Interaction between the C8α-γ and C8β Subunits of Human Complement C8: Role of the C8β N-Terminal Thrombospondin Type 1 Module and Membrane Attack Complex/Perforin Domain†

Primrose Musingarimi, Mnason E. Plumb, and James M. Sodetz*

Department of Chemistry and Biochemistry and School of Medicine, University of South Carolina, Columbia, South Carolina 29208

Received May 28, 2002; Revised Manuscript Received July 19, 2002

ABSTRACT: Human C8 is one of five complement components (C5b, C6, C7, C8, and C9) that interact to form the cytolytic membrane attack complex (MAC). It is an oligomeric protein composed of a disulfide-linked C8α-γ heterodimer and a noncovalently associated C8β chain. C8α and C8β are homologous; both contain an N-terminal thrombospondin type 1 (TSP1) module, a low-density lipoprotein receptor class A (LDLRA) module, an extended central segment referred to as the membrane attack/perforin (MACPF) domain, an epidermal growth factor (EGF) module, and a second TSP1 module at the C-terminus. In this study, the segment of C8β that confers binding specificity toward C8α-γ was identified using recombinant C8β constructs in which the N- and/or C-terminal modules were deleted or exchanged with those from C8α. Constructs were tested for their ability to bind C8α-γ in solution and express C8 hemolytic activity. Binding to C8α-γ was found to be dependent on the TSP1 + LDLRA + MACPF segment of C8β. Within this segment, the TSP1 module and MACPF domain are principally involved and act cooperatively to mediate binding. Results from activity assays suggest that residues within this segment also mediate binding and incorporation of C8 into the MAC.

Human C8 is one of five components (C5b, C6, C7, C8, and C9) that interact to form the cytolytic membrane attack complex of complement (*1*, *2*). It is composed of an α (64 kDa), β (64 kDa), and γ (22 kDa) subunit, which are products of different genes (*3*, *4*). Within C8, these subunits form a disulfide-linked C8α-γ heterodimer that is noncovalently associated with C8β. C8α and C8β are homologous and together with C6, C7, and C9 form the MAC[1] family of proteins (*5*, *6*). These proteins are similarly organized, and all have a modular structure. C8α and C8β contain tandemly arranged N-terminal TSP1 and LDLRA modules, an extended central segment referred to as the MACPF domain, an EGF module, and a second TSP1 module at the C-terminus. By contrast, C8γ is unrelated and is a member of the lipocalin family of proteins that bind small, hydrophobic ligands, e.g., retinol, pheromones, and odorants (*7*−*9*). Although a ligand has not been identified, a similar binding function for C8γ is suggested from its crystal structure (*10*).

Self-assembly of the MAC on target cells requires a sequential and highly specific interaction between the constituent proteins (*1*, *6*). Despite speculation about their role in MAC formation, few studies have addressed whether the modules confer specificity or if they primarily function to stabilize binding. C8 is an excellent model for investigating this because the affinity between C8α-γ and C8β is high and mediated principally by interactions involving C8α and C8β (*11*). The role of the modules in this interaction was recently investigated using recombinant forms of C8α in which the modules were deleted or exchanged with those from C8β (*12*). All constructs containing the MACPF portion of C8α retained the ability to bind C8β, including one composed of the MACPF segment alone. This suggests that specificity for C8β is determined solely by the C8α MACPF domain and the modules themselves function to stabilize the interaction.

In the present study, we examined the requirement for the C8β modules in binding C8α. Recombinant forms of C8β in which the N- and/or C-terminal modules were deleted or exchanged with those from C8α were characterized for their ability to bind C8α-γ. Results show that specificity is not determined solely by the C8β MACPF domain but requires an apparent cooperative interaction between this domain and the N-terminal TSP1 module. Our results also suggest that the LDLRA module of C8β has a role in mediating C8 incorporation into the MAC.

EXPERIMENTAL PROCEDURES

Materials. Human C8 was purified from plasma fraction III (Bayer Corp., Clayton, NC) (*13*). C8α-γ and C8β were

† Supported by NIH Grant GM 42898.

