Identity of the Residues Responsible for the Species-restricted Complement Inhibitory Function of Human CD59

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The membrane-anchored glycoprotein CD59 inhibits assembly of the C5b-9 membrane attack complex (MAC) of human complement. This inhibitory function of CD59 is markedly selective for MAC assembled from human complement components C8 and C9, and CD59 shows little inhibitory function toward MAC assembled from rabbit and many other non-primate species. We have used this species selectivity of CD59 to identify the residues regulating its complement inhibitory function: cDNA of rabbit CD59 was cloned and used to express human/rabbit CD59 chimeras in murine SV-T2 cells. Plasma membrane expression of each CD59 chimera was quantified by use of a 5'-TAG peptide epitope, and each construct was tested for its ability to inhibit assembly of functional MAC from human versus rabbit C8 and C9. These experiments revealed that the species selectivity of CD59 is entirely determined by sequence contained between residues 42 and 58 of the human CD59 polypeptide, whereas chimeric substitution outside this peptide segment has little effect on the MAC inhibitory function of CD59. Substitution of human CD59 residues 42–58 into rabbit CD59 resulted in a molecule that was functionally indistinguishable from native human CD59, whereas the complementary construct (corresponding residues of rabbit CD59 substituted into human CD59) was functionally indistinguishable from rabbit CD59. Based on the solved solution structure of CD59, these data suggest that selectivity for human C8 and C9 resides in a cluster of closely spaced side chains on the surface of CD59 contributed by His44, Asn48, Asp49, Thr51, Thr52, Arg53, and Gln58 of the polypeptide.

CD59 is a 77-residue glycosylphosphatidylinositol (GPI)-anchored plasma membrane glycoprotein that serves to protect human blood and vascular cells from injury by the C5b-9 components of the complement system present in plasma (1–6). The complement inhibitory function of CD59 resides in its capacity to interrupt formation of lytic pores in the plasma membrane by binding to segments of the C8α and C9 polypeptides that become exposed during their assembly into the complement C5b-9 membrane attack complex (MAC) (7–13). The amino acid sequence and solution structure of CD59 conform to those of the Ly6/cardiotxin protein superfamily in which five internal disulfide bonds stabilize the polypeptide into a discoid structure consisting of a three-stranded β-sheet apposed to a two-stranded β-finger (14–17). In CD59, the GPI anchor is attached to the C-terminal Asn77, and Asn18 provides a site of N-linked glycosylation (14, 15, 18). Whereas the carbohydrate attached to Asn18 occludes most of one face of the discoid surface of the protein, these glycosidic residues do not contribute to the MAC inhibitory properties of CD59 (14, 15, 18–20). This suggests that amino acid side chains exposed on the non-glycosylated face of the protein provide this function.

The complement inhibitory function of CD59 exhibits marked selectivity for MAC assembled from C8 and C9 of human or primate origin, and CD59 shows little inhibitory function toward these same complement components of rabbit and several other non-primate species (8, 10, 13, 21–23). Conversely, whereas rabbit erythrocytes are well protected from lysis by rabbit C5b-9, they are exquisitely sensitive to the lytic action of C5b-9 assembled from human C8 or C9 (24, 25). This implies that the CD59 homologue expressed on the surface of rabbit cells is also selective for homologous complement and cannot effectively interact with the human C8 and C9 components to inhibit MAC. We have previously taken advantage of the species selectivity inherent to the interaction of CD59 with C8 and C9 to map the peptide-binding sites within the human C8 and C9 proteins that are recognized by human CD59 (7, 8, 26). In those studies, the capacity of human CD59 to inhibit MAC assembly was measured using recombinant human/rabbit chimeras of C8 and C9 in order to identify the residues within the C8α and C9 polypeptides that are specifically required to confer recognition of these complement components by human CD59. Using a similar strategy of functional mapping in human/rabbit CD59 chimeras expressed on the surface of transfected cell lines, we now report the identity of the residues of plasma membrane CD59 that confer its capacity to selectively inhibit the pore-forming activity of human MAC.

