 \times 10^4 )</td>
</tr>
<tr>
<td>C7</td>
<td>C5</td>
<td>30</td>
<td>( 0.6 \times 10^4 )</td>
<td>( 2 \times 10^4 )</td>
</tr>
<tr>
<td>C7</td>
<td>rC5-C345C</td>
<td>30</td>
<td>( 3 \times 10^4 )</td>
<td>( 10 \times 10^4 )</td>
</tr>
<tr>
<td>rC5-C345C</td>
<td>C7</td>
<td>3</td>
<td>( 3 \times 10^4 )</td>
<td>( 0.9 \times 10^4 )</td>
</tr>
<tr>
<td>rC5-C345C</td>
<td>rC7-FIMs</td>
<td>50</td>
<td>( 4 \times 10^4 )</td>
<td>( 2 \times 10^4 )</td>
</tr>
<tr>
<td>rC7-FIMs</td>
<td>C5</td>
<td>40</td>
<td>( 1 \times 10^4 )</td>
<td>( 4 \times 10^4 )</td>
</tr>
<tr>
<td>rC7-FIMs</td>
<td>rC5-C345C</td>
<td>100</td>
<td>( 5 \times 10^4 )</td>
<td>( 5 \times 10^4 )</td>
</tr>
</tbody>
</table>

\(a\) All measurements were made in 0.01 M HEPES (pH 7.4), 0.15 M NaCl, and 0.005% surfactant P20, at 25°C, and in two orientations, with members of each binding pair serving both as the ligand (immobilized on the SPR surface) and as the analyte (free in solution).

\(b\) From Ref. 15.

**FIGURE 5.** Representative normalized SPR sensorgrams showing the effects of pulsing C5 (left panel labeled C5) and rC5-C345C (right panel; CC), from \( t = 150 \) s to \( t = 450 \) s at different concentrations over a surface bearing 1000 RU of rC7FIMs. Concentrations ranged in 2-fold increments from 2 to 130 nM for C5, and from 23 to 1.5 \( \mu \)M for rC5-C345C. In this experiment, the theoretical global fit responses gave \( K_d = 37 \) nM, \( k_{on} = 1.4 \times 10^4 \) M\(^{-1}\)s\(^{-1}\), and \( k_{off} = 5.2 \times 10^{-4} \) s\(^{-1}\) for rC7-FIMs binding to C5; and \( K_d = 124 \) nM, \( k_{on} = 4.4 \times 10^4 \) M\(^{-1}\)s\(^{-1}\), and \( k_{off} = 5.5 \times 10^{-3} \) s\(^{-1}\) for binding to rC5-C345C, with \( x^2 \) values of 0.1 and 1.5, respectively.

**FIGURE 6.** SPR sensorgrams showing that rC7-FIMs inhibits binding of C7 to both C5 and rC5-C345C. For the experiment with C5 (left panel), solutions containing 0.25 \( \mu \)M C5 and rC7-FIMs at the concentrations indicated were pulsed over a surface bearing 6000 RU of immobilized C7. In the right panel, solutions of 0.3 \( \mu \)M rC5-C345C containing rC7-FIMs at the concentrations indicated were pulsed over 3200 RU of immobilized C7. In both experiments, rC7-FIMs in the unlabeled scan was at 0.15 \( \mu \)M. rC7-FIMs mediates binding of C7 to C5 and rC5-C345C.

To confirm that the FIMs in C7 mediate its binding to C5 and rC5-C345C, we tested the ability of rC7-FIMs to block binding of C7 to C5. Fig. 6 shows the effects of rC7-FIMs in solution on binding of C5 (panel labeled C5) and rC5-C345C (panel CC) also in solution, to immobilized C7 (6000 and 3000 RU, respectively). The concentrations of C5 and rC5-C345C were constant at 0.25 and 0.3 \( \mu \)M, respectively, whereas the concentration of rC7-FIMs was varied from 0 to 2.4 \( \mu \)M as indicated in Fig. 6. These results demonstrate that rC7-FIMs inhibits binding of both C5 and rC5-C345C to C7.

**Discussion**

This report describes the expression of the two tandem FIMs of C7 in a bacterial system and the interaction of the recombinant product (rC7-FIMs) with C5 and its recombinant C345C domain. The electrophoretic mobilities of the reduced and oxidized forms of rC7-FIMs, its lack of reactive cysteines, and an atypical circular dichroism spectrum that is similar to that of the C6 FIMs are all consistent with a structure representative of the FIMs within C7. However, the strongest evidence of the proper structure is the recombinant module’s specific binding to C5 but not C3, with an affinity similar to intact C7, and its ability to inhibit binding of C7 to both C5 and rC5-C345C. C7 and rC7-FIMs bind to C5 and to rC5-C345C with nearly identical affinities, indicating that the FIMs alone mediate reversible binding of C7 to C5, with negligible contributions from the C7 SCRs. In summary, the present results provide compelling evidence that the reversible binding of C5 to C7 that was reported over 30 years ago by Müller-Eberhard and his colleagues (3, 4) involves direct contact between the C345C domain in C5 and one or both FIMs in C7.

