Structural Comparison of Fucosylated and Nonfucosylated Fc Fragments of Human Immunoglobulin G1

Shigeki Matsumiya1, Yoshiki Yamaguchi2, Jun-ichi Saito1, Mayumi Nagano2, Hiroaki Sasakawa3, Shizuo Otaki1, Mitsuo Satoh4, Kenya Shitara4 and Koichi Kato2,3,5

Removal of the fucose residue from the oligosaccharides attached to Asn297 of human immunoglobulin G1 (IgG1) results in a significant enhancement of antibody-dependent cellular cytotoxicity (ADCC) via improved IgG1 binding to Fcγ receptor IIIa. To provide structural insight into the mechanisms of affinity enhancement, we determined the crystal structure of the nonfucosylated Fc fragment and compared it with that of fucosylated Fc. The overall conformations of the fucosylated and nonfucosylated Fc fragments were similar except for hydration mode around Tyr296. Stable-isotope-assisted NMR analyses confirmed the similarity of the overall structures between fucosylated and nonfucosylated Fc fragments in solution. These data suggest that the glycoform-dependent ADCC enhancement is attributed to a subtle conformational alteration in a limited region of IgG1-Fc. Furthermore, the electron density maps revealed that the traces between Asp280 and Asn297 of our fucosylated and nonfucosylated Fc crystals were both different from that in previously reported isomorphous Fc crystals.

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Keywords: immunoglobulin G1; fucose; antibody-dependent cellular cytotoxicity; X-ray crystal structure analysis; NMR spectroscopy

Introduction

The Fc portion of immunoglobulin G (IgG) possesses one conserved glycosylation site at Asn297 in each of the Cγ2 domains, where complex biotennary type oligosaccharides are expressed. These carbohydrate moieties are essential for promotion of effector functions.1,2 Namely, IgG molecules with an aglycosylated Fc portion retain little...
ability in activating complements and binding to Fcγ receptors (FcγRs). The carbohydrate moieties of IgG-Fc exhibit various microheterogeneities resulting from the presence or absence of fucose (Fuc), bisecting N-acetylgalactosamine (GlcNAc), galactose, and sialic acid residues, depending on species, aging, pathological states, and culture condition of Ig-producing cells.23–13 Positive roles in the FcγR-mediated activities of the terminal galactose and bisecting GlcNAc residues have been reported but remain controversial.14–19

It has recently been shown that lack of Fuc on human IgG1 oligosaccharide enhances the binding to Fcγ receptor IIIa (FcγRIIIa) and thereby improves (>50-fold) antibody-dependent cellular cytotoxicity (ADCC).19 Hence, control of IgG fucosylation can be one of the most promising technologies to improve the efficacy of therapeutic antibodies.27 Indeed, low Fuc chimeric anti-CCR4 (chemokine (C-C motif) receptor 4) antibody has been demonstrated to have a higher antitumor activity than the highly fucosylated antibody in a murine xenograft model employing a CCR4-positive T-cell lymphoma and human peripheral blood mononuclear cells.22

To address the underlying mechanisms of affinity improvement upon the Fuc depletion, Okazaki et al. characterized the human IgG1–FcγRIIIa interaction by isothermal titration calorimetry and surface plasmon resonance experiments.23 The isothermal titration calorimetry data indicated that the IgG1–FcγRIIIa interaction was driven by favorable binding enthalpy but opposed by unfavorable binding entropy change. Fuc depletion from IgG1 enhanced the favorable ΔH, leading to an increase in the binding constant of IgG1 for the receptor by a factor of 20–30. The surface plasmon resonance analyses showed that the increase in affinity is mainly attributed to an enhanced association rate.

The crystal structures of human IgG1–FcγRIIIa interaction with soluble FcγRIIIa expressed by Escherichia coli indicate that the carbohydrate moieties expressed at Asn297 are packed between the two Cγ2 domains of Fc with the Fuc residues close to the FcγR-binding surface, but the Fuc and other oligosaccharide residues make little contact with the receptor.28–31 Recently, glycome-dependent conformational alteration of human IgG1–FcγRIIIa interaction has been revealed by stable-isotope-assisted NMR spectroscopy as well as X-ray crystallography.32,33 The crystal structures have shown that removal of the outer GlcNAc and the mannose (Man) residues of the Fc glycan induces the conformational change in the C/E loop (Gln295-Thr299) that contains the N-glycosylation site, resulting in the mutual approach of the Cγ12 domains. The NMR data demonstrated that cleavage at the GlcNAcβ1-4GlcNAc glycosidic linkage perturbs the structure of the lower hinge region, which forms the major FcγR-binding site. Although these structural studies have underlined the contribution of the non-Fuc residues of the carbohydrate chains to the structural integrity of IgG-Fc, no structural basis has so far been provided for the improvement of ADCC upon defucosylation.