EXPERIMENTAL PROCEDURES

Materials—Rabbit whole blood and chicken whole blood in acid citrate dextrose were from Cocalico Biologics, Inc. (Reamstown, PA). Human serum, rabbit serum, human serum depleted of complement protein C8 (C8D), human complement proteins (C5b67, C8, and C9), and rabbit complement proteins (C8 and C9) were purified and assayed as described previously (8, 10, 27, 28). CHAPS, phenylmethylsulfonyl fluoride, dimethyl sulfoxide, and bovine serum albumin were from Sigma. Nonidet P-40 and Triton X-100 were from CalBiochem. Tween 20 was from Fisher. The TA cloning kit, the mRNA purification kit, Escherichia coli strain TOP10, and pcDNA3 vectors were obtained from Invitrogen (San Diego, CA). All restriction endonucleases were from New England Biolabs Inc. (Beverly, MA). T4 ligase, Hank’s balanced salt solution, Dulbecco’s modified Eagle’s medium, and DNA primers were purchased from or synthesized by Life Technologies, Inc. The Wizard DNA purification kit was from Promega (Madison, WI). Advanced KlenTaq enzymes, the cDNA library construction kit, and the Marathon racing kit...
were from CLONTECH (Palo Alto, CA). The Sequenase Version 2.0 kit was from U. S. Biochemical Corp. The SV-T2 cell line (ATCC 163.7) was obtained from American Type Culture Collection (Rockville, MD). BCECF/FAM dye was purchased from Molecular Probes, Inc. (Eugene, OR). Fetal bovine serum, calf serum, cell dissociation buffer, L-glutamin, nonessential amino acids, phytohemagglutinin, trypan blue, and the Transduction Labs FACS buffer FACS were from CLONTECH (Palo Alto, CA). Silver stain, Coomassie Blue, and BCA protein assay reagents were from Pierce. All other chemicals were reagent grade or better.

Isolation of Rabbit CD59—Rabbit erythrocyte ghost membranes were prepared as described previously for human CD59 purification and suspended to a final volume representing 1.5 times the original volume of packed erythrocytes (4). The ghost suspension was brought to 150 mM NaCl, 1 mM phenylmethylsulfonfyl fluoride, and 1-butanol added slowly to 20% (v/v). Following stirring (3 h at 4 °C) and centrifugation (10,000 × g, 30 min at 4 °C), the butanol-saturated aqueous phase was collected, and CHAPS was added to a final concentration of 0.1% (v/v) and dialyzed against 20 mM Tris (pH 7.4). The dialyzed extract was applied to a 2.5 × 10-cm DEAE-Sepharose Fast Flow column (Sigma) equilibrated in the same buffer and eluted with 500 mM of a linear NaCl gradient (40–400 mM). Fractions were tested for MAC inhibitory function using chicken erythrocyte target cells as described previously, substituting rabbit C8 and C9 for human C8 and C9 (10). Fractions containing rabbit MAC inhibitory activity were pooled, and NaCl was added to final concentration of 300 mM. The pool was applied to a 1.6 × 8-cm phenyl-Sepharose column (Sigma) equilibrated with 300 mM NaCl, 0.05% CHAPS, and 20 mM Tris (pH 8.0). Following washing with the same solution, protein was eluted with a linear gradient representing 0.05–1% CHAPS and 300 to 0 mM NaCl in 20 mM Tris (pH 8.0). The active fractions were pooled and further purified on a 0.5 × 5-cm Mono Q HR column (Pharmacia Biotech Inc., Uppsala, Sweden) using a gradient of 0–400 mM NaCl in 0.5% CHAPS and 20 mM Tris (pH 7.4). Active fractions were concentrated by step elution on a Mono Q column and further purified by SDS-polyacrylamide gel electrophoresis using a 10% NuPAGE gel (Novex, San Diego, CA) run under nonreducing conditions. The protein band at 20 kDa (~8 μg of total protein from the original 300 ml of packed rabbit erythrocytes) was eluted from the gel slice into 0.1% CHAPS and 20 mM Tris (pH 7.4), and the inhibitory activity of the eluted protein toward rabbit MAC was confirmed by functional assays. Fusion chromatography procedures were performed at room temperature on a BioCAD 20 perfusion chromatography workstation (PerSeptive Biosystems, Framingham, MA). N-terminal sequence was then obtained through 40 cycles of Edman degradation (Protein and Carbohydrate Structure Facility, University of Michigan, Ann Arbor, MI), yielding SLMCYHC-LLISIPNCSTVTNCTPNIHDACLTVSGPRYVRC. CD59 cDNA—DNA oligonucleotides were constructed based on protein sequence and used to amplify a rabbit lymphocyte cDNA library (5′-RACE, Marathon kit) from which a 200-base pair PCR product was obtained. Specific primers based on this 200-base pair cDNA clone were designed and used to amplify the rabbit lymphocyte cDNA library by 3′-RACE. Full-length cDNA of rabbit CD59 was obtained by linking the PCR products from 5′- and 3′-RACE using PCR. The sequence of this cDNA clone was deposited in GenBank™ (accession number AF040387), and the deduced amino acid sequence of the predicted open reading frame is shown in Fig. 1. The predicted translation product consists of 124 residues, including a 24-residue signal peptide before the N-terminal Ser of the mature protein. The unusual N-terminal Ser of the mature rabbit CD59 protein was confirmed at both the protein and DNA levels (see Fig. 1). Construction of Plasmids Encoding Human/Rabbit CD59 Chimeras—The 467-base pair insert encoding human CD59 was released from pUC18 using EcoRI restriction sites and subcloned into the EcoRI site in the pcDNA3 expression vector. The vector with correct CD59 cDNA orientation was selected and used as template for PCR. A 33-base pair oligonucleotide (corresponding to the TAG peptide sequence from 5′-SEEDLN) was added between the leading sequence of CD59 and the N-terminal amino acid residue (Leu1) of the mature protein using PCR. Rabbit TAG-CD59 in the pcDNA3 vector was made by replacing the sequence in human TAG-CD59 pcDNA3 with the sequence encoding mature rabbit CD59 and the rabbit CD59 C-terminal signal using HindIII and XbaI sites in pcDNA3. cDNAs encoding the human/rabbit chimeric CD59 constructs depicted in Fig. 2 (panels I–VI) were prepared using PCR amplification by procedures previously described (29).