* Corresponding author. Phone: 803-777-6625. Fax: 803-777-9521. E-mail: Sodetz@mail.chem.sc.edu.

[1] Abbreviations: MAC, membrane attack complex or C5b-9; TSP1, thrombospondin type 1; LDLRA, low-density lipoprotein receptor class A; EGF, epidermal growth factor; MACPF, membrane attack complex/perforin; pRAHC8α-γ, rabbit anti-human C8α-γ antiserum; pRAHC8β, rabbit anti-human C8β antiserum; MabT1β, mouse monoclonal antibody specific for the N-terminal TSP1 module of C8β; MabNTα, mouse monoclonal antibody specific for an epitope located within residues 1−102 of C8α; wt, wild type; EAC1−7, sensitized sheep erythrocytes carrying human complement C1−C7. Module abbreviations follow recommendations by the 1994 International Workshop on Sequence, Structure, Function and Evolution of Extracellular Protein Modules (available in ref *16* and at SWISS-PROT).

10.1021/bi026207h CCC: $22.00
Published on Web 08/21/2002

separated by gel filtration in high ionic strength buffer and their molar concentrations determined from published $\epsilon_{280}^{1\%}$ values (*14*). Goat antiserum against human C8 and rabbit antiserum against human C8α-γ or C8β were prepared by standard methods. Goat anti-human C8α and C8β antibodies were affinity purified as described (*12*). A purified mouse monoclonal antibody specific for the TSP1 module of human C8β and one specific for an epitope within the N-terminal region of C8α were a gift from Dr. Peter J. Sims, Scripps Research Institute. Epitope specificity was determined using a panel of human C8α and C8β deletion constructs expressed as fusion proteins in *Escherichia coli*.[2]

Expression Constructs. Constructs containing wt C8α or wt C8β cDNA in the COS cell expression vector pcDNA3 (Invitrogen) were prepared as described previously (*15*). To produce C8βc1, a chimeric primer and a C8β primer were used with a wt C8β template to generate a PCR fragment encoding residues 202−444 of C8β and residues 463−465 of C8α. In a separate reaction, a similar strategy was used with a wt C8α template to generate a fragment corresponding to residues 442−444 of C8β and residues 463−554 of C8α. In second round PCR, these self-annealing fragments were used to generate a chimeric cDNA fragment encoding residues 202−444 of C8β and residues 463−554 of C8α. The product was digested with restriction enzymes *Spe*I and *Apa*I. This fragment was ligated to a *Bam*HI/*Spe*I fragment from wt C8β, which contains the N-terminal modules and a portion of the MACPF domain, and subsequently cloned into pcDNA3. The C8βt1 construct was created by overlap extension PCR using wt C8β as the template. Primers were designed to generate a fusion at the junction between the codon for amino acid 444 and the termination codon TAG.

Constructs C8βc2, C8βc4, and C8βc5 were prepared using a strategy similar to that for C8βc1. For C8βc2, chimeric primers were used with wt C8α or wt C8β as templates to generate self-annealing 5′ and 3′ PCR fragments with an overlap at the LDLRA/MACPF boundary. These fragments were used with flanking universal primers in a second round of PCR to generate a single chimeric fragment. The fragment was digested with *Eco*RV and *Apa*I and substituted into wt C8β which had been digested with the same enzymes. The same strategy was used to create C8βc4; self-annealing 5′ and 3′ fragments with an overlap at the TSP1/LDLRA boundary were created from wt C8α and wt C8β templates. Following combination of the fragments with universal primers in a second round of PCR, the product was digested with *Hin*dIII and *Bsm*BI and exchanged with the corresponding fragment in C8βc2. To produce C8βc5, a C8β fragment encoding the leader sequence as well as residues 1−65 was generated by PCR using a wt C8β template. A second fragment containing residues 59−65 of C8β, residues 64−102 of C8α, and residues 104−537 of C8β was produced using a chimeric primer and C8βc2 as the template. Second round PCR produced a full-length fragment containing residues 1−65 of C8β (TSP1), residues 64−102 of C8α (LDLRA), and residues 104−537 of C8β. C8βc6 was produced by substituting the *Hin*dIII/*Bsm*BI fragment from C8βc1 with the corresponding fragment from C8βc5. C8βc3 was prepared by using PCR to introduce a $Cys^{164} \rightarrow Gly^{164}$ into the previously described chimera C8αc3 (*12*). All constructs were subjected to dideoxy sequencing to confirm the fidelity of PCR-generated products and the integrity of junction sites.