This was largely due to the lack of an appropriate method to produce large amounts of nonfucosylated IgG with a homogeneous glycoform that is adequate for structural studies.

In this circumstance, we employed the FUT8−/− CHO cell lines to produce nonfucosylated IgG glycoproteins for structural analyses. By virtue of their abilities to produce almost completely nonfucosylated IgG molecules in large quantity, we report the first coordinated X-ray crystallographic and NMR spectroscopic studies of nonfucosylated human IgG1-Fc and compare the results with those of its fucosylated counterpart. Inspection of the crystallographic and NMR data suggests that the ADCC enhancement upon defucosylation is attributed to a subtle conformational alteration in a limited region of IgG1-Fc, if any. Furthermore, during this study, we noted that the traces for the glycosylated segments of our fucosylated and nonfucosylated Fc crystals were both different from those in previously reported isomorphous Fc crystals. We will discuss a cause of these differences based on electron density maps.

Results

Crystal structures of fucosylated and nonfucosylated Fc

For X-ray crystallographic analyses, we used KM3060 (chimeric anti-CCR4 antibody produced by CHO cells) and KM3575 (anti-CD20 chimeric antibody produced by FUT8−/− CHO cells) as fucosylated and nonfucosylated IgGs, respectively, because they could be produced in large quantities with tailored glycoforms and an identical amino acid sequence in their Fc portions carrying the human IgG1 isotype. Prior to papain digestion, both oligosaccharide profiling using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry and monosaccharide analysis by trifluoroacetate digestion on the two chimeric antibodies were performed, and both >99% fucosylation in KM3060 and no fucosylation in KM3575 were confirmed. Oligosaccharide analysis also revealed similar distributions of terminal galactosylation pattern; the molar ratio of agalactosyl glycan:monogalactosyl glycan:digalactosyl glycan was 44.6:48.9:6.5 for KM3060 and 65.9:31.7:2.4 for KM3575, each calculated as the distribution among the major composition with biantennary complex-type glycoform. Hence, the covalent structures of these two Fc preparations differed solely in their oligosaccharide structure, predominantly in Fuc contents. The Fc fragments prepared from KM3060 and KM3575 will be simply designated Fuc (+) and Fuc (−), respectively. These Fc fragments were subjected to crystallographic analyses.

The unit cell dimensions, space group, and molecular packing motifs of Fuc (+) and Fuc (−) crystals are nearly identical with each other and with those of previously reported Fc crystals obtained by
microdialysis. The asymmetric units of the Fc crystals were composed of two glycosylated peptide chains that form a horseshoe-shape dimer. Although these crystals differed in quality judging from maximum resolution and overall temperature factors presumably due to some differences in cryocrystallographic handling, the conformations of Fuc (+) and Fuc (−) were very similar: Superposition of Fuc (+) and Fuc (−) using all backbone atoms (1668 pairs) yielded RMS deviation of 0.32 Å. In the orthorhombic Fc crystals atoms belonging to the C122 domain of chain B have higher B-factors than those in the other domains. In this study Fuc (+) and Fuc (−) crystal structures were refined with a translation/libration/screw (TLS) option that can divide B-factors into contributions from rigid body movement of each domain and intrinsic mobility of individual atoms. Although the gross B-factors calculated by TLSANL exhibited the same trend with previous crystals, the residual B-factors of all domains were not very different within the crystal (Table 1 and Figure 1). This direct evidence confirms the supposition that the higher gross B-factors in the C122 domain of chain B are caused by large domain motion.32 The topology of each carbohydrate chain connected to Asn297 was determined from electron density (Figure 2). Fuc (−) could be easily distinguished from Fuc (+) through the absence of the blob attached to GlcNAc1. We located eight sugar residues (GlcNAc1–GlcNAc5, Man7, GlcNAc8, and Fuc) on both chains of Fuc (+). In the case of Fuc (−) we observed six on chain A (GlcNAc1–Man4, Man7, and GlcNAc8) and seven on chain B (GlcNAc1–GlcNAc5, Man7, and GlcNAc8). In the chain A of Fuc (−), GlcNAc5 could not be placed because of poor electron density, presumably due to high mobility of the distal residue. Although a considerable amount of the carbohydrate chains contained galactose at either end of the branched chains, we could not locate these galactose residues on the electron density maps of Fuc (+) or Fuc (−). Because of the large domain motion of the C122 domain, electron density maps around the glycosylation site of chain B were deteriorated compared with those of chain A in both crystals. In the better-defined electron density maps of chain A, two and one peaks that could be attributed to water oxygen atoms were unambiguously observed around Tyr296 for Fuc (+) and Fuc (−), respectively (Figure 3). The B-factors of these water molecules converged to reasonable values, i.e., 31.5 and 41.9 for Wat1 and Wat2, respectively, for Fuc (+) and 48.0 for Fuc (−), with several interatomic distances interpretable as hydrogen bonds. Thus, water molecules mediate the polypeptide chain and the carbohydrate chain with different manners between Fuc (+) and Fuc (−).