The pcDNA3 plasmids containing human or rabbit CD59 sequence were used as template to generate the cDNA encoding chimeric CD59 proteins. The chimeric cDNA was then inserted into the pcDNA3 vector using HindIII and XbaI sites. Human, rabbit, and human/rabbit chimeric CD59 constructs were used to transfected E. coli strain TOP10. Constructs from independent colonies were sequenced in their entirety in both directions by automated DNA sequencing (Applied Biosystems, Inc.) or by dyeoxy sequencing using the Sequenase Version 2.0 kit. Plasmids containing the desired constructs without nucleotide error were selected and amplified for expression in the SV-T2 cell line.

Expression of CD59 Constructs in SV-T2 Cells—SV-T2 cells were transfected with human, rabbit, or chimeric TAG-CD59 pcDNA3 electroporated as described previously (29). After 48 h, stable transfecants were selected with Dulbecco’s modified Eagle’s medium containing 1 mg/ml Geneticin for 10 days. If episomal replication in the transfected cells was desired, 8 × 10⁶ SV-T2 cells were transfected with 120 μg of plasmid DNA by electroporation using Gene Pulse (Bio-Rad) at 360 V and 500 microfarads (29). Geneticin-selected cells were stained with murine monoclonal antibody 9E10 against the TAG epitope followed by fluorescein isothiocyanate-conjugated goat anti-mouse IgG and sorted by flow cytometry (FACStar Plus, Becton Dickinson). Individual clones were then obtained by limiting dilution in Dulbecco’s modified Eagle’s medium containing 0.5 mg/ml Geneticin. Comparison was made to normal cell lines derived by transfection with pcDNA3 lacking insert (vector-only controls).

Measurement of Cell-surface TAG-CD59—The cell-surface expression of each TAG-CD59 construct in transfected SV-T2 cells was quantified by the binding at saturation of murine monoclonal antibody 9E10 (against the TAG epitope) as described previously (29). Following growth to near confluence, cells were detached and incubated for 30 min at 23 °C with murine monoclonal antibody 9E10 (100 μg/ml) in Hank’s balanced salt solution containing 1% bovine serum albumin. After washing with Hank’s balanced salt solution containing 1% bovine serum albumin, the cells were incubated (20 min at 23 °C) with fluorescein isothiocyanate-conjugated goat anti-mouse IgG at a final concentration of 10 μg/ml. The fluorescence was determined by flow cytometry (FACScan, Becton Dickinson) as described previously (29). Assay of MAC Inhibitory Function—The complement inhibitory activity of recombinant CD59 expressed on the transfected SV-T2 cells was evaluated by a minor modification of methods previously described (29, 30). For cell clones expressing each chimeric CD59 construct, human C5b67 was deposited on the plasma membrane, and susceptibility to the lytic activity of either human C8 and C9 or rabbit C8 and C9 was measured and compared with identically treated clones expressing wild-type human or rabbit CD59. Briefly, SV-T2 cells grown to 80% confluence were washed and loaded with BCECF/FAM dye. C5b67 complexes were deposited on the cells using 40% human C8D serum as complement source. After two washes, the C5b67 cells were incubated in serum-free medium either with 2 μg/ml human C8 and 5 μg/ml human C9 or with 2 μg/ml rabbit C8 and 5 μg/ml rabbit C9. MAC-mediated cell lysis was determined from the measured release of BCECF dye from the cytoplasm, with correction for nonspecific dye leakage from matched controls omitting C8 and C9, as described previously (29). Under these conditions, MAC-mediated lysis of the vector-only SV-T2 controls not expressing recombinant CD59 ranged from 75 to 90%.