Expression in COS Cells. COS-7 cells were transfected and cultured as described (*12*). Control media were prepared from nontransfected cells. After harvesting, media were concentrated ∼60−80-fold and dialyzed as described below. Expression efficiency was evaluated by immunoblotting. Samples were precipitated with 10% (w/v) TCA and subjected to 10% SDS−PAGE. Depending on the construct, blots were developed with pRAHC8α-γ and/or pRAHC8β as the primary antibody and a horseradish peroxidase conjugate of goat anti-rabbit IgG as the secondary antibody (Bio-Rad). Proteins were visualized with Super Signal chemiluminescent substrate (Pierce).

Quantitation of Recombinant C8β. Following dialysis of the media, the concentration of expressed C8β product was determined by ELISA or immunoblotting. Quantitation by ELISA was performed as previously described with the following modifications (*12*). To quantitate C8β chimeras, a mixture of goat anti-human C8α and C8β antibodies was plated onto microtiter plates. To quantitate wt C8β and the truncate C8βt1, only C8β antibodies were plated. Medium containing wt C8β was quantitated against a control of purified human C8β and used to generate a standard curve. Samples containing C8βc1, C8βt1, C8βc3, C8βc5, or C8βc6 were added to the appropriate microtiter plate and the bound products detected using MabT1β as the primary antibody. For C8βc2, bound product was detected with MabNTα as the primary antibody, and the results were compared to a standard curve of purified C8α-γ. A horseradish peroxidase conjugate of goat anti-mouse IgG was used as the secondary antibody (Bio-Rad). For C8βc4, concentrations were estimated from immunoblots because the construct is not recognized by MabNTα. Purified C8β was used as a standard and pRAHC8β as the primary antibody.

Density Gradient Binding Assays. Binding between the expressed C8β constructs and C8α-γ was measured by sucrose density gradient centrifugation. Concentrated media were dialyzed into low ionic strength buffer (5 mM imidazole, 33.6 mM NaCl, 0.15 mM $CaCl_2$, 0.5 mM $MgCl_2$, pH 7.4) and quantitated as described above. The final concentration of wt C8β and the various C8β constructs ranged from ∼4 to 14 pmol/mL. Purified human C8β was diluted to a comparable concentration in buffer and used as a control. Purified human C8α-γ was added at the desired molar excess over the C8β construct, and the mixture was incubated for 1 h at 25 °C. A volume of 200 μL was applied to a 4 mL 5−15% (w/v) sucrose density gradient prepared in the same buffer supplemented with 1 mg/mL BSA (*12*). Gradients were centrifuged for 2 h at 4 °C in a Sorvall VTi65 rotor at 202000*g*. Samples were fractionated, and the sedimentation position of each C8β construct was determined by an ELISA. These were performed as above using goat anti-human C8β as the capture antibody and pRAHC8β as the primary antibody.

Activity Assays. Concentrated expression media and control medium were dialyzed against a buffer of intermediate ionic strength (5 mM imidazole, 72.7 mM NaCl, 0.15 mM $CaCl_2$, 0.5 mM $MgCl_2$, pH 7.4). After quantitation, each recombinant form of C8β was incubated with an excess of purified

[2] T. Barber and J. M. Sodetz, unpublished results.

