

Crystal Structure of the Extracellular Domain of a Human Fc γ RIII

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Summary

Fc receptors play a major role in immune defenses against pathogens and in inflammatory processes. The crystal structure of a human immunoglobulin receptor, Fc γ RIIIb, has been determined to 1.8 Å resolution. The overall fold consists of two immunoglobulin-like domains with an acute interdomain hinge angle of approximately 50°. Trp-113, wedged between the N-terminal D1 and the C-terminal D2 domains, appears to further restrict the hinge angle. The putative Fc binding region of the receptor carries a net positive charge complementary to the negative-charged receptor binding regions on Fc. A 1:1 binding stoichiometry between the receptor and Fc was measured by both the equilibrium and nonequilibrium size-exclusion chromatography. Two separate parallel dimers are observed in the crystal lattice, offering intriguing models for receptor aggregation.

Introduction

Because of their ability to bind the Fc portion of antibodies, Fc receptors provide a link between humoral and cellular immune responses. There are Fc receptors for each Ig isotype. Except for Fc ϵ RII (CD23), which is C-type lectin-like, all other known Fc receptors are members of the immunoglobulin superfamily (Daeron, 1997). Among them, Fc γ RI and Fc ϵ RI are high-affinity receptors with dissociation constants ranging from 10⁻⁸

to 10⁻¹⁰ M, whereas the other receptors, such as Fc γ RII and Fc γ RIII, are low-affinity receptors with dissociation constants ranging from 10⁻⁵ to 10⁻⁷ M (Hulett and Hogarth, 1994; Galon et al., 1997; Powell et al., 1999).

Fc γ RIII, also known as CD16, is the type III Fc γ receptor. It exists as two isoforms in humans, Fc γ RIIIa and Fc γ RIIIb, sharing 96% sequence identity in their extracellular immunoglobulin binding regions. Fc γ RIIIa is expressed on macrophages, mast cells, and natural killer cells as a transmembrane receptor. Present exclusively on neutrophils, Fc γ RIIIb is the only Fc receptor anchored by a glycosyl-phosphatidylinositol (GPI) linker to the plasma membrane. Despite the lack of a signaling component, Fc γ RIIIb plays an active role in triggering Ca²⁺ mobilization and neutrophil degranulation (Kimberly et al., 1990; Unkeless et al., 1995). In addition, in conjunction with Fc γ RIIa, Fc γ RIIIb activates phagocytosis, degranulation, and oxidative burst, which lead to the clearance of opsonized pathogens by neutrophils. Recently, a soluble form of Fc γ RIIIb was reported to activate the complement receptor CR3-dependent inflammatory process (Galon et al., 1996). The immunoglobulin binding affinities of Fc γ RIIIb were reported to be 1 × 10⁻⁶ and 2 × 10⁻⁶ M for human IgG1 and IgG3, respectively (Galon et al., 1997).

The recent crystal structures of a Fc ϵ RI α , Fc γ RIIa, and Fc γ RIIIb revealed a conserved immunoglobulin-like (Ig-like) structure, particularly the small hinge angle between the two Ig-like domains that is unique to the Fc receptors (Garman et al., 1998; Maxwell et al., 1999; Sondermann et al., 1999a). Several models of receptor activation/oligomerization have been proposed, based on the crystal lattice packings and molecular modeling. However, discrepancies exist among these models and none appear to form an oligomeric state. We report here a 1.8 Å crystal structure of a human Fc γ RIIIb and its implication to the receptor oligomerization, based on our observed crystal lattice packing.

Results and Discussion

Overall Structure of Fc γ RIII

The sequence of human Fc γ RIII is 49% identical to Fc γ RIIa and 39% identical to Fc ϵ RI α (Figure 1). Two forms of the Fc γ RIII receptor crystal were obtained. The structure was initially determined on the lower resolution crystal form to 2.3 Å resolution by multiwavelength anomalous dispersion (MAD) method using a HgCl₂ derivative (Table 1). The refined R values are 0.24 and 0.29 for R_{cryst} and R_{free}, respectively, for this crystal form. The two receptor molecules, A and B, in each crystallographic asymmetric unit were refined independently without noncrystallographic symmetry (NCS) constraints. A second form of the receptor crystal, which relates to the first crystal form by converting the non-crystallographic two-fold into a crystallographic two-fold symmetry, was obtained under slightly different crystallization conditions. The refined receptor model from the first crystal form was further refined to 1.8 Å

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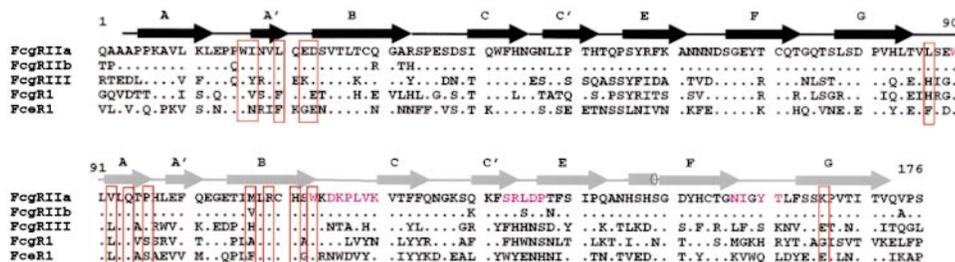