RESULTS

Cloning of Rabbit CD59—The predicted translation product of cDNA encoding rabbit CD59 consists of 124 residues, including a 24-residue signal peptide, a predicted GPI attachment site, and a 23-residue signal peptide including a transmembrane domain C-terminal to the predicted transamidase cut site (Fig. 1) (31, 32). N-terminal sequencing of protein purified from rabbit erythrocytes and analysis of the signal peptide sequence at the transamidase cleavage site of the transamidase domain C-terminal to the predicted transamidase cut site from the mouse CD59 cDNA indicate an additional Ser residue before the highly conserved N-terminal Leu3 found in all other CD59 homologues that have been sequenced to date. Therefore, to simplify discussion of the aligned residues of human and rabbit CD59 in various chimeric constructs, we have renumbered residues of the mature rabbit CD59 polypeptide commencing with N-Ser⁰-Leu¹-Met²-Cys³, etc. (Fig. 1). All references to amino
acids in rabbit C59 are based on this renumbering of residues in the mature polypeptide. Whereas Gly76 is a predicted transamidase cut site for GPI attachment, the possibility of cleavage at another residue (e.g. Asp174) cannot be excluded.

Species-Selective Activity of Human and Rabbit CD59—SV-T2 cell lines expressing various levels of cell-surface CD59 (human or rabbit) were produced through stable transfection with the TAG-CD59 pcDNA3 plasmid. Each cell line was then tested for its capacity to resist lysis by C5b-9. As has been previously described, cells transfected to express human CD59 were nearly completely protected from lysis by human C5b-9, and this protective effect of human CD59 was not observed when rabbit C8 and C9 substituted for human C8 and C9 in the C5b-9 complex (plotted curves in Fig. 2, panels I–VI). On the other hand, rabbit CD59 expressed on the surface of this murine cell line conferred a selective resistance to lysis by C5b-9 assembled from rabbit C8 and C9, whereas we observed virtually no inhibition of the lytic action of MAC when human C5b-9 components were used. These data confirm that recombinant rabbit CD59 shows the same homologous species-selective complement inhibitory function as was inferred from the differential susceptibility of rabbit erythrocytes to lysis by human versus rabbit complement (24, 25). As previously noted for the human TAG-CD59 construct (29), these data also suggest that the rabbit TAG-CD59 fusion protein retains the properties of native rabbit CD59 that is expressed in the rabbit erythrocyte membrane.

Human/Rabbit CD59 Chimeras—To probe which residues of human CD59 conferred its ability to selectively inhibit lysis by human C5b-9, we next constructed chimeric proteins in which segments of the rabbit and human CD59 polypeptides were interchanged. Our choice of constructs reflected (i) identity of amino acid residues exposed on the surface of human CD59 that were not conserved in the aligned polypeptide sequence of rabbit CD59 (selection was based on the reported solution structure of the glycosylated protein, utilizing surface residues considered not to be occluded by the N-linked carbohydrate (1, 14, 15)); (ii) an attempt to group these various nonconserved amino acid side chains into contiguous clustered spatial arrays; and (iii) a consideration of prior data relating to potential identity of the active-site residues in human CD59 as deduced from peptide studies, site-directed mutagenesis in the protein, or analysis of other CD59 chimeras (see “Discussion”) (20, 21, 29, 33–35). Based upon these considerations, we chose to analyze six human/rabbit CD59 chimeras (and the six complementary rabbit/human CD59 chimeras) constructed so as to replace the surface-exposed side chains contributed by (i) residues 8, 10, 12, and 14 (chimeras Ch1 and Ch1R; Fig. 2, panel I); (ii) residues 5, 37, and 38 (chimeras Ch2 and Ch2R; panel II); (iii) residues 20–22 and 41 (chimeras Ch3 and Ch3R; panel III); (iv) residues 60 and 62 (chimeras Ch4 and Ch4R; panel IV); (v) residues 29, 30, 60, and 62 (chimeras Ch5 and Ch5R; panel V); or (vi) residues 44, 48, 49, 51, 52, 55, and 58 (chimeras Ch6 and Ch6R; panel VI).