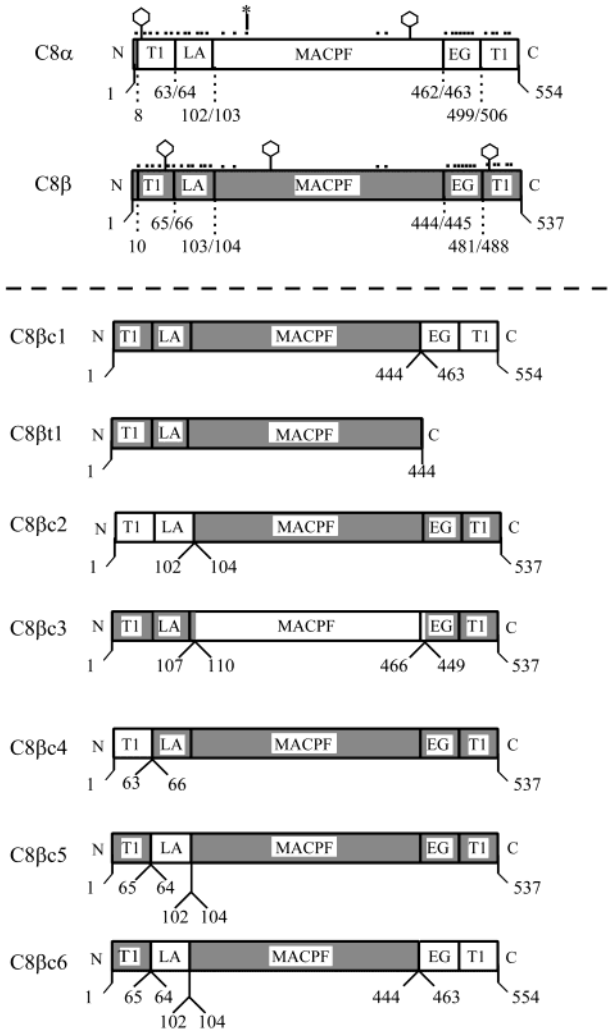

FIGURE 1: Chimeric and truncated forms of C8β. Upper panel: Full-length C8α and C8β. Modules are designated as recommended (*16*) TSP1 (T1), LDLRA (LA), and EGF (EG). Residue numbers identify module boundaries as defined in the SWISS-PROT Protein Sequence Data Bank. Cys residues are indicated as dots above each map. All are conserved and all form internal disulfide bonds except Cys^{164} (*), which is normally linked to C8γ. Hexagons identify potential N-glycosylation sites. Lower panel: Chimeric and truncated C8β. Residues are numbered in accordance with their location in full-length C8α or C8β. Chimeric junctions in C8βc3 correspond to conserved Cys and therefore differ slightly from the module boundaries (*12*).

C8α-γ for 30 min at 25 °C, serially diluted in isotonic buffer (5 mM imidazole, 72.7 mM NaCl, 0.15 mM $CaCl_2$, 0.5 mM $MgCl_2$, 2.5% glucose, 0.05% gelatin, 1 mg/mL BSA, pH 7.4), and assayed for hemolytic activity toward sheep EAC1−7 as described previously (*15*).

RESULTS

C8β constructs used to identify segments that confer specificity toward C8α are described in Figure 1. Each was expressed and secreted when transfected into COS-7 cells (Figure 2). Epitopes from each subunit vary among the chimeric constructs; therefore, immunoblots were developed with a mixture of C8α-γ and C8β antibodies to achieve maximum signal intensity. Immunoblots probed separately with each antibody confirmed the presence of the appropriate segment of each subunit, although the signal was predictably weak in some cases.

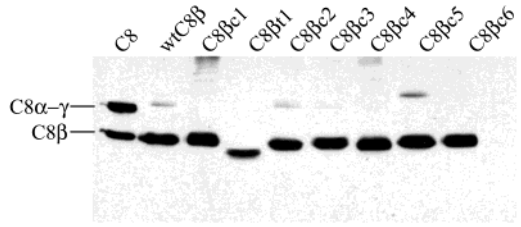

FIGURE 2: Expression of recombinant forms of C8β. COS-7 cells were transfected with wt C8β, chimeric C8β, or a truncated form of C8β. Expression media were harvested and subjected to SDS−PAGE under nonreducing conditions. Immunoblotting was performed using a mixture of pRAHC8α-γ and pRAHC8β antiserum. Control medium from nontransfected cells produced no detectable signal. The location of C8α-γ and C8β in a human C8 standard is indicated.