Figure 1. Sequence Alignment of Human Fc Receptors

Amino acids identical to the sequence of Fc γ RIIIa are not shown. The secondary structure elements of Fc γ RIII are shown above the sequence with the D1 domain in black and the D2 domain in gray. Residues involved in the interdomain hinge packing are boxed in red. Residues in the putative Fc binding region are shown in magenta.

resolution in the second crystal form with the final R values of 0.18 and 0.21 for R_{cryst} and R_{free} , respectively. The refined model contains amino acids 3–174 of the mature sequence of Fc γ RIII ligand binding domain. The electron density is well defined throughout the molecule in the refined ($2F_o - F_c$) map. The overall structure of Fc γ RIII consists of two immunoglobulin-like domains (Figure 2A), similar to those of Fc γ RIIIa, Fc γ RIIIb, and Fc ϵ RI α (Garman et al., 1998; Maxwell et al., 1999; Sondermann et al., 1999a). Different from the C2-like hematopoietic growth factor receptor fold, the first β strand of both domains contains a switch from A to A', pairing with strand B and G, respectively. This strand switch is centered at Pro-14 and Pro-96 of the D1 and D2 domains and is also observed in the structure of killer cell immunoglobulin-like receptors (KIR) (Fan et al., 1997; Maenaka et al., 1999; Snyder et al., 1999). Pro-14 is in the *cis* conformation, whereas Pro-96 is in the *trans* conformation and can be replaced by a Ser at this location, as in the structure of Fc ϵ RI and in the sequence of Fc γ RI. The structure of the N-terminal D1 domain of

Fc γ RIII can be readily superimposed to the C-terminal D2 domain, resulting in a root-mean-square (rms) difference of 1.2 Å between 73 pair of C α atoms.

The refined structures of the two receptor molecules in the first crystal form are nearly identical to each other and to that of the second crystal form, with the differences among the individual domains less than 0.7 Å in rms deviation.

Among members of known Ig superfamily receptors, the structure of Fc receptors is most similar to that of killer cell immunoglobulin-like (KIR) receptors, despite the existence of only 18% sequence identity. They not only share the same fold, but the individual domains can be readily superimposed, resulting in rms differences of 1.9 and 1.5 Å among their individual D1 (72 superimposed C α atoms) and D2 (71 superimposed C α atoms) domains, respectively.

The Interdomain Hinge Angle

A feature common to all known Ig superfamily Fc receptors is their acute hinge angle between the N- and

Table 1. Data Collection and Refinement Statistics

Data Set	Wavelength λ (Å)	Resolution (Å)	Unique Reflections	Completeness (%)	$I/\sigma(I)$	R_{sym}^a	Phasing ^c Power	R_{cullis}^b iso/ano	f.o.m. ^d
Native 1	1.040	2.3	19133	96.7 (90.0)	31.2 (4.3)	0.043 (.25)			
Native 2	1.54	1.8	36180	95.8 (80.1)	29.5 (6.9)	0.054 (.21)			
HgCl-3	1.0078	2.35	34036	98.8 (97.6)	21.1 (3.4)	0.038 (.25)	1.8	0.64/0.83	0.51
HgCl-4	1.0246	2.35	34340	99.1 (98.1)	20.9 (3.7)	0.039 (.27)	1.5	0.67/0.92	
HgCl-5	1.0093	2.35	33830	98.1 (96.4)	22.0 (3.4)	0.037 (.28)	–	–/0.92	
Refinement ^e									
	Resolution (Å)	Solvent Molecules	R_{cryst}	R_{free}	Average B Factor	Root-Mean-Square Deviation			
						Bond (Å)	Angle (°)	Dihedral (°)	
Native 1	10.0–2.3	299	0.242	0.290	53.6	0.01	2.2	26.3	
Native 2	20.0–1.8	223	0.184	0.216	24.1	0.013	1.73	27.6	

^a $R_{\text{sym}} = \sum |I_h - \langle I_h \rangle| / \sum I_h$, where $\langle I_h \rangle$ is the mean intensity for multiple measurements of symmetry-related reflection h . The values in parentheses for the completeness, I/σ , and R_{sym} correspond to those of the highest resolution shells, which are 2.38–2.3 Å for the Native 1, 1.86–1.80 for the Native 2, and 2.43–2.35 Å for the Hg derivative data sets.

^b R_{cullis} is calculated as the ratio of the lack of closure and the isomorphous or anomalous differences. HgCl-3, -4, and -5 data sets correspond to the calculated peak, remote, and edge wavelengths of Hg L_{III} dispersion edge. HgCl-5 is treated as the reference data set throughout the phase refinement.

^c The overall phasing power is calculated as the average of (F_o/F_c lack of closure) and includes both centric and acentric reflections.

^d The figure of merit is calculated for all three wavelength data sets.

^e Refinement is carried out against the native data set. R_{free} is calculated using 5% randomly selected test reflections. The percentage residues in the four Ramachandran Plot regions (the most favored, additional allowed, generously allowed, and disallowed) are 78.3, 16.1, 4.0, and 1.6 for Native 1; and 86.6, 12.1, 1.3, and 0.0 for Native 2.