Individual clones expressing each of these recombinant proteins were obtained, and the expression level on the cell surface was determined from the N-terminal TAG epitope common to each construct. In each case, two separate clones were expanded for independent assay of MAC inhibitory function.

MAC Inhibitory Function of Recombinant CD59 Chimeras—SV-T2 clones expressing the chimeric constructs were analyzed for their capacity to restrict lysis mediated by MAC. The species selectivity of the complement inhibitory function of each construct was tested using human versus rabbit C8 and C9 to assemble the C5b-9 complex. In each case, results for the chimeric constructs were compared with those obtained for transfected SV-T2 cells expressing full-length CD59 (human or rabbit) and for vector-transfected SV-T2 cells lacking the CD59 insert (Fig. 2, panels I–VI). As the data of this figure reveal, the species-selective inhibitory function of either human CD59 or rabbit CD59 was unaffected by the amino acid substitutions contained in chimeras Ch1/Ch1R, Ch2/Ch2R, Ch4/Ch4R, or Ch5/Ch5R (Fig. 2, panels I, II, IV, and V). This implies that residues 1–19, 29, 30, 37, 38, 60, and 62 of the CD59 polypeptide do not directly contribute to its selective avidity for homologous C8 and C9. The results we obtained for the Ch1/Ch1R chimeras are consistent with recent observations made with human CD59/Ly6E chimeras, which had suggested that the N-terminal residues of the human CD59 polypeptide do not contribute to its MAC inhibitory function (see “Discussion” (33)).

By contrast to the results above, substitution of human CD59 residues 42–58 into rabbit CD59 resulted in a protein (chimera Ch6) that was functionally indistinguishable from native human CD59, whereas the complementary construct (rabbit CD59 residues 42–58 substituted into human CD59; chimera Ch6R) was functionally indistinguishable from rabbit CD59 (Fig. 2, panel VI). These data imply that the amino acid side chains contributed by residues contained between Lys12 and Glu58 in human CD59 are responsible for the selective avidity of this complement inhibitor for human MAC (see “Discussion”). In the case of chimeras Ch3 and Ch3R (Fig. 2, panel III),
Fig. 2. MAC inhibitory function of recombinant CD59 containing human/rabbit chimeric substitutions. Human CD59 (open circles), rabbit CD59 (open triangles), and the human/rabbit chimeric CD59 constructs (Ch1–Ch6 and Ch1R–Ch6R; closed symbols) were each expressed...
a partial loss of the complement inhibitory function of CD59 was observed. Replacement of residues 20–22 and 41 in rabbit CD59 with the corresponding residues from human CD59 reduced the inhibitory activity of rabbit CD59 toward MAC assembled with rabbit C8/C9, but this substitution did not confer upon rabbit CD59 the capacity to inhibit human MAC. Similarly, substitution of human CD59 residues 20–22 and 41 with the corresponding residues from rabbit CD59 reduced the inhibitory activity of human CD59 toward human MAC, but this substitution did not confer upon human CD59 the capacity to inhibit MAC assembled with rabbit C8/C9.

