Decay-accelerating factor (CD55), a regulator of the alternative and classical pathways of complement activation, is expressed on all serum-exposed cells. It is used by pathogens, including many entroviruses and uropathogenic Escherichia coli, as a receptor prior to infection. We describe the x-ray structure of a pathogen-binding fragment of human CD55 at 1.7 Å resolution containing two of the three domains required for regulation of human complement. We have used mutagenesis to map biological functions onto the molecule; decay-accelerating activity maps to a single face of the molecule, whereas bacterial and viral pathogens recognize a variety of different sites on CD55.

The innate immune system provides the first line of defense against invading pathogens. A key aspect of this defense is the complement system; this consists of classical, alternative, and lectin pathways that converge, via a well characterized cascade of protein intermediates, to target cells for destruction. Regulators of complement activation (RCA's) proteins, acting at key points in the cascade, have evolved to prevent the pathological consequences of inappropriate activation.

Decay-accelerating factor (CD55) is a glycophsphatidylinositol-anchored member of the RCA protein family that protects autologous cells from complement damage (1–3). CD55 regulates all three pathways by binding and consequently accelerating the decay of the C3 convertases (2), the enzymes that link the three activation pathways and the downstream effects. This central role in regulating complement has led to the development of transgenic animals that express human CD55 as a potential source of organs for xenotransplantation.

A number of additional activities have been associated with CD55, which is the location of the Cr and Tc blood group antigens (4). Recent studies have shown that it is involved in coordinating the innate immune response to bacterial pathogens by enhancing natural killer cell activity (5). Additionally, CD55 binds CD97, a member of the epidermal growth factor (EGF)-TM7 protein family, the expression of which is rapidly up-regulated upon activation of T and B cells (6).

Perhaps as a consequence of its ubiquitous expression, CD55 has been exploited as an attachment molecule by bacterial and viral pathogens (7). The fimbriae of uropathogenic strains of E. coli bind CD55, as do many echoviruses (EV) and other members of the enterovirus genus of the Picornaviridae, a family of small, non-enveloped positive-strand RNA viruses associated with a range of human diseases including meningitis, pneumonia, exanthema, respiratory disease, and acute hemorrhagic conjunctivitis.

CD55, like the related complement control proteins CD46 and CR1, is composed of a series of short consensus repeat (SCR) domains, each of ~60 residues folded into a characteristic β-strand structure held together by two disulfide bridges (8). CD55 has four SCR domains, attached to the cell membrane by a glycosylphosphatidylinositol anchor and separated from it by a heavily O-glycosylated serine/threonine-rich “stalk” (3, 9).

Deletion of individual SCRs and exchange with homologous domains from related proteins has broadly defined the regions of CD55 necessary for the various biological functions. Complement control requires SCR2–4 (10), whereas CD97 binding only involves CR1 (6). Among the CD55-binding enteroviruses, there are clear differences in the SCR domains bound (a few exclusively need CR1 (11, 12)), whereas all the echoviruses require at least paired SCR domains, consisting of SCR3 linked to either SCR2 and/or SCR4 (13).

Understanding the functional biology of CD55 requires a more detailed knowledge of the three-dimensional structure. Since the majority of CD55 interactions involve multiple domains, the relative spatial orientation of each SCR must be critical. This cannot be accurately modeled from the existing structural data base. For this reason, we have determined the structure of the two membrane-proximal SCR domains of CD55 (SCRs 3 and 4; designated CD5534) in a range of different crystal environments to a maximal resolution of 1.7 Å. A panel of CD55 mutants were produced based upon this structure, and the ability of the variant proteins to function in complement assays and pathogen interactions was determined. This struc-

Received for publication, December 10, 2002
Published, JBC Papers in Press, January 13, 2003, DOI 10.1074/jbc.M212561200
### Table I
Structure solution

| Wavelength (Å) | Pt-1 | Pt-2 | Pt-3 | Au | Native |
|---------------|------|------|------|----|--------|
| 1.0714        | 1.0717 | 1.060 | 0.87 | 1.488 | 0.87 |
| Resolution (outer shell) (Å) | 27.0–2.0 (2.1–2.0) | 27.0–2.0 (2.1–2.0) | 27.0–2.0 (2.1–2.0) | 40.0–2.4 (2.5–2.4) | 20.0–1.7 (1.75–1.7) |
| Total observations | 72589 | 65968 | 72823 | 32464 | 685390 |
| Unique observations | 14562 | 14302 | 14314 | 8519 | 44162 |
| R(merge)Å (%) | 8.8 (46.9) | 8.4 (55.7) | 9.1 (53.7) | 9.4 (27.2) | 6.2 (35.0) |
| Completeness (%) | 97.0 (96.3) | 97.0 (94.9) | 96.9 (96.0) | 95.2 (95.1) | 100.0 (92.0) |
| Average I/SigI | 5.7 (1.0) | 6.5 (1.0) | 6.8 (1.1) | 2.9 (1.1) | 10.4 (1.5) |
| Redundancy | 3.7 | 3.4 | 3.7 | 3.8 | 15.5 |
| Riso (%) (20–2.6 Å) | 24 | 24 | 24 | 20 | |
| Bruoulis (%) (20–2.6 Å) | 25 | 40 | 25 | 35 | |