Solution structures of fucosylated and nonfucosylated Fc

To examine the conformational alteration induced by defucosylation in solution, we performed comparative NMR analyses using the Fc fragments from KM3060 and KM3416 with an amino acid sequence identical to that of Fuc (+) and Fuc (−), respectively. To avoid spectral complexity resulting from galactosylation heterogeneity,33 the isotopically labeled Fc fragments were subjected to galactosidase treatment prior to NMR measurements. HPLC profiling of the pyridylaminated oligosaccharides derived from the IgG1-Fc preparations confirmed that degalactosylation was achieved for both fucosylated (>96%) and nonfucosylated Fc (>99%).

We have made assignments of 131H–15N heteronuclear single-quantum coherence (HSQC) peaks originating from the polypeptide backbone of Fuc (+),33 which correspond to 66% of the amino acid residues except for the N-terminal and Pro residues and are used as spectroscopic probes to assess the conformational alteration upon depletion of Fuc. As an example, Figure 4 shows the 1H–15N HSQC spectra of Fuc (+) and Fuc (−) in which the amide groups of Tyr residues were selectively labeled with 15N. Among the nine Tyr residues, a significant chemical shift difference was observed solely for the HSQC peak from Tyr296. Similarly, spectral comparisons were carried out for Ala, Arg, Cys, Gly, His, Ile, Leu, Lys, Met, Phe, Ser, Thr, Trp, Tyr, and Val residues. In Figure 5, the amino acid residues showing and not showing significant chemical shift differences [(0.2δN + δ1H)/2]>0.1 ppm] between Fuc (+) and Fuc (−) were mapped on the crystal structure of Fuc (+). The spectral analyses of the anomeric regions of the 1H–13C HSQC spectra of the Fuc (+) and Fuc (−) glycoforms, in which the carbohydrate moieties were labeled with 13C, indicated that the Fuc residues have little influence on structure and dynamics of the Fc glycans (data not shown). These data indicated that Fuc depletion has an impact on quite limited residues in close spatial proximity thereof, i.e., Val1240, Tyr296, and Thr299. Tyr296 exhibited a significant broadening of the 1H–15N HSQC peak only for the Fuc (−) glycoform (Figure 4).

Table 1. Comparison of average B-factors (Å²)

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<th>Glycoform</th>
<th>Fuc (+)</th>
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<td>All Cα</td>
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<tr>
<td>Carbohydrates C1</td>
<td>26.1</td>
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</tr>
</tbody>
</table>

* Lys340 and precedent residues.
* Gly341 and the following residues.
* Glc296–Thr299.
* Asn325–Pro331.
indicating some chemical exchange process around this residue.

**Discussion**

**Comparison of the environments around glycosylation sites of Fuc (+) and Fuc (−)**

The crystal structures of the fucosylated and nonfucosylated Fc fragments were similar. The stable-isotope-assisted NMR analyses confirmed the similarity of the overall structures between fucosylated and nonfucosylated Fc fragments in solution. These data suggest that ADCC enhancement by defucosylation is attributed to a subtle conformational alteration in a limited region of IgG1-Fc, if any.