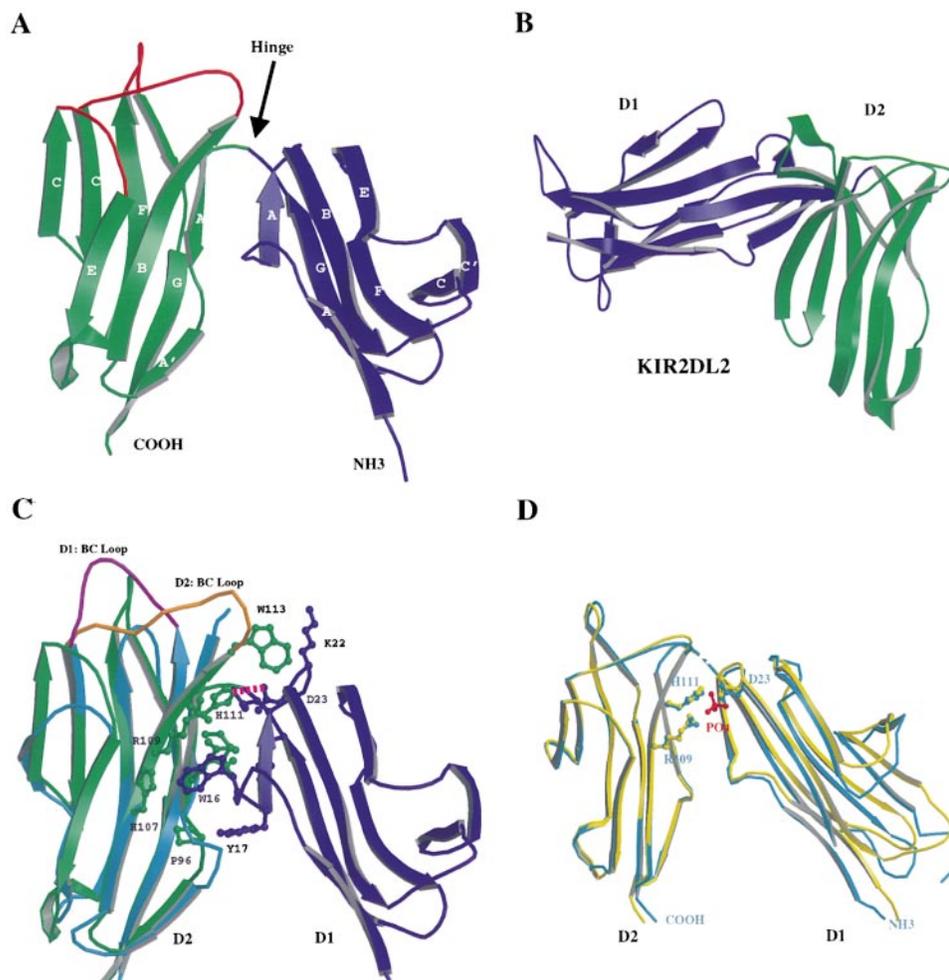


Figure 2. Structure of Fc γ RIII

(A) The N-terminal D1 and the C-terminal D2 domains are colored in blue and green, respectively. The putative ligand binding loops of Fc γ RIII are colored in red. The strands for the D1 domain are A (5–12), A' (17–20), B (22–33), C (39–45), C' (47–52), E (55–61), F (66–73), and G (77–85) and the strands for the D2 domain are A (91–95), A' (98–101), B (104–113), C (120–125), C' (130–134), E (136–143), F (149–157), and G (163–172). Residues 146–149 form a short α helix.

(B) Orientation of the D1 and D2 domains in KIR. The D2 domain of KIR is in a similar orientation to that of Fc γ RIII.

(C) The interdomain hinge region. The D1–D2 domain of Fc γ RIII and their respective interdomain packing residues are shown in dark blue and green. The BC loop of the D2 domain is colored in orange. The dashed line indicates the salt bridge between Asp-23 and His-111. The superposition of the D1 domain on the D2 domain is shown in light blue and its BC loop in purple.

(D) Superposition of the molecule A and B of Fc γ RIII in the asymmetric unit. A bound phosphate ion is found in molecule B but not in A. All ribbon figures are prepared using the program MOLSCRIPT and Raster3D (Kraulis, 1991; Merritt and Bacon, 1997).

C-terminal Ig domains. This angle was reported between 52° and 70° for Fc γ RIIa and Fc γ RIIb, respectively, a discrepancy most likely caused by the different algorithms used in estimating the angles (Maxwell et al., 1999; Sonderrmann et al., 1999a). The hinge angle of Fc γ RIIa and Fc γ RIIb are calculated to be 55° and 52°, respectively, using the HINGE program (Snyder et al., 1999). The interdomain hinge angle is calculated to be 51°, 47°, and 49° for receptor A, receptor B of the first crystal form, and the receptor of the second crystal form, respectively. Compared to other Ig-like structures, such as the hematopoietic receptors, KIR receptors, and adhesion receptors, Fc receptors not only have smaller hinge angles, but the position of their N-terminal (D1) domain with respect to the C-terminal (D2) domain

is also opposite to other receptors (Figures 2A and 2B). Detailed examination of the hinge region reveals the molecular basis for the unique interdomain hinge angle common to all Fc receptors. First, as noted in the Fc γ RIIa structure, the interdomain linker for all Fc receptors consists of two residues; that is shorter than the five-residue linker observed in KIR and growth hormone receptor. The KIR-like D1–D2 arrangement requires a longer interdomain linker, while the linker for Fc receptors appears too short to adopt the KIR-like interdomain arrangement. Second, the BC loop from the D2 domain, residues 113–118, protrudes away from the core of the D2 domain toward the D1 domain instead of assuming a typical up-and-down direction (Figure 2C) as in the BC loop of the D1 domain. As a result, this BC loop acts as a cap