### Refinement

- R = \( \frac{\sum(|F_{o}| - |F_{c}|)^2}{\sum|F_{o}|^2} \) \times 100
- \( R_{free} = \frac{\sum(|F_{o}| - |F_{c}|)^2}{\sum|F_{o}|^2} \) \times 100
- Number of residues in model
- R.M.S.D. bonds
- R.M.S.D. angles
- Mean protein B
- Mean non-protein B
- Tilt/Twist angles relating SCR3 and SCR4 (21, 34)

|                     | P1 | C2a | C2b | P2.2.2 |
|---------------------|----|-----|-----|--------|
| \( a = 31.6 \text{Å} \) | 31.6 | 125.7 | 126.9 | 31.6 |
| \( b = 38.8 \text{Å} \) | 38.8 | 30.4 | 30.7 | 38.9 |
| \( c = 45.3 \text{Å} \) | 45.3 | 52.7 | 41.8 | 107.5 |
| \( \alpha \) | 96.6 | 110.5 | 111.5 | 99.4 |
| \( \beta \) | 108.9 | 110.5 | 111.5 | 99.4 |

\( ^a \) \( R(I) = \frac{\sum|I_{o} - I_{c}|}{\sum I_{o}} \times 100 \)

\( ^b \) \( R = \frac{\sum|F_{o} - F_{c}|}{\sum F_{o}} \times 100 \)

\( ^c \) R.M.S.D., root mean square deviation.

\( ^d \) Grouped B factor refinement, two groups per residue (main chain and side chain groups).

\( ^e \) All Bs set to value obtained from Wilson plot for experimental data.
ture and the functional analysis of variant CD55 proteins provide detailed insights at high resolution into the domain architecture of CD55, the natural interactions of the protein in the complement system, and the range of pathogen interactions.

EXPERIMENTAL PROCEDURES

Protein Production and Crystallization—C-terminally histidine-tagged CD55$_{34}$ was expressed in yeast, and crystals were grown from sitting drops equilibrated against a mother liquor containing polyethylene glycol 4000 and NaCl at low pH as described previously (13, 14). Mutants were expressed in Chinese hamster ovary cells as soluble, dimeric, glycosylated, Fc-linked proteins containing CD55$_{34}$ and purified to high homogeneity using affinity chromatography (>95%). All proteins displayed a wild-type phenotype in at least one biological assay (convertase decay, virus binding (see “Results”), or interaction with domain-specific monoclonal antibodies (data not shown)), demonstrating that the mutations were localized in influence and did not grossly disturb SCR folding.

Crystallographic Analysis—Multisubject anomalous dispersion x-ray diffraction data to 2.0 Å were collected on European Synchrotron Radiation Facility, Grenoble, France, BM14 from a two-site platinum (K$_2$PtCl$_4$) derivatised P1 crystal frozen at 100 K. 360 degrees of data were collected at three wavelengths chosen with respect to the platinum edge. A three-site gold derivative data set (NaAuCl$_4$) was collected at Synchrotron Radiation Source, Daresbury, UK, beamline 9.6. A highly redundant 1.7-Å native data set was collected from a single crystal at 100 K at SRS beamline 14.1. Data were processed using the CCP4 suite of programs (15). Phases were calculated using SHARP (16). Multiple cycles of rebuilding in Xfit (17) and refinement in CNS (18) led to the models described below. For crystal form P2$_1$2$_1$2$_1$, two SCRs from molecule P were used independently for sequential molecular replacement into the other crystal forms using program EPMR (19). For the two crystal forms at lower resolution (C2h and P2$_1$2$_1$2$_1$), SCR3 was rotated and translated into position followed by a search for SCR4. In both cases, the top solutions were seen to position the C terminus of SCR3 and N terminus of SCR4 could be trivially joined. Rigid body refinement where the two SCRs were allowed to move independently was followed by least squares minimization and either grouped B-factor refinement (two Bs per residue for crystal form C2h) or setting of all atom Bs to the Wilson B value for crystal form P2$_1$2$_1$2$_1$. Limited rebuilding in Xfit and further refinement led to the models described below. For crystal form C2h, the higher resolution of the data (2.0 Å) allowed a full rebuild and refinement using Xfit and CNS. The final model statistics for the different crystal forms are as shown in Table I. Contact distances were calculated using the CCP4 program suite.

