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Short communication

Room temperature structure of human IgG4-Fc from crystals analysed *in situ*

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ABSTRACT

The Fc region of IgG antibodies (Cγ2 and Cγ3 domains) is responsible for effector functions such as antibody-dependent cell-mediated cytotoxicity and phagocytosis, through engagement with Fcγ receptors, although the ability to elicit these functions differs between the four human IgG subclasses. A key determinant of Fcγ receptor interactions is the FG loop in the Cγ2 domain. High resolution cryogenic IgG4-Fc crystal structures have revealed a unique conformation for this loop, which could contribute to the particular biological properties of this subclass. To further explore the conformation of the IgG4 Cγ2 FG loop at near-physiological temperature, we solved a 2.7 Å resolution room temperature structure of recombinant human IgG4-Fc from crystals analysed *in situ*. The Cγ2 FG loop in one chain differs from the cryogenic structure, and adopts the conserved conformation found in IgG1-Fc; however, this conformation participates in extensive crystal packing interactions. On the other hand, at room temperature, and free from any crystal packing interactions, the Cγ2 FG loop in the other chain adopts the conformation previously observed in the cryogenic IgG4-Fc structures, despite both conformations being accessible. The room temperature human IgG4-Fc structure thus provides a more complete and physiologically relevant description of the conformation of this functionally critical Cγ2 FG loop.

1. Introduction

IgG effector functions, such as antibody-dependent cell-mediated cytotoxicity, antibody-dependent cellular phagocytosis and complement activation, are mediated by the antibody Fc region (Cγ2 and Cγ3 domains). The IgG4 subclass binds certain Fcγ receptors with lower affinity than IgG1 and IgG3 (Bruhns et al., 2009), and does not activate complement (van der Zee et al., 1986). Antibody determinants that influence the affinity for Fcγ receptors include sequence variation in the Cγ2 domain and hinge region, and the composition of the oligosaccharide moiety attached to the Cγ2 domain (Canfield and Morrison, 1991; Shields et al., 2002).

The Cγ2 domain FG loop (residues 325–331) plays a crucial role in the interaction with Fcγ receptors, in which Pro329 from the FG loop forms a hydrophobic "proline sandwich" interaction with two tryptophan residues from the receptor (Sondermann et al., 2000), and is also involved in the interaction between IgG1/3 and C1q (Canfield and Morrison, 1991; Tao et al., 1991, 1993; Idusogie et al., 2000). While the conformation of the Cγ2 domain FG loop is conserved in IgG1, high resolution cryogenic crystal structures of IgG4-Fc revealed a different, unique conformation for the Cγ2 FG loop, which would disrupt the interaction with Fcγ receptors (Davies et al., 2014b). Subsequent cryogenic crystal structures of IgG4-Fc (Davies et al., 2014a) and intact IgG4 (Scapin et al., 2015) revealed that the IgG4 Cγ2 FG loop could also adopt the conserved IgG1-like conformation. However, the role of the unique loop conformation in modulating the biological properties of IgG4, and whether one, or both, conformations could be adopted at physiological temperature, and in solution, remains unclear.

Using a technique to collect data from crystals *in situ* (Axford et al., 2015), we solved a 2.7 Å resolution room temperature (RT) structure of recombinant human IgG4-Fc. The RT IgG4-Fc structure

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reveals conformational diversity in the Cγ2 FG loop. In contrast to the cryogenic structure, the FG loop adopts the IgG1-like conformation in one Cγ2 domain, with substantial changes to the crystal packing interactions at the higher temperature which would preclude the unique conformation due to steric clashes. On the other hand, the FG loop from the other Cγ2 domain is able to adopt either conformation – in fact it adopts the unique, IgG4-like conformation at room temperature, a conformation that would disrupt the interaction with Fcγ receptors.

2. Materials and methods

2.1. Protein production and crystallisation

Recombinant, glycosylated human IgG4-Fc was produced and crystals were grown as described previously (Davies et al., 2014b), with the following modification: a Greiner Bio-One CrystalQuick^{TM} X plate was set up using a reservoir volume of 20 μL, and drops comprising 0.5 μL protein (3 mg/mL) and 0.5 μL reservoir. Crystals typically started to appear after one day.