Table 2. Interactions between D1 and D2 Domains of Fc γ R1II

Hydrogen Bonds and Salt Bridges		
D1	D2	Distance ^a (Å)
Ser-18 O	Gln-94 N ϵ 2	3.0
Asp-23 O δ 1	His-111 N ϵ 2	2.9
O δ 2	His-111 N ϵ 2	3.1
Hydrophobic Contacts ^b		
Trp-16	Gln-94, Ala-95, Pro-96, His-107, Arg-109	
Tyr-17	Ala-95, Trp-98	
Ser-18	Leu-92	
Val-19	Leu-92	
Leu-20	Leu-92, His-111, Ser-112, Trp-113	
Glu-21	Trp-113	
Lys-22	Trp-113, Lys-114	

^a Hydrogen bond distance cutoff is 3.5 Å.

^b Carbon-carbon contacts < 4.0 Å.

situated on top of the D1 domain, restricting its position. A similar conformation is also found in the BC loop from the structure of Fc ϵ RI. Third, a conserved tryptophan residue, Trp-113, (also referred to as Trp-110 in the structures of Fc γ R1IIb and Fc ϵ RI) (Figure 1) from the BC loop of the D2 domain inserts itself into one end of

the D1 domain as a wedge to further restrict the hinge movement (Figure 2C). Any increase in the hinge angle would create steric hindrance between Trp-113 and the backbone of the AB loop in the D1 domain. A Trp-113 to Ala mutation in Fc ϵ RI, which would likely result in a conformational change, has been shown to affect the IgE binding dramatically (Hulett et al., 1999). In addition, there are also favorable interdomain interactions to stabilize the tight D1–D2 packing (Table 2). One such interaction is a conserved salt bridge between Asp-23 of the D1 domain and His-111 of the D2 domain. There is also a small core of hydrophobic residues, consisting of Trp-16, Leu-20, Leu-92, Gln-94, and Trp-113, in the hinge region. Hydrogen bonds and van der Waals interactions among hydrophilic residues are also found in the hinge region providing additional stability to the hinge angle. The D1–D2 interface buries 1290 Å² solvent-exposed area. The small hinge angle difference between molecules A and B is most likely the result of a bound phosphate ion that is found in the hinge region of molecule B but not in A (Figure 2D). The existence of a bound phosphate ion was also evident from mass spectrometry of Fc γ R1II (data not shown). Although the biological relevance of the phosphate-bound and phosphate-free forms of the receptor remains unclear, the difference in

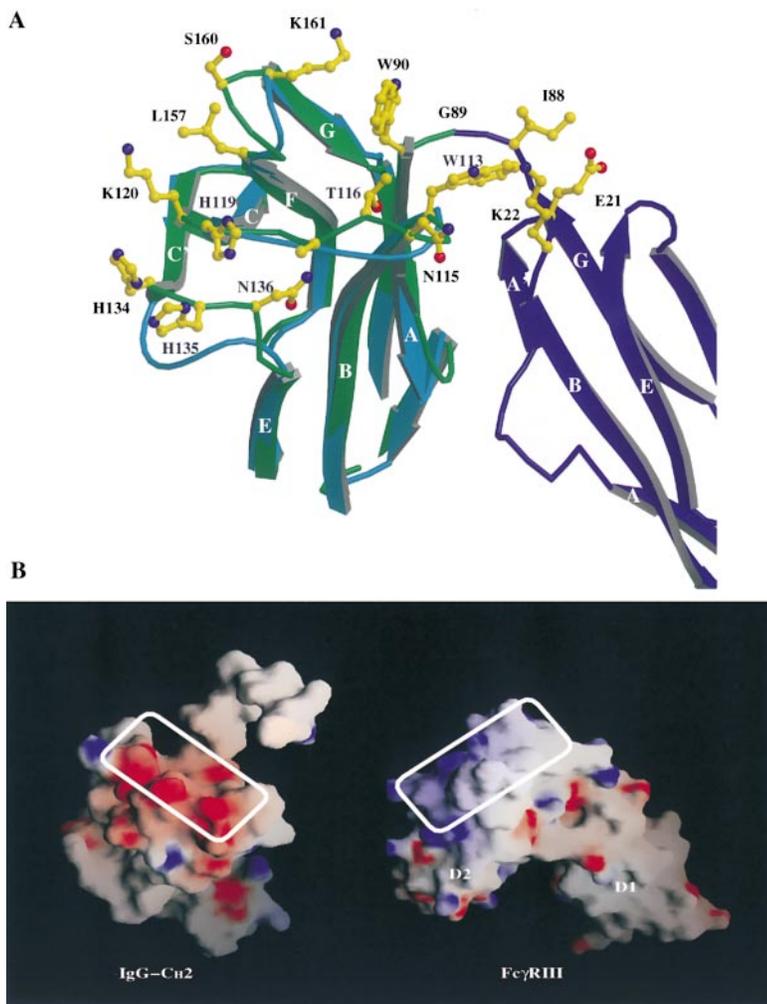


Figure 3. The Structure of Fc Binding Region (A) The putative Fc binding region. The D1 and D2 domains of Fc γ R1II are shown in dark blue and green, respectively. Residues in the putative ligand binding region of Fc γ R1II are shown in ball-and-stick model. The D2 domain of Fc ϵ RI superimposed onto the D2 domain of Fc γ R1II is shown in light blue. (B) The Grasp representation of the electrostatic potential distribution at the putative interface of Fc γ R1II and the C ϵ 2 domain of IgG. Positive potentials are colored in blue, and negative potentials are in red. The putative interface regions are highlighted. The coordinates for Fc are taken from the structure of IgG2a, Protein Data Bank entry 1IGT.