Immunological Assays (Complement, HA, and Antibody Binding)—Classical and alternative pathway complement assays were conducted essentially according to Harris et al. (20). Classical pathway activity is given as the mean percentage of wild-type (WT) activity ± S.D. if it differs from WT by at least three standard deviations, otherwise as WT. Activities were determined from a minimum of three experiments. Alternative pathway activity was determined in duplicate and is shown as WT (activity did not differ from WT by more than a factor of 2) or factor difference from WT activity. Echovirus binding was quantified by a hemagglutination inhibition (HAI) assay as described previously (13). Input virus was normalized to 4 HA units (where 1 HA unit is the amount of virus required to hemagglutinate 50 μl of 0.5% human erythrocytes) and incubated with serial dilutions of purified dimeric Fc-linked CD55. HA endpoints were scored visually and were repeated at least three times and are presented in the same manner as the alternative pathway data.

RESULTS

CD55$_{34}$ was crystallized in a number of different spaces (14) from identical crystallization conditions. The overall structure of CD55$_{34}$ (Fig. 1A) contains two copies of the SCR fold observed in other RCA proteins (8), each consisting of five antiparallel β strands with strands 1 and 4b and strands 3 and 5b being “pulled together” by a conserved disulfide at opposing ends of the domains. Strand 1 does not meet the strict criteria for definition as a β-strand in these structures.

The crystals yielded five independent, differently packed copies of CD55$_{34}$ with only one crystal contact being conserved between two of the crystal forms (see the supplemental data available online). Little structural variation is seen at the interface between SCR3 and -4, implying that this junction is relatively rigid. This conclusion is supported by numerical calculation of tilt and twist angles, which define the orientation of SCR3 with respect to SCR4 (21, 34). The tilt for all molecules is 8 ± 8° (Table I). The packing of the SCR3 strand 3–4 loop against the SCR3-SCR4 linker appears fundamental to maintaining the relative orientation of the SCR domains. Additional stability is probably provided by the packing of the SCR4 strand 4–5 loop (Fig. 2), although precise definition of the determinants of the interdomain rigidity is not trivial. Although hydrogen bonds are important for internal stabilization of the loops (Fig. 2C), only the SCR3 strand 3–4 loop is tied to the linker via a hydrogen-bonding network since the linker forms a continuation of strand 5b.

A panel of SCR3 and -4 mutations was generated to functionally probe the various faces of the molecule revealed by the structure. These mutants were tested in complement control and virus binding assays and provide further evidence supporting the importance of the observed SCR3–4 organization to the function of CD55. Prior to analysis, all mutants were screened with domain-specific monoclonal antibodies to confirm that there was no gross disruption of structure.

The mutant proteins were found to have a range of abilities to regulate the alternative and classical pathways of complement (Table II and Fig. 3). Our data confirm the role for residues $^{125}$KKK$^{127}$ and $^{147}$LF$^{148}$ reported earlier (22). In light of our structure (see “Discussion”), we interpret these mutations as acting by altering the packing of domains 2 and 3 rather than directly mapping the site of convertase interaction. Our data also demonstrate that changes to several other residues on the “back” face of CD55 (as defined in Fig. 3) are able...
to alter the ability of the molecule to regulate both pathways of complement activation.

Echoviruses agglutinate erythrocytes by binding CD55 (13, 23), and hemagglutination inhibition (HAI) assays provide a means to readily quantify the interaction of virus and receptor. Earlier work (13) has grouped CD55-binding echoviruses into three broad categories based on specific SCRs required for virus binding. A virus from each of these groups was assayed