**DISCUSSION**

Our data suggest that residues 42–58 of human CD59 contain the segment of the protein that is responsible for its species-restricted MAC inhibitory function. As shown in Fig. 2 (panel VI), substitution of human CD59 residues 42–58 into rabbit CD59 resulted in a protein that was functionally indistinguishable from human CD59, whereas the complementary construct (rabbit CD59 residues 42–58 substituted into human CD59) was functionally indistinguishable from rabbit CD59. Within this portion of the polypeptide, residues 43, 45, 46, 47, 53, 54, 56, and 57 are identically conserved between human and rabbit. From the solved solution structure of human CD59, the side chains of residues 42 and 50 are buried. This implies that the residues of human CD59 that dictate its selective ability to bind to human C8 and C9 are localized to a cluster of amino acid side chains exposed on the surface of the protein that are contributed by His44, Asn48, Asp49, Thr51, Thr52, Arg55, and Glu58. In addition to these residues, data for Ch3/Ch3R (chimeric substitution of residues at positions 20–22 and 41) imply that the side chains of one or more of these residues can also influence the species selectivity of human CD59 (Fig. 2, panel III). Among these residues, it is noteworthy that the side chain of Lys41 (replaced by Arg41 in rabbit CD59) projects in close proximity to the side chain of His44 (replaced by Asp44 in rabbit CD59), a residue contained within the functionally dominant segment identified by the Ch6/Ch6R chimeras (cf. Fig. 2, panels III and VI). The relatively conservative Lys → Arg substitution in SV-T2 cells. Clonal cell lines were obtained, and the level of expression of each protein on the cell surface was determined as detailed under "Experimental Procedures" (absissa). MAC-induced lysis of each cell line was then determined by BCECF dye release assay (ordinate). In each experiment, either human (plot A) or rabbit (plot B) C8 and C9 were used to assemble the C9 complex. Closed triangles indicate results for CD59 chimeras Ch1 (panel I), Ch2 (panel II), Ch3 (panel III), Ch4 (panel IV), Ch5 (panel V), and Ch6 (panel VI). Closed circles indicate results for the corresponding complementary chimeric constructs, Ch1R–Ch6R. Error bars represent the mean ± S.D. (n = 3–6). Data are the cumulative results of experiments performed on separate days. In each panel, the amino acid substitutions made in each chimera are depicted both in a linear presentation of the mature human (Hu; black) and rabbit (Rb; white) CD59 polypeptides and in a space-filling model of human CD59 (Rasmol program) in which only the nonconserved surface residues are highlighted (shaded atoms).
substitution of a side chain located in proximity to the functionally dominant region of the protein may explain why a relatively small loss of activity toward homologous MAC was observed for the Ch3/Ch3R constructs and why this was not accompanied by a comparable gain in MAC inhibitory function toward the heterologous complement proteins.

Our results are generally consistent with data recently reported by Yu et al. (21, 33) relating to the identity of peptide segments within the CD59 polypeptide that contribute to function. These authors noted that MAC inhibitory function was retained in CD59/Ly6E chimeras in which CD59 residues 1–16 and 57–77 were each replaced by the corresponding sequence of murine lymphocyte antigen Ly6E, a structural homologue of CD59 that lacks any complement inhibitory properties (33). These data imply that the active-site residues in CD59 are contained in the central portion of the polypeptide and C9 polypeptides to which CD59 attaches has given rise to a diversity in this binding motif among the various species. Both models assume the same selective pressure to retain the capacity of CD59 to protect homologous cells from injury by complement, through inhibition of MAC. Whereas definitive identification of the residues forming the active site of CD59 awaits a direct demonstration of which side chains of the protein actually contact the C8 and C9 components of MAC, it is of interest to note the following. (i) In the case of both human C8α and C9 polypeptides, the peptide segment within each protein that has been directly shown to provide the specific binding site for attachment of human CD59 to these complement components represents a distinct region within each polypeptide that is most notable for marked divergence in aligned sequence when human C8α and C9 are each compared with its corresponding polypeptide of rabbit and other species (7–9, 26). This suggests that the motif within each of these complement proteins to which CD59 attaches has itself undergone considerable evolutionary drift relative to other portions of the polypeptide, implying a similar evolutionary drift of the complementary binding site in CD59 as would be required to maintain interaction between the proteins. (ii) The residues we now identify in human CD59 to confer its species-selective interaction with human C8α and C9 (i.e. His44, Asn48, Asp49, Thr51, Thr52, Arg55, and Glu56) form a distinct cluster on the non-glycosylated surface of the protein and would presumably be available for a binding function. By contrast, alignment of CD59 homologues for which sequence is now available does not suggest a single motif that is both conserved among all species and also available on the surface of the protein: of those residues in the human CD59 polypeptide that are conserved among the other species (Fig. 3), only Tyr4, Ser20, Asp24, Thr29, Glu32, Tyr36, Lys41, Asn46, Tyr61, and Asn70 are identified in the NMR structure to be exposed on the surface, and in general, these residues are widely scattered across the exposed face of the protein (Fig. 4) (1, 14, 15).

Recent evidence suggests that complement inhibitors specifically directed against human MAC have potential clinical use in preventing hyperacute rejection of transplanted organs and in reducing the pathological consequences of complement acti-
vation in various immune and inflammatory diseases (23, 36–40). Identification of the specific protein motif that is responsible for the selective inhibitory action of CD59 toward the pore-forming and cytolytic properties of human MAC promises to permit rational design of small molecules that can mimic the protective effect of this natural cell-surface complement inhibitor.

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