



Crystal structure of the human IgG4 C_H3 dimer reveals the role of Arg409 in the mechanism of Fab-arm exchange

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ABSTRACT

Antibodies of the human IgG4 subclass uniquely undergo a process of Fab-arm exchange in which the heavy-chains of antibodies of different specificities can dissociate and then recombine. The mechanism by which the resulting functionally monovalent but bi-specific antibodies are formed is not only key to understanding their biological role, but is also important for the design of therapeutic monoclonal antibodies. Both the hinge region and the C_H3 domain interface are known to be involved, and of the residues that differ between human IgG1 and IgG4 in C_H3, residue 409, the only difference at the interface itself, has been implicated. We report the high resolution (1.8 Å) structure of the C_H3 domain dimer of IgG4, and find that Arg409 in IgG4, when compared with Lys409 observed in high resolution IgG1 structures, disrupts a network of water-mediated hydrogen bonding that is conserved in IgG1. Other conformational differences were detected that