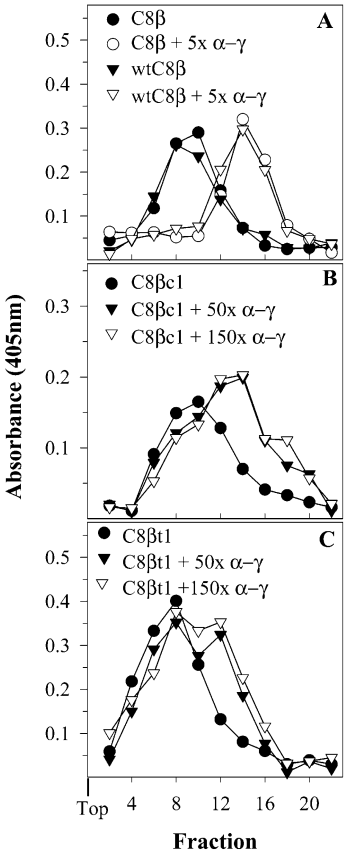

FIGURE 3: Role of the C8β C-terminal modules in binding C8α-γ. The indicated molar excess of C8α-γ (x) was added to expression media containing either wt C8β or the C-terminal mutant forms of C8β (C8βc1 and C8βt1) and analyzed for binding by sucrose density gradient centrifugation. The sedimentation position of each construct was identified by ELISA as described in the text. Controls prepared with media from nontransfected cells or with C8α-γ alone produced no detectable signal when fractionated and assayed. Results are representative of those obtained with media from three different expressions. Panel A: Control experiments comparing C8α-γ binding to purified C8β and wt C8β. Panels B and C show corresponding results for C8βc1 and C8βt1, respectively.

The requirement for the C-terminal modules in binding C8α-γ was examined in experiments shown in Figure 3. Constructs in which the C8β C-terminal modules were exchanged with the corresponding pair in C8α (C8βc1), and a truncated form in which the modules were deleted (C8βt1), were assayed for their ability to bind C8α-γ in solution. Formation of a complex with C8α-γ was determined by density gradient centrifugation followed by an ELISA to

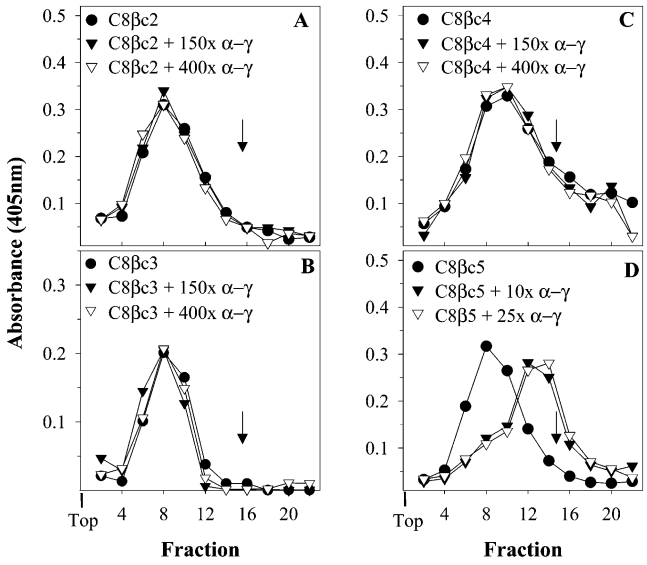

FIGURE 4: Role of the C8β N-terminal modules and MACPF domain in binding C8α-γ. Experiments similar to those in Figure 3 were performed using C8β constructs in which the N-terminal module pair (A), the MACPF domain (B), or the individual N-terminal modules (C and D) were exchanged for those in C8α. Arrows identify the sedimentation position of a C8 marker formed with wt C8β and C8α-γ.