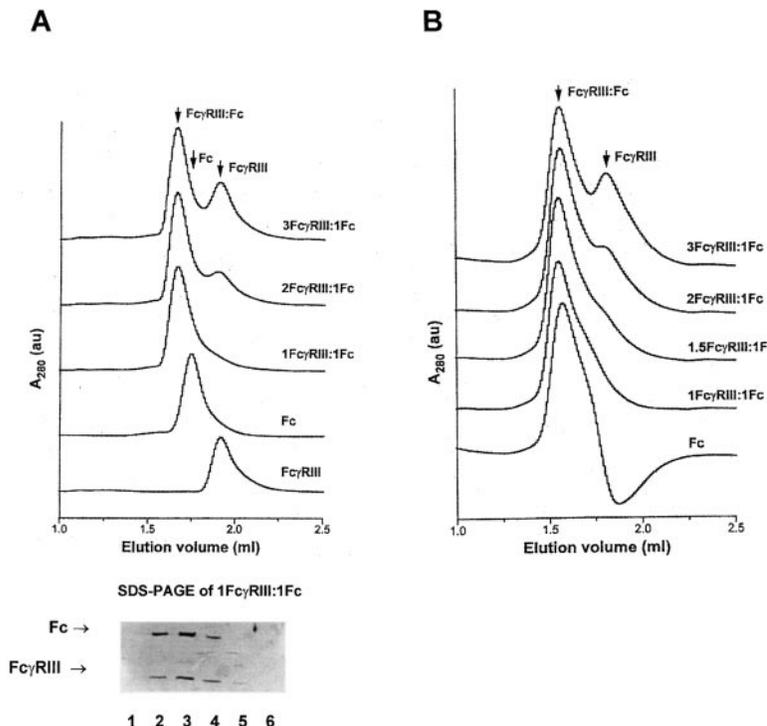


Figure 4. Analysis of the Fc γ RIII and Fc Complex by Size-Exclusion Chromatography under Both Nonequilibrium and Equilibrium Conditions

The apparent molecular weights of Fc γ RIII, Fc, and their complex are 25, 51, and 63 kDa, respectively. The predicted molecular masses for Fc γ RIII and Fc are 21.0 and 50.4 kDa, respectively.

(A) Nonequilibrium gel filtration. The injection sample contains 10 μ M Fc γ RIII or 10 μ M Fc for the Fc γ RIII and Fc-only runs. The complexes were prepared with 10 μ M Fc and various amounts of the receptor to give rise to the 1:1, 2:1, and 3:1 receptor:Fc molar ratio. (B) Equilibrium gel filtrations were carried out using a buffer containing 50 mM NaCl, 50 mM Tris (pH 8.0), and 10 μ M Fc γ RIII. Samples contain 10 μ M Fc with various amounts of Fc γ RIII as indicated in each curve. The elution volumes of Fc γ RIII and the Fc γ RIII:Fc complex are marked.

hinge angle observed between the two molecules in the asymmetric unit suggests that the hinge angle can vary.

The Putative Ligand Binding Region

Extensive mutational analysis on Fc receptors in recent years has identified regions of the receptors that are important for the binding of immunoglobulins. In particular, the construction of Fc γ RII and Fc ϵ RI chimeric receptors and the use of site-directed mutagenesis have identified residues on the BC, C'E, and FG loops of the D2 domain that are important for the function of Fc receptors (Hulett et al., 1994, 1995; Cook et al., 1997; Henry et al., 1997). However, the details of molecular recognition between Fc and Fc receptors remain to be elucidated. Structurally, these loops encompass a rather extensive area and together with the hinge loop (residues 88–90) and the AB loop of the D1 domain (residue 21–23) they form a putative ligand binding region (Figure 3A). There are a total of 16 residues in this region. With six potential positively charged amino acids and only one negatively charged amino acid, the putative ligand binding region of Fc γ RIII appears overwhelmingly positively charged. Interestingly, the sequence alignment of the immunoglobulin Fc region shows that the presumed Fc receptor binding site, the lower hinge region, and the loops nearby carry a net negative charge (Figure 3B) (Kato et al., 2000). This results in an apparent charge complementarity between the receptor and Fc.

Among Fc receptors, Fc ϵ RI and Fc γ RI are high-affinity receptors, whereas Fc γ RII and Fc γ RIII are low-affinity receptors. It has been proposed that a Trp ladder observed in the structure of Fc ϵ RI is critical to its ligand affinity (Garman et al., 1998). In addition, the superposition of the receptor D2 domains between Fc γ RIII and Fc ϵ RI shows that significant structural differences in the

BC, C'E, and FG loops exist between the two receptors, while the β strands are closely superimposed (Figure 3A). In particular, the C'E loop differs as much as 5 Å between the two receptors, with the loop adopting an up conformation in Fc γ RIII and down conformation in Fc ϵ RI. It is possible that this conformational difference contributes to part of the difference in ligand binding affinity.