2.2. Data collection, structure determination and refinement

Data were collected at room temperature (293 K) at beamline I03 at the Diamond Light Source (Harwell, UK) from crystals *in situ*. Small wedges (typically 3°–6°) of data were collected from different crystals, or spatially distinct regions from a single crystal, using an oscillation per image of 0.2°. For multiple datasets collected from a single crystal, the oscillation start angle for each dataset was incremented by 2°. Over 200 partial datasets from 48 isomorphous crystals were collected in this manner. Integration was performed with XDS (Kabsch, 2010) within the *xia2* package (Winter, 2010) and further processing was carried out using POINTLESS (Evans, 2011), SORTMTZ, AIMLESS (Evans and Murshudov, 2013) and TRUNCATE (French and Wilson, 1978) from the CCP4 suite (Winn et al., 2011). Only the first 10 images (2° of data) from each partial dataset that had been successfully integrated with XDS, with Batch R_{merge} values of 40% or less, were typically used for scaling, with 129 runs of data finally included. The structure was solved by molecular replacement with PHASER (McCoy et al., 2007) using protein atoms from PDB: 4C54 as a search model, with residues 325–331 omitted from the model. Refinement was performed with PHENIX (Adams et al., 2010), using the "Optimize X-ray/stereochemistry weight" and "Optimize X-ray/ADP weight" options, and manual model building was performed with *Coot* (Emsley et al., 2010). For both chains of the asymmetric unit, the Cγ2 domain FG loop conformation was validated by inspection of $2F_o$-F_c and F_o-F_c electron density maps following refinement with residues 325–331 omitted from the model (Fig. 1). Structure quality was assessed with MolProbity (Chen et al., 2010) within PHENIX. Data processing and refinement statistics are presented in Table 1. Interfaces were analysed with PISA (Krissinel and Henrick, 2007) and figures were produced with PyMOL (The PyMOL Molecular Graphics System, Version 1.1r1, Schrödinger, LLC).

3. Results and discussion

3.1. Overall structure

The asymmetric unit of the room temperature (RT) recombinant human IgG4-Fc crystal structure solved from crystals *in situ* contains one Fc molecule, comprising two chains (A and B). Residues Gly236-Ser444 and Gly237-Ser444 were built for chains A and B, respectively. A heptasaccharide core, covalently linked to Asn297 in the Cγ2 domain, was modelled for each chain. Each oligosaccharide moiety additionally contains a fucose residue attached to the first N-acetylglucosamine residue. The quality of the electron density map is illustrated for the oligosaccharide moiety from chain A in Fig. 2A.

The RT structure belongs to the same crystal form (space group $P\,2_1\,2_1\,2_1$) previously reported for the cryogenic recombinant IgG4-Fc crystal structure (Davies et al., 2014b), and the overall domain topology is comparable. However, the Cγ2 domains adopt a slightly more "open" conformation at room temperature *i.e.* they are further apart from one another compared with their position in the cryogenic structure (Fig. 2B). For example, the Cα atoms for Val323 are 34.8 Å and 36.0 Å apart in the cryogenic and RT structures, respectively.

Despite belonging to the same crystal form, the *b* and *c* unit cell dimensions in the RT structure ($b = 81.93$ Å, $c = 103.88$ Å) are ~3 Å and 6 Å longer, respectively, than those in the cryogenic recombinant IgG4-Fc structure ($b = 78.97$ Å, $c = 97.88$ Å). The longer unit cell dimensions at room temperature are mostly attributed to a conformational difference in the Cγ2 FG loop in chain B.

3.2. Crystal packing interactions for the Cγ2 domain of chain A

With the exception of residues Asp280-Val282, crystal packing interactions for the Cγ2 domain from chain A are similar in both

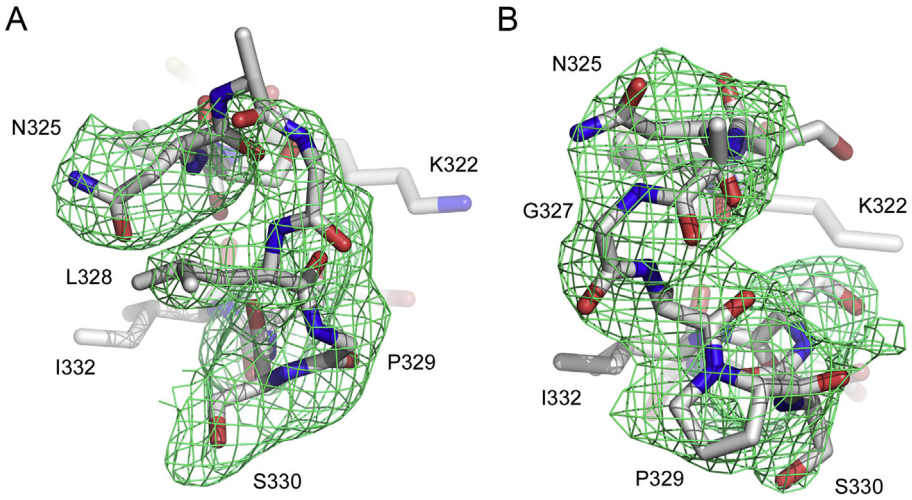

Fig. 1. Electron density for the Cγ2 domain FG loop. (A) Cγ2 FG loop from chain A. (B) Cγ2 FG loop from chain B. F_o-F_c maps are shown, contoured at 2.5σ. Residues 325–331 were omitted from the model prior to refinement.