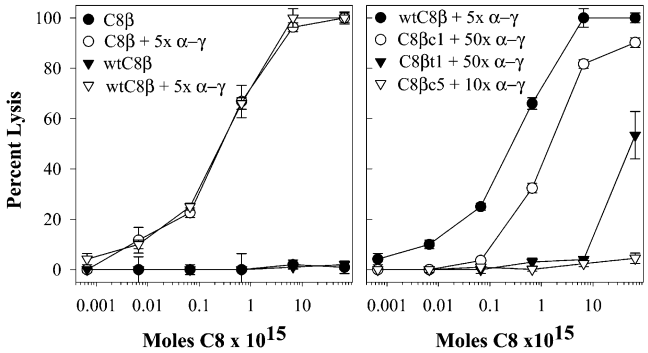

FIGURE 5: Hemolytic activity of recombinant forms of C8β. Left: Control experiment in which excess C8α-γ was added to purified C8β or wt C8β and assayed for hemolytic activity. Right: C8β constructs with the capacity to bind C8α-γ were assayed for hemolytic activity after adding excess C8α-γ. For all assays, moles of C8 are based on each form of C8β as the limiting reagent. Controls containing expression media or C8α-γ alone exhibited no measurable activity. Error bars show the deviation from an average of three different experiments.

locate the sedimentation position of each C8β construct. Although binding constants have not been determined, previous studies indicate that purified C8β has a high affinity for C8α-γ. Formation of a complex at physiological ionic strength requires only low concentrations (<0.1 μg/mL) and low molar ratios of subunit (~1:2) (*15*, *17*). As expected, control experiments in Figure 3 show that binding to purified C8β or wt C8β is complete with only a 5-fold excess of C8α-γ (panel A). When corresponding experiments were performed with C8βc1, complex formation was also observed but at a higher molar excess of C8α-γ (50-fold) (panel B). Similar results were obtained with the respective truncate C8βt1 (panel C). For both C8βc1 and C8βt1, binding appeared incomplete as indicated by the presence of residual monomer at excesses as high as 150-fold C8α-γ. Observing the same amount of residual monomer at 50- and 150-fold excess suggests that this is a population of misfolded construct that is incapable of binding C8α-γ. Nevertheless, a significant percentage of both constructs has the ability to bind C8α-γ, indicating that the C8β C-terminal module pair is not required for binding.

To investigate whether specificity toward C8α-γ is determined solely by the N-terminal module pair or the MACPF domain in C8β, constructs were prepared in which these segments were exchanged for the corresponding segments in C8α. Results in Figure 4 show that substituting either the N-terminal module pair (panel A) or the MACPF domain (panel B) abrogates binding to C8α-γ. No evidence of complex formation was observed at C8α-γ levels as high as 400-fold excess. This suggests that both the N-terminal modules and MACPF domain have essential roles in binding C8α-γ.

To investigate the contribution of each N-terminal module, C8β was modified by separately exchanging the TSP1 or LDLRA module for the corresponding modules in C8α and assaying for binding activity. Results in Figure 4 show that substituting the TSP1 module abolishes binding activity (panel C) whereas substituting the LDLRA module has little effect on the affinity for C8α-γ (panel D). Complete binding to the latter construct required only a 10-fold molar excess of C8α-γ, an amount comparable to the 5-fold excess required for C8β or wt C8β. Thus, the LDLRA module appears to make only a minor contribution to specificity and binding affinity.

Constructs capable of binding C8α-γ were also assayed for their ability to express C8 hemolytic activity. Hemolytic activity is dependent on the ability of C8 to bind to C5b-7, a precursor of the MAC which is assembled from C5b, C6, and C7 on erythrocytes and serves as a receptor for C8. Within C8, the C8β subunit is recognized by C5b-7 and mediates C8 incorporation into the MAC. The C8α subunit is necessary for normal hemolytic activity because it facilitates C9 binding and formation of a functional MAC on the cell membrane. Results from hemolytic assays are shown in Figure 5. Control experiments show that wt C8β has hemolytic activity that is comparable to that of purified C8β. The chimeric construct C8βc1 also exhibits significant hemolytic activity. When compared at 50% lysis, this construct has ~20% of the activity of wt C8β. The corresponding truncate C8βt1 is also active when combined with C8α-γ. The activity is comparatively low (~1−2%) but significant relative to controls. Incubation of either construct with higher amounts of C8α-γ (150-fold) did not result in increased activity (not shown). The lower activities relative to wt C8β are likely due in part to the population of construct which does not bind C8α-γ. Interestingly, construct C8βc5, which has near-normal affinity for C8α-γ, had no measurable hemolytic activity, even when assayed with a 25-fold excess of C8α-γ. This suggests that, within C8, the LDLRA module of C8β is essential for binding C5b-7.