Stoichiometry of Fc and Fc γ RIII Recognition

Recent solution binding studies suggest the binding of IgG be 1:1 for both Fc γ RIII and Fc γ RII (Ghirlando et al., 1995; Kato et al., 2000), although both 1:1 and 2:1 receptor–ligand binding modes have been proposed (Metzger, 1992; Maxwell et al., 1999; Sondermann et al., 1999b). We have examined the binding of Fc γ RIII with an Fc derived from a human IgG1, using both analytical ultracentrifugation (AUC) and size-exclusion chromatography techniques. The affinity between the receptor and Fc measured by the AUC experiments is 0.4 μ M. The receptor–ligand binding stoichiometry, however, could not be confirmed from the AUC experiments. To determine the receptor–ligand binding stoichiometry, we have used both the nonequilibrium and the equilibrium size-exclusion chromatography techniques (Hummel and Dreyer, 1962; Martin and Bjorkman, 1999; Sondermann et al., 1999b). Under the nonequilibrium conditions, a series of gel filtration experiments were carried out using 10 μ M Fc mixed with 10, 20, and 30 μ M Fc γ RIII to form the 1:1, 2:1, and 3:1 receptor–Fc molar ratio of the complex (Figure 4A). The apparent molecular weights of the receptor, Fc, and their complex are 25, 51, and 63 kDa, respectively. The appearance of excess receptor in the 3:1 and 2:1 but not 1:1 molar ratio is

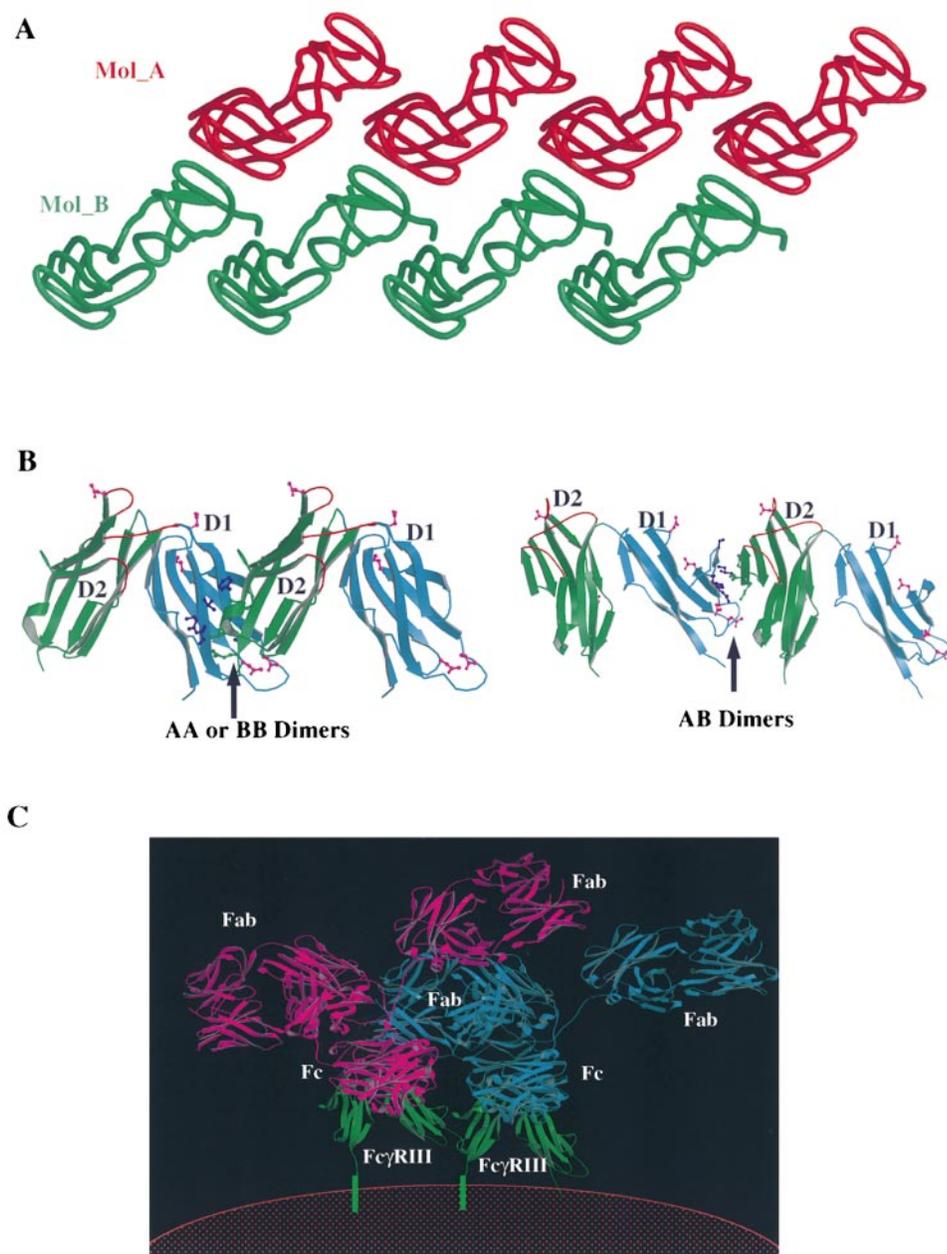


Figure 5. Fc Receptor Dimerization and Aggregation Model

(A) Crystal lattice packing of molecule A (in red) and B (in green).