A marked difference around glycosylation sites other than fucosylation was found in the hydration mode (Figure 3). Chain A of Fuc (+) held two waters between Gln295, Tyr296, and Fuc through hydrogen bonds of <3.0 Å (Figure 3(a)). In particular, Wat1+
was strongly bound with O4 of Fuc and Wat2+ was kept on the plane formed by Gln295 O$_\varepsilon$, Tyr296 N, and Wat1+. On the other hand, hydrogen bond lengths between Wat1- and surrounding atoms (Tyr296 N, Asn297 N, and GlcNAc1 O5) of Fuc (−) exceed 3.0 Å. Also, the NMR data indicate that there

Figure 2. 2F$_{o}$−F$_{c}$ electron density maps around the carbohydrate moieties of IgG1-Fc fragments superimposed with refined molecular structures ((a) and (b)) and the carbohydrate sequence attached to Asn297 of Fc fragments (c). (a) Chain A of Fuc (+), contour level=0.25 eÅ$^{-3}$ (1.2 $\sigma$). (b) Chain A of Fuc (−), contour level=0.15 eÅ$^{-3}$ (1.2 $\sigma$).

Figure 3. Environment around Tyr296. Possible hydrogen bonds are indicated by blue broken lines accompanied by interatomic distance (Å). (a) Chain A of Fuc (+). (b) Chain A of Fuc (−).
exists a significant difference in the environment surrounding Tyr296 between Fuc (+) and Fuc (−), which might be attributed to the hydration mode variances found in the crystal structures.

Re-examination of the structures of the residues around the glycosylation site

After the pioneering work of Deisenhofer and co-workers, the molecular structures of Fc fragments in uncomplexed crystals have been described as having the folded down side-chain of Tyr296 beside Fuc and the C/C′ loop (Asp280–Ala287), a hook-shape trace at Gln283–Ala287 (residue 283 was Glu in our Fc samples), and inappropriate main chain conformations at Gln283–Tyr296.28,34 On the other hand, recent reports on Fc–Peptide complexes35,36 describe Fc fragments as having a straight and shorter trace at the C/C′ loops, and the hydroxyphenyl groups of Tyr296 tip up from the carbohydrate moieties. We call the former and the latter type of structures “classic” and “complex-type” models, respectively.

In the case of our Fc crystals the omit maps calculated from the diffraction data unambiguously revealed that the complex-type model is appropriate (Figure 6). For example, the shorter trace at Glu283–His285 (and the discontinuity at the region where His285 has been located in the former structures), the bulge corresponding to the five-member ring of Pro291 and the protrusion at Tyr296 were difficult to interpret with the classic model. Likewise, the real space R-factors at Asp280–Thr299 increase when the classic model was applied for our diffraction data (Table 2). Especially, the real space R-factors of His285 escalate from 7.0–12.6% for the complex-type model to 47.2–55.8% for the classic model. In addition, good Ramachandran plot statistics also support this interpretation (Table 3).

Sondermann and co-workers were aware of the inconsistency between their classic models and electron density maps at the C/C′ loops, but they ascribed this inconsistency to the intermolecular contacts within the crystals.32 In the orthorhombic Fc crystals, the C/C′ loop of each chain contacts the B/C′ loop (His268–Val273) of the neighboring molecule. Therefore, we cannot exclude the possibility that a slight difference in crystal contact affected the molecular structure, causing the conformational discrepancy between their crystals and ours. However, the conformational soundness, better fit to electron density and similarity with other crystalline forms, suggests that the native structure of Fc at Glu/Gln283–Tyr296 should be of the complex type. In addition, we surveyed electron density maps of IgG1 Fc archived at the Uppsala Electron Density Server37 and Protein Data Bank Japan. We found that 13 (1ADQ,38 1FCC,39 1H3T,32 1H3U,32 1H3V,32 1H3W,32 1H3X,32 1H3Y,32 1HZH,40 1I1A,41 1L6X,35 1OQO, and 1OQX (Raju et al., unpublished data)) of 14 maps exhibited a shorter trace at region 283–285, irrespective of amino acid sequences, ligand species, crystal packing, or deposited atomic coordinates. The only exception was 2GJ7,42 which was determined at 5.0 Å and had a hard-to-interpret map. Because these two archives calculate a map using atomic coordinates and structure factors deposited with the Protein Data Bank,43 the map is
Implications for the Fc–FcγRIII interaction

Using the classic Fc as the search model, two crystal structures of Fc–FcγRIII complexes have so far been solved for hexagonal (PDB ID: 1E4K)\textsuperscript{29} and orthorhombic (PDB ID: 1T83)\textsuperscript{30,31} forms, respectively. The Fc fragment in 1E4K shows a structure similar to that of the search model, whereas the Fc fragment in the 1T83 assumes a structure similar to that of Fuc (+) and Fuc (–): Modes of intermolecular interactions including the side-chain of Tyr296 are different between 1E4K and 1T83 (Figure 7). As in the case of uncomplexed Fc, 1E4K contains residues with inappropriate phi/psi angles at Gln283–Ser298, while most of the corresponding residues in 1T83 are conformationally acceptable.