DISCUSSION

This study extends our understanding of the role of the modules in mediating interactions between the MAC proteins. Results show that C8β specificity toward C8α is not determined by any single module or the MACPF domain. Recognition and binding primarily involve residues located

within the TSP1 + LDLRA + MACPF segment. Binding was not observed with chimera C8βc2, which lacks the N-terminal modules but contains the remaining portion of C8β. Similar results were obtained with C8βc3, which contains the modules but lacks the MACPF domain. This indicates that neither the N-terminal module pair nor the MACPF domain alone has the capacity to form a stable complex with C8α-γ.

Results for chimeras in which the individual N-terminal modules were exchanged (C8βc4 and C8βc5) suggest that the N-terminal TSP1 module and MACPF domain act cooperatively to confer binding specificity. Substitution of the TSP1 module abolishes binding to C8α-γ whereas substitution of the LDLRA module has little effect (Figure 4). This suggests that the LDLRA module is not directly involved in binding. Alternatively, homology between the LDLRA modules in C8α and C8β may be sufficient to allow either to function effectively in a C8β framework. This seems unlikely since they exhibit only 53% sequence identity and 58% similarity.

To determine if the C8β N-terminal TSP1 module and MACPF domain together are sufficient to bind C8α-γ, a chimera (C8βc6) containing only these segments of C8β was prepared and expressed (Figures 1 and 2). In experiments not shown, this construct exhibited neither binding nor hemolytic activity when incubated with C8α-γ excesses as high as 400-fold. Although not essential for recognition, other portions of the C8β structure apparently enhance binding affinity and contribute to the stability of the complex. In this respect, the C-terminal modules appear to be more important than the LDLRA module. Exchanging or deleting the C-terminal modules (C8βc1 and C8βt1) had a greater effect on binding than exchanging the LDLRA module (C8βc5). It should also be noted that a negative result with C8βc6 suggests that the binding seen with C8βc5 is not due to structural homology between the C8α and C8β LDLRA modules. If this were so, then one would expect C8βc6 to exhibit binding characteristics and activity similar to those of C8βc1.

The requirement for cooperative interaction between the C8β N-terminal TSP1 module and MACPF domain in forming a binding site for C8α is in marked contrast to what is known about binding sites in C8α (*12*, *18*). The ability to independently express the C8α MACPF segment is compelling evidence that it is a self-folding domain within C8α. This domain retains the ability to associate intracellularly with C8γ and form a disulfide-linked heterodimer that has substantial affinity for C8β. Thus, in C8α the MACPF domain alone confers specificity toward both C8β and C8γ whereas the principal function of the modules is to stabilize the complex.

Results from activity assays suggest that the N-terminal modules and MACPF domain of C8β are also important for binding C5b-7. Of the constructs capable of binding C8α-γ, only chimera C8βc1 and to a lesser extent truncate C8βt1 were hemolytically active. Chimera C8βc5, which lacks the LDLRA module but has near-normal affinity for C8α-γ, was not active. This module may have a direct role in binding C5b-7, or alternatively, C8 formed with this construct may be incapable of binding C9 to produce a functional MAC. The latter possibility seems unlikely since C8α provides the requisite binding site for C9 within the MAC.

In summary, we conclude that specificity of C8β toward C8α is determined by a cooperative interaction between the N-terminal TSP1 module and the MACPF domain. These two segments appear to form a specific binding site that mediates the initial interaction with C8α; however, these segments alone are not sufficient for stable complex formation. While other portions of C8β, and the C-terminal modules in particular, are not essential for binding, they clearly contribute to stability, presumably through secondary interactions. Our findings also suggest that determinants of specificity within the MAC protein family are not likely to consist of a single, small segment of primary structure but more likely involve interactions between distant regions of each protein. Furthermore, the observation that the C8α MACPF domain alone has a binding site for C8β suggests that structural determinants of specificity may vary among family members and not necessarily involve the modules.