(B) AA, BB, and AB parallel dimers. The dimers are formed through translational symmetry. The view is 90° from that of panel (A). D1 and D2 domains are colored in light blue and green. The interface residues are shown as ball-and-sticks in dark blue and green for the D1 and D2 domains, respectively. The predicted glycosylation sites are shown in magenta. The putative Fc binding loops are colored red. AB parallel dimer is formed between the two molecules in each asymmetric unit.

(C) A hypothetical model of receptor aggregation based on the observed parallel AB dimer (in green) in crystal lattice. The antibody coordinates, colored in magenta and blue, are from Protein Data Bank entry 1IGT.

consistent with a 1:1 receptor–ligand binding stoichiometry. The composition of Fc γ RIII and Fc in the complex peak was also estimated by N-terminal peptide sequencing. The result is also consistent with a 1:1 molar ratio between Fc γ RIII and Fc.

The equilibrium gel filtration experiments were performed with the column equilibrated using a buffer containing 10 μ M Fc γ RIII (Figure 4B). A trough corresponding to the receptor was observed when the sample

contained only Fc (at 10 μ M concentration), indicating a depletion of the receptor in running buffer. The trough disappeared at the receptor–ligand ratio of 1:1 and developed into a peak at the receptor–ligand ratios of 2:1 and 3:1. Similar gel filtration profiles were also observed when the column was equilibrated with 1 μ M of Fc instead of the receptor. The results from both the non-equilibrate and equilibrate gel filtration experiments suggest a 1:1 receptor–ligand stoichiometry.

Receptor Dimerization and Aggregation in Crystal
Ligand-induced receptor aggregation is presumed to be a common mechanism for initiating receptor-mediated signaling. Structurally, one of the best known examples is the human growth hormone-induced receptor dimerization, in which one molecule of growth hormone or erythropoietin binds to two molecules of their receptors (de Vos et al., 1992; Livnah et al., 1999). The situation between Fc and Fc receptors is less clear due to the 1:1 receptor–ligand complex. In these crystals of Fc γ R1II, we observed two forms of receptor dimers among symmetry molecules (Figures 5A and 5B). The first dimer is formed between molecule A or B and their equivalent molecules in adjacent unit cells, the AA or BB dimer (Figures 5A and 5B). They are parallel dimers in which monomers of the same orientation associate through a D1–D2 interaction. The interface of this AA dimer, burying 860 Å² solvent-exposed surface, consists of portions of the B and E β strands from the D1 domain of one molecule and portions of the F and G β strands from the D2 domain of another molecule. The putative ligand binding region is in identical orientation in the dimer, and the predicted glycosylation sites are located outside the dimer interface. An ordered oligomeric aggregate is formed based on the AA dimer throughout the crystal, in which each receptor interacts with a preceding receptor via its D1 domain and a successive receptor via its D2 domain, using the same D1–D2 interface (Figure 5A). The second dimer, the AB dimer, observed in the crystal of Fc γ R1II is formed between receptors A and B of each asymmetric unit. It is also a parallel dimer but with a different interface than in the AA and BB dimers (Figures 5A and 5B). The interface of AB dimer, burying 500 Å² solvent-exposed surface, is formed between the C' strand of the receptor A D2 domain and the C, C' strands of the receptor B D1 domain. The predicted glycosylation sites are located outside the interface area. Since the parallel orientations relating AA, BB, and AB dimers are incompatible with the two-fold symmetry possessed within the immunoglobulin Fc region, it predicts that the parallel dimers are consistent only with a 1:1 receptor–ligand stoichiometry. The existence of Fc γ R oligomers on the cell surface remains to be proven. However, since Fc γ R1I and Fc γ R1II are low-affinity IgG receptors whose activation requires antigen-bound immune complexes, it is tempting to speculate that these parallel receptor dimers may resemble the oligomers formed upon binding to immune complexes. This would increase the avidity of IgG–FcR interaction and facilitate the triggering of cellular responses. Figure 5C depicts a hypothetical receptor aggregation model with a 1:1 stoichiometry based on the parallel dimer observed in our current crystal.

Experimental Procedures

Protein Expression and Purification

The extracellular ligand binding domain of a human Fc γ R1IIb, residues 1–174 of the mature sequence, was subcloned into a Novagen pET-28b vector using the NcoI and NotI restriction sites and an *E. coli* BL21(DE3) strain. Two additional amino acids (Met–Ala) were added to the 5' end of the gene, and a histidine tag (Ala–Ala–Ala–Leu–Glu–His₆) was added to the 3' end to facilitate the expression and purification (Snyder et al., 1999). The protein was first expressed in an inclusion body form and then reconstituted in vitro. In brief,

cells containing the Fc γ R1IIb-expressing plasmid were grown in a 10 liter New Brunswick Bioflo 3000 bioreactor vessel and induced with 0.5 mM IPTG at an approximate OD₆₉₅ of 10.0 for 5 hr. Once harvested, the inclusion bodies were isolated by repeated washing with 2 M urea solution and then redissolved in 8 M urea prior to refolding. The refolding reaction was initiated by a quick dilution of the urea-dissolved inclusion bodies of Fc γ R1IIb into a refolding buffer consisting of 0.5 M arginine, 5 mM CaCl₂, 5 mM cysteamine, 5 mM cystamine, 10 μ g/ml AEBSF, and 50 mM Tris (pH 8.0) and then dialyzed thoroughly against water. The renatured Fc γ R1IIb was concentrated on a Ni-NTA affinity column and further purified on a Superdex 200 size-exclusion column. The integrity of the refolded receptor was analyzed both by N-terminal amino acid sequencing and by electrospray ionization mass spectrometry (ESI-MS).