| Mutation        | Classical pathway decay acceleration | Alternative pathway decay acceleration | Echovirus binding |
|-----------------|-------------------------------------|--------------------------------------|-------------------|
| WT              | 100%                                | 100%                                 | WT                |
| E134A           | 65 ± 5                              | WT                                   | WT, down 4-fold   |
| F169A           | WT                                  | No activity detectable              | WT                |
| A195P           | WT                                  | WT                                   | WT                |
| K161A G3D       | WT                                  | WT                                   | WT                |
| L147F/L148F     | 0 ± 0                               | down 8-fold                          | WT                |
| L147F/L148F P78S| 0 ± 0                               | down 8-fold                          | WT                |
| T218M N220H     | 155 ± 10                            | WT                                   | WT                |
| S165V           | WT                                  | WT                                   | WT, up 4-fold     |
| S165A           | 125 ± 5                             | WT                                   | WT                |
| T166A           | 165 ± 10                            | WT                                   | WT                |
| K125A/K126A/K127A| 80 ± 5                             | down 8-fold                          | WT, down 4-fold   |
| AGM             | WT                                  | ND                                   | No binding detectable |
|                 |                                     |                                      |                   |
| a WT, activity is wild-type within standard errors. |
| b ND, not detectable. |
for its ability to bind to the panel of CD55 mutant proteins by HAI. Substitutions at the SCR2–3 interface (125KKK127 or 147LF148) had little or no influence on virus binding (with the exception of EV11, which was somewhat sensitive to the 125KKK127 change), implying that either the SCR2–3 interdomain angle or SCR2 makes only minor contributions to virus interactions. SPR analysis of CD55 binding by EV11 also supports the latter conclusion (24). However, despite the close genetic relationship between decay-accelerating factor-binding enteroviruses (13), EV12 and EV7 were independently influenced by substitutions on opposite faces of CD55 (Fig. 3C and Table II). Further differences were apparent in the interaction of EV with African green monkey (AGM) CD55, which differs by 42 residues within SCR1–4. EV7 binds to human and AGM CD55 indistinguishably, whereas EV11 cannot bind AGM CD55, and EV12 binding to AGM CD55 is significantly reduced (Table II). These results are in agreement with our earlier SPR and HAI analysis using SCR-deleted forms of CD55 to broadly map the domains of CD55 that interact with these viruses (13, 23, 24). They also demonstrate that viruses that share the same SCR specificity for binding may actually interact with distinctly different faces of the molecule.

**DISCUSSION**

**Molecular Architecture of CD55**—Using the extended linear structure of CD5534 to predict the topology of the four SCR domains in the full molecule produces a model that projects ~300 Å above the cell membrane (including an estimated ~100 Å for the serine/threonine-rich stalk). This is significantly extended, by almost 50%, when compared with previous models (22, 25). Alternative topologies are possible if the observed linearity of the SCR34 interface is not repeated at the junctions of the other SCR domains. A recent low resolution structure of CD55 complexed with EV7 (26) is essentially linear along the SCR2–3 junction but exhibits a distinct angle at the interface between SCR1 and -2. Until a high resolution structure of a four SCR domain CD55 molecule is determined, it is important that the functional data concerning SCRs 1 and 2 are interpreted in the light of a variety of architectural models, of which our structure-based model and earlier homology models of Kuttner-Kondo et al. (25) represent the extremes available.

**Mapping Complement Regulation**—Alignment of mammalian CD55 sequences has identified two highly conserved regions in SCR3, a positively charged block (consisting of 125KKK127) and a hydrophobic patch (147LF148). Previous studies have shown that modification of these residues grossly perturbs complement control, and based on structural modeling, may form the primary sites of convertase interaction (22). Our data confirm the importance of these residues in influencing both classical and alternative pathway decay acceleration (Table II) and show that these regions are juxtaposed at the membrane-distal end of SCR3. Based on the observed linearity of the SCR3–4 interface (Fig. 1), the structure of the SCR3...
interstrand loops located in this region, and the apparent linearity of the SCR2–3 junction (see above and Ref. 26), we predict that these mutations are buried at the SCR2–3 interface and exert their effect by altering the relative positions of the two domains (Fig. 3B). Based on the functional analysis of our mutants, we propose that the back face of CD55a (as defined in Fig. 3) is the most likely site of direct contact with the convertases. Our mutational data suggest that the conversion interactions of both the classical and alternative pathway are broadly similar with the exception of E134A and F169A, which respectively solely affect the classical or alternative pathways. The E134A mutation does not significantly disrupt CD55 structure as the protein has native activity in alternative complement pathway assays, in virus binding (see below), and in binding domain-specific antibodies (data not shown). Our mapping of Glu134 to the "front" of the molecule suggests that CD55 may interact via an extended interface with the classical pathway convertase (which at 230 kD is much larger than CD55), the convertase wrapping around CD55 to contact both faces of the regulator.