ACKNOWLEDGMENT

The authors thank Drs. Elizabeth Summer and Robert J. Dunlap for their assistance with preparing the C8β constructs.

REFERENCES

1. Müller-Eberhard, H. J. (1988) Molecular organization and function of the complement system, *Annu. Rev. Biochem. 57*, 321−347.
2. Esser, A. F. (1994) The membrane attack complex of complement: assembly, structure and cytotoxic activity, *Toxicology 87*, 229−247.
3. Steckel, E. W., York, R. G., Monahan, J. B., and Sodetz, J. M. (1980) The eighth component of human complement: purification and physiochemical characterization of its unusual subunit structure, *J. Biol. Chem. 255*, 11997−12005.
4. Ng, S. C., Rao, A. G., Howard, O. M., and Sodetz, J. M. (1987) The eighth component of human complement (C8): evidence that it is an oligomeric serum protein assembled from products of three different genes, *Biochemistry 26*, 5229−5233.
5. Hobart, M. J., Fernie, B. A., and DiScipio, R. G. (1995) Structure of the human C7 gene and comparison with the C6, C8A, C8B, and C9 genes, *J. Immunol. 154*, 5188−5194.
6. Plumb, M. E., and Sodetz, J. M. (1998) Proteins of the membrane attack complex, in *The Human Complement System in Health and Disease* (Volanakis, J. E., and Frank, M. M., Eds.) pp 119−148, Marcel Dekker, New York.
7. Flower, D. R. (1996) The lipocalin protein family: structure and function, *Biochem. J. 318*, 1−14.
8. Flower, D. R., North, C. T., and Sansom, C. E. (2000) The lipocalin protein family: structural and sequence overview, *Biochim. Biophys. Acta 1482*, 9−24.
9. Schreck, S. F., Parker, C., Plumb, M. E., and Sodetz, J. M. (2000) Human complement protein C8γ, *Biochim. Biophys. Acta 1482*, 199−208.
10. Ortlund, E., Parker, C. L., Schreck, S. F., Ginell, S., Minor, W., Sodetz, J. M., and Lebioda, L. (2002) Crystal structure of human complement protein C8γ at 1.2 Å resolution reveals a lipocalin fold and a distinct ligand binding site, *Biochemistry 41*, 7030−7037.
11. Brickner, A., and Sodetz, J. M. (1985) Functional domains of the α-subunit of the eighth component of human complement: identification and characterization of a distinct binding site for the γ chain, *Biochemistry 24*, 4603−4607.
12. Plumb, M. E., Scibek, J. J., Barber, T. D., Dunlap, R. J., Platteborze, P. L., and Sodetz, J. M. (1999) Chimeric and truncated forms of human complement protein C8α reveal binding sites for C8β and C8γ within the membrane attack complex/perforin region, *Biochemistry 38*, 8478−8484.

13. Esser, A. F., and Sodetz, J. M. (1988) Membrane attack complex proteins C5b-6, C7, C8, and C9 of human complement, *Methods Enzymol. 162*, 551−578.
14. Rao, A. G., and Sodetz, J. M. (1984) Purification of functional subunits of the eighth component of human complement (C8) under nondenaturing conditions, *Complement 1*, 182−186.
15. Schreck, S. F., Plumb, M. E., Platteborze, P. L., Kaufman, K. M., Michelotti, G. M., Letson, C. S., and Sodetz, J. M. (1998) Expression and characterization of recombinant subunits of human complement component C8: further analysis of the function of C8α and C8γ, *J. Immunol. 161*, 311−318.
16. Bork, P., and Bairoch, A. (1995) Extracellular protein modules, *Trends Biochem. Sci. 20* (Suppl.), C03.
17. Monahan, J. B., and Sodetz, J. M. (1980) Binding of the eighth component of human complement to the soluble cytolytic complex is mediated by its β subunit, *J. Biol. Chem. 255*, 10579−10582.
18. Plumb, M. E., and Sodetz, J. M. (2000) An indel within the C8α subunit of human complement C8 mediates intracellular binding of C8γ and formation of C8α-γ, *Biochemistry 39*, 13078−13083.

BI026207H