In both crystal structures, the C′/E loop of Fc is directly involved in the intermolecular interaction with FcγRIII. Especially, the crystal structure 1T83 indicates interactions of Tyr296 with Lys125 and Asp126 of FcγRIII, which apparently require some conformational rearrangement of the C′/E loop including Tyr296 upon binding to FcγRIII. The present NMR data suggest a local conformational fluctuation around Tyr296 caused upon depletion of the Fuc residue (Figure 4). Inspection of the hydrogen bond patterns and dynamics in solution suggests that the conformation of Tyr296 is more flexible and thereby adjustable for FcγRIII in Fuc (–) than in Fuc (+). Our previous kinetic analyses...
revealed that an increase in affinity by defucosylation was mainly attributed to an enhanced association rate. The conformational fluctuation of Tyr296 may positively contribute to this process. In addition, our calorimetric analyses demonstrated that the affinity improvement by the Fuc depletion of IgG1 is characterized by favorable $\Delta H$, but opposed by unfavorable $\Delta S$. While the favorable $\Delta H$ indicates a more extensive formation of noncovalent interactions using the C'/E loop of Fuc (−) than using that of Fuc (+), the unfavorable $\Delta S$ observed for Fuc (−) may be ascribed, at least partially, to the reduction of conformational flexibility of Tyr296 upon complex formation with FcyRIII.

It has been reported that glycosylation of FcyRIIIa may affect affinity to IgG. A recent report suggested the possibility that the carbohydrate chain attached to Asn162 of FcyRIIIa interacts with the IgG1-Fc surface that is only accessible when Fc lacks the Fuc residue and thereby contributes to the affinity for Fc. This hypothetical model should be carefully examined because it has been based on the classic Fc crystal structure. Also, the dynamic property of Tyr296 and rearrangements of the water

**Figure 6.** Structural superposition of Val282–Asn297 of the classic model (Protein Data Bank code 1H3X) (cyan) and our Fuc (+) (yellow) rendered on the total omit map of Fuc (+) calculated using SFCHECK (CCP4) with a contour level of 0.40 eÅ$^{-3}$ (0.6 $\sigma$). (a) Val282–Lys288 of chain A. His268 of chain A (0.5 $\pm$ x, 0.5 $\mp$ y, 1 $\mp$ z) is shown in magenta. (b) Asn286–Asn297 of chain A. The carbohydrate chain is shown in green.
Table 2. Comparison of average real space R-factors (%)

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<th>Fuc (+) Chain B</th>
<th>Fuc (−) Chain A</th>
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* Lys340 and precedent residues.
* Gly341 and the following residues.
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* Lys288–Glu294.
* His285–Thr299.

Table 3. Crystallographic data and refinement statistics of the Fc fragments

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Materials and Methods

Establishment of human IgG1-producing cell lines

The Chinese hamster ovary (CHO)/DG44 cell line was kindly provided by Dr. Lawrence Chasin (Columbia University, NY). pKANTEX2160, an expression vector encoding an anti-CCR4 chimeric antibody with human IgG1/κ constant regions, was transfected into CHO/DG44 or FLUT8−/− cell line Ms704.19 High producing transfectants were selected as reported previously, and monoclonal antibodies purified from the culture supernatants of the CHO/DG44 and Ms704 cells were designated KM3060 and KM3416, respectively. Similarly, an expression vector encoding anti-CD20 chimeric IgG1/κ antibody was transfected into Ms704 and the antibody produced was thereby designated KM3575.

Protein expression and stable-isotope labeling for NMR study

Fucosylated and nonfucosylated human IgG1 were prepared by cultivating the transfectants with Nissui NYSF 404 medium supplemented with 2% dialyzed fetal bovine serum.47 Amino acid selective labeling of IgG1 for NMR analyses was performed as described previously.35,48 Selective 13C-labeling of oligosaccharides attached to Asn297 of IgG1-Fc was carried out using the reported protocol.49 After cell growth, the supernatant was purified using a protein A column.

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The Chinese hamster ovary (CHO)/DG44 cell line was kindly provided by Dr. Lawrence Chasin (Columbia University, NY). pKANTEX2160, an expression vector encoding an anti-CCR4 chimeric antibody with human IgG1/κ constant regions, was transfected into either CHO/DG44 or FLUT8−/− cell line Ms704.19 High producing transfectants were selected as reported previously, and monoclonal antibodies purified from the culture supernatants of the CHO/DG44 and Ms704 cells were designated KM3060 and KM3416, respectively. Similarly, an expression vector encoding anti-CD20 chimeric IgG1/κ antibody was transfected into Ms704 and the antibody produced was thereby designated KM3575.