CD55-Pathogen Interactions—Many euviruses use CD55 as a receptor, and earlier work (13) has grouped these viruses into families on the basis of which the CD55 domain(s) is critical for binding. Our results demonstrate that specific euvirus types bind to different sites on CD55 (even when the viruses are dependent on the same SCR for recognition); it is therefore likely that the binding determinants on the virus capsid will also differ (either in sequence or location). The cryo-electron microscopy-determined structure of EV7 complexed with CD55 shows the receptor binding across the 2-fold axes of symmetry (26), whereas previous mapping of EV11 mutations with the loss of CD55 binding has implicated a platform surrounding the 5-fold axes (27). Taken together, these observations further support the hypothesis that CD55 receptor usage may have evolved independently in these enteroviruses rather than being an ancestral trait (13).

The use of CD55 as a receptor has been coupled with symptomatic infections by a variety of uropathogenic E. coli (28, 29) with recent evidence showing a link between the use of CD55 and stimulation of the host immune response via induction of MICA expression (5). The influence on E. coli binding of the two known single amino acid polymorphisms within the SCR domains has been examined previously (28, 30) and suggests that those bacteria sensitive to a change in SCR3 are insensitive to changes in SCR4 (and vice versa). This strongly implies multiple, independent binding sites on CD55 for different bacterial strains. Our structure shows that these two polymorphisms lie on opposite faces of the molecule (Fig. 3D). Our structure-based mapping of CD55 biology provides strong support for the hypothesis that convergent evolution is responsible for the range of pathogens that bind CD55, implying a distinct selective advantage for those that bind this molecule. The precise nature of the evolutionary pressure for both viral and bacterial pathogens to bind CD55 remains to be determined. The high level expression of CD55 on serum-exposed cells means it could provide an important initial attachment for the pathogen before interaction with additional cell entry determinants (12, 31, 33). However, there are many other widely expressed cell surface proteins, and an attractive alternative hypothesis is that pathogens have evolved to exploit the cellular role(s) of the molecule, perhaps to gain immunological advantage. Our structure provides the basis for a better understanding of the functional domains of CD55 and the interactions with complement control proteins, bacterial fimbriae, and enteroviruses. We are, however, aware that hypotheses proposed on the basis of mapping mutational data will require validation by determination of the structures of complexes of CD55 with its many ligands.

Acknowledgments—We thank Bob Sim for discussion of complement assay data, Martin Noble for assistance with figure preparation, David Stuart for thoughtful reading of an earlier version of this manuscript and the staff of the SRS, Daresbury, UK and ESRF, Grenoble, France for assistance with data collection.

REFERENCES
1. Lublin, D. M., Lemons, R. S., Le Beau, M. M., Holfers, V. M., Tykocinski, M. L., Medof, M. E., and Atkinson, J. P. (1987) J. Exp. Med. 165, 1731–1736
2. Lublin, D. M., Kinoshita, T., and Nussenzweig, V. (1984) J. Exp. Med. 160, 1558–1578
3. Medof, M. E., Walter, E. L., Roberts, W. L., Haas, R., and Rosenberry, T. L. (1986) Biochemistry 25, 6740–6747
4. Telen, M. J., Rao, N., Udani, M., Thompson, E. S., Kaufman, R. M., and Lublin, D. M. (1994) Blood 84, 3205–3211
5. Tieng, V., Le Bouguenec, C., du Merle, L., Bertheau, P., Desreumaux, P., Jamin, A., Charron, D., and Toutou, A. (2002) Nat. Acad. Sci. U. S. A. 99, 2977–2982
6. Hamann, J., Vogel, B., van Schijndel, G. M., and van Lier, R. A. (1996) J. Immunol. 156, 1185–1189
7. Lindahl, G., Sjoberg, U., and Jonsson, E. (2000) Curr. Opin. Immunol. 12, 44–51
8. Barlow, P. N., Baron, M., Norman, D. G., Day, A. J., Willis, A. C., Sim, R. B., and Campbell, I. D. (1999) Biochemistry 38, 1097–1098
9. Medof, M. E., Lublin, D. M., Kinoshita, T., and Nussenzweig, V. (1984) J. Exp. Med. 160, 1558–1578
Mapping CD55 Function: THE STRUCTURE OF TWO PATHOGEN-BINDING DOMAINS AT 1.7 Å
Pamela Williams, Yasmin Chaudhry, Ian G. Goodfellow, Jason Billington, Robert Powell, O. Brad Spiller, David J. Evans and Susan Lea

J. Biol. Chem. 2003, 278:10691-10696.
doi: 10.1074/jbc.M212561200 originally published online December 22, 2002

Access the most updated version of this article at doi: 10.1074/jbc.M212561200

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

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

Supplemental material:
http://www.jbc.org/content/suppl/2003/03/17/278.12.10691.DC1

This article cites 34 references, 16 of which can be accessed free at http://www.jbc.org/content/278/12/10691.full.html#ref-list-1