What is the role of reversible binding of C5 to C6 and C7 in complement function?

In a previous study, we found using SPR that the reversible binding reactions of C5 with C6 and C7 are characterized by very high affinities, with \( K_d = 0.4 \) and 0.1 nM, respectively (15). The plasma concentrations of C5, C6, and C7 are all \( \sim 500 \) nM (9), or a 1000-fold higher than these dissociation constants. Therefore, we suggested that complexes of C5 bound to C6 or C7 probably exist in plasma, and that these complexes could facilitate MAC assembly by increasing the local concentrations of C6 and C7 before C5 activation. However, our results also indicated that C5 binds preferentially to C7, with C7 readily displacing C6; this was problematic of course, because C6 precedes C7 in MAC assembly.
The present results indicate that the affinity of C5 for C7 is actually substantially lower, with $K_D \approx 50 \text{ nM}$. As discussed earlier, our assumption that this is the more accurate affinity is somewhat arbitrary, being based only on strength of numbers: measurements in six of eight cases gave affinities in this range, whereas the other two measurements, with $C7$ in solution, gave the much higher affinities reported earlier (Table I). This lower affinity also agrees with the previously reported $K_D = 100 \text{ nM}$ for binding of $C5$ to the $C6$ FIMs (8). Whatever the absolute affinity for $C7$ might be, the relative affinity for $C6$ is unequivocally much lower, because $C7$ displaces $C6$ from $C5$ when both are present in solution, and binds with 3- to 10-fold higher affinity in parallel SPR measurements with immobilized $C5$ and $C5-C345C$ (15). Arroyave and Müller-Eberhard (3) reported a similar preference for $C7$ when both $C6$ and $C7$ were presented to $C5$. However, even with $K_D$ in the range of 50 to 100 nM, more than half of the $C5$ in plasma should still be bound reversibly to $C6$ or $C7$. But this dissociation constant applies at 25°C with purified proteins, and the affinities under physiological conditions may be much lower. Therefore, the present results substantially undermine the case for our earlier suggestion that complexes composed of $C5$ bound to $C6$ or $C7$ exist in plasma. Furthermore, the enhancing effect of the FIMs on $C6$ activity (8) does not appear to require preactivation association with $C5$, because these complexes should not be formed in the dilute serum used to measure classical pathway hemolytic activity.

What roles do the FIMs play in $C6$ and $C7$ function?

There appears to be a general consensus that the FIMs in $C6$ enhance, but are not essential for activity, and are probably not involved in any significant interactions within the MAC. The FIMs in mouse $C6$ may serve a similar facilitating but nonessential purpose as well, because although $C6A$ is active, the $B$ form is much more active and is larger by an amount consistent with the addition of two FIMs: $C6B$ comigrates with human $C6$ on an SDS-polyacrylamide gel (7). However, there are reasons to believe that the FIMs in $C7$ play a more prominent role. For example, 6 of the 15 mutations that have been found to cause $C7$ deficiency cluster in or very near to the FIMs, whereas those causing $C6$ deficiency are more evenly distributed across the $C6$ gene (23). In addition, it seems unlikely that the strong, specific interaction between the $C7$ FIMs and $C5$ serves no purpose.

Based on available information, we suggest the following model (illustrated in Fig. 7). The $C345C$ module of newly activated $C5b$ binds to the $C6$ FIMs during formation of $C5b6$, and this association is maintained within the heterodimer. This interaction facilitates binding, but provides a relatively small amount of the overall association energy and does not greatly increase the stability of $C5b6$. We assume that most of the binding energy comes from a second interaction, at the metastable binding site of $C5$ (labeled mbs), that is exposed transiently after $C5$ activation. Subsequent binding of $C7$ to $C5b6$ results in displacement of the $C6$-FIMs by the higher affinity $C7$-FIMs; this would accommodate the somewhat puzzling observations that the affinity of $C5$ for $C7$ is several times greater than for $C6$, and $C7$ easily displaces $C6$ from $C5$ (3, 15). The $C6$-FIMs may then associate with another region of $C5$, with $C7$, or remain free. We assume in this picture that the resulting strong bond between the $C5-C345C$ domain and the $C7$-FIMs is an essential component of the interaction between $C7$ and $C5b6$, and that this contact is maintained within the fully assembled MAC. We also assume that there are other interacting sites between $C7$ and $C5b6$. An attractive idea is that the conformational change that exposes a membrane-binding site in $C7$ when it binds to $C5b6$ (1), also exposes a latent site for $C6$ binding. Multiple specific protein-protein interactions are likely in general to account for the nonreversible character of each step leading to the MAC. The specific reversible associations first described by Kolb et al. (4) doubtless reflect a subset of these interactions.

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References