Analytical Ultracentrifugation Experiments

Sedimentation equilibrium profiles were obtained in a Beckman XL-A, using absorbance optics at wavelengths of 230, 250, and 280 nm. One hundred and fifty microliters of samples in 5 mM phosphate buffer, at different concentrations of Fc and CD16, was used at rotor speeds of 14,000 and 18,000 rpm. Sedimentation equilibrium profiles of both CD16 and the Fc dimer were well described by that of thermodynamically ideal monomeric species, with buoyant molar masses consistent with those calculated from the amino acid sequence. Analysis of the mixtures was performed by a global fit of several equilibrium distributions at multiple rotor speeds, protein concentrations, and molar ratios.

Nonequilibrium and Equilibrium Gel Filtration Experiments

For nonequilibrium gel filtration experiments, Fc γ R1II was incubated with Fc (Fc concentration was kept at 10 μ M) at 1:1, 2:1, and 3:1 molar ratios in a running buffer of 50 mM Tris (pH 8.0) and 50 mM NaCl for at least 30 min. A sample of 25 μ l was injected onto a Superdex 200 PC 3.2/30 gel filtration column (Amersham Pharmacia Biotech) equilibrated with the same buffer at a flow rate of 0.1 ml/min. The absorbance of the eluent was monitored at 280 nm, and fractions in 0.1 ml volume were analyzed by SDS-PAGE. N-terminal peptide sequencing by Edman degradation method was used to quantify the amount of Fc γ R1II and Fc in the isolated receptor–Fc complex.

For equilibrium gel filtration experiments, the Superdex 200 PC 3.2/30 gel filtration column (Amersham Pharmacia Biotech) was first equilibrated with a buffer containing 50 mM Tris (pH 8.0), 50 mM NaCl, and 10 μ M Fc γ R1II. Samples (25 μ l) with molar compositions of 10 μ M Fc γ R1II:10 μ M Fc, 15 μ M Fc γ R1II:10 μ M Fc, 20 μ M Fc γ R1II:10 μ M Fc, and 30 μ M Fc γ R1II:10 μ M Fc were incubated at room temperature in the same buffer for at least 30 min before injection onto the column at a flow rate of 0.1 ml/min.

Crystallization, Data Collection, and Structure Determination

Two forms of Fc γ R1II crystals were obtained by hanging-drop methods. The first form was grown from a buffer of 10% PEG 4000, 50 mM Sodium HEPES (pH 7.0), and a protein concentration of \sim 7 mg/ml. Crystals grew to a size of 0.1 \times 0.2 \times 0.5 mm³ in about 1 month and diffracted to 2.3 Å resolution. A HgCl₂ derivative was identified using a mass spectrometry-assisted heavy atom screening method (Sun and Hammer, 2000). The mercury-derivative crystal was prepared by soaking crystals in a well solution containing 0.5% saturated HgCl₂ for 12 hr. Both the native (Native 1) and the mercury derivative data were collected at the Brookhaven NSLS X9B beamline under cryofreezing temperature (-180° C) and processed using HKL2000 (Table 1) (Otwinowski and Minor, 1997). The crystals belong to a space group P2₁2₁2 with the unit cell dimensions of a = 78.9, b = 147.8, and c = 36.4 Å and two molecules in each asymmetric unit. Multiwavelength anomalous dispersion (MAD) data were collected at the L_{III} edge of Hg (Table 1). The heavy atom binding sites were determined by both inspection of the difference Patterson maps and by using the SHELX program and were refined by using the program MLPHARE (Sheldrick, 1990; Otwinowski, 1994). The initial chain tracing and all subsequent model building were done using the program O, version 6.2.1 (Kleywegel and Jones, 1996). The structure was refined with CNS, version 0.9, using a maximum likelihood target function (Brunger et al., 1998) and without NCS

constraints. The refinement started with rigid body refinement for each domain and was followed by alternating positional and grouped B factor refinement. Maps were calculated and the model was revised using the program O at the end of each round of positional and B factor refinement. After all protein residues were built and refined, water molecules were added to peaks higher than 2–3 σ in the residual difference map and with likely hydrogen bonding partners. The second form of Fc γ R1II crystals was obtained under slightly different condition with 10% PEG 8000 and 50 mM sodium HEPES (pH 6.5) as the precipitation reagent. The crystals belong to the same space group, P2₁2₁2, as the first form but with different cell dimensions of a = 67.4, b = 85.7, and c = 36.3 Å and only one receptor molecule in the crystallographic asymmetric unit. A native data (Native 2, Table 1) was collected on an in-house Raxis IV imaging plate detector to 1.8 Å resolution. The interdomain hinge angle is defined as the angle between the principal ellipsoid axes of the D1 and D2 domains of the receptor and is calculated using the program HINGE (Snyder et al., 1999).

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Protein Data Bank ID Codes

The coordinates of Fc γ RIII have been deposited in the Protein Data Bank under the ID code 1FNL.