Protein expression and stable-isotope labeling for NMR study

Fucosylated and nonfucosylated human IgG1 were prepared by cultivating the transfectants with Nissui NYSF 404 medium supplemented with 2% dialyzed fetal bovine serum.47 Amino acid selective labeling of IgG1 for NMR analyses was performed as described previously.35,48 Selective 13C-labeling of oligosaccharides attached to Asn297 of IgG1-Fc was carried out using the reported protocol.49 After cell growth, the supernatant was purified using a protein A column.
NMR measurements

The Fc fragment was dissolved in 0.5 ml of 5 mM sodium phosphate buffer, pH 6.0, containing 50 mM NaCl in \( H_2O:2H_2O=9:1 \). Final sample concentration was 0.1–1.5 mM. Two and three-dimensional heteronuclear NMR experiments were carried out on Bruker DMX-500, Avance-600, and JEOL ECA-920 spectrometers equipped with 5-mm triple-resonance probes at 1H frequencies of 500, 600, and 920 MHz, respectively. The probe temperature was set to 52 °C. Spectral assignments were made as described.1 H chemical shifts were given from external sodium 2,2-dimethyl-2-silapentane-5-sulfonate, while 15N chemical shifts were from liquid ammonia at 21.6 ppm.

Preparation of IgG1-Fc

The Fc fragments of IgG1s were prepared by papain digestion according to the literatures.50,51 Purity of the isolated Fc fragment was checked by SDS-PAGE. Galactosidase treatment of the Fc fragment was carried out as described.33 Briefly, Fc (2 mg/ml) dissolved in 50 mM sodium acetate buffer, pH 5.5, was incubated at 37 °C for 72 h in the presence of 0.02 units/ml of \( \beta \)-galactosidase with 10 mM MnCl\(_2\). The reaction mixture was neutralized with 1.5 M Tris–HCl (pH 8.5) and then applied onto a protein A column for purification of Fc.

N-glycosylation profiling of Fc was carried out by an HPLC mapping technique, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, and monosaccharide analysis as described previously.33,52

Crystal structure analyses

The crystals of Fc fragments were prepared by microdialysis against purified water. The crystals were sequentially dialyzed overnight against 5, 10, 15, 20, 25, and 30% (v/v) 2,3-butanediol solutions for cryoprotection. X-ray diffraction data were collected at the Pharmaceutical Industry Beamline BL32B2 of SPring-8 at a temperature of 100 K. The diffraction images were taken using synchrotron radiation of 1.0000 Å wavelength and a Rigaku/MSC Jupiter210 CCD detector. The diffraction images were processed by MOSFLM, SCALA, and TRUNCATE programs in the CCP4 program suite to yield 50.00 to 2.00-Å data for Fuc (+) and 50.00 to 2.20-Å data for Fuc (−). The difference in redundancy was derived from the oscillation range, which was wider for Fuc (+) than for Fuc (−). Molecular replacement calculations were performed using PHASER54 with the crystal structure of the Fc fragment of human IgG1 Cri32 (Protein Data Bank ID: 1H3X) as the search model. The initial models obtained from molecular replacements were iteratively refined and rebuilt to fit electron density maps using CNX (Accelrys) and O55 and waters were picked up from the difference Fourier map using CNX. The resulting structures were divided into six TLS groups (CH2 domain (Lys340 and preceding residues), CH3 domain (Gly341 and the following residues), and carbohydrate chain for each molecule) subjected to the re-
refinement using REFMAC5\(^55\) with the TLS refinement option. After refinements, atomic temperature factors (B-factors) were recalculated with TLSANL (in the CCP4 suite) to estimate the amplitude of domain motions. Ramachandran plots and residue-by-residue real space R-factors were calculated using PROCHECK\(^57\) and CNX, respectively. Unit cell dimensions and data statistics are summarized in Table 3. Molecular structure drawings were generated using RasMol\(^58\) and DINO (A. Philippsen).\(^\dagger\)

**Protein Data Bank accession numbers**

The atomic coordinates and structure factors have been submitted to the Protein Data Bank (ID: 2DTQ for fucosylated Fc and 2DTS for nonfucosylated Fc).

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\(\dagger\)http://www.dino3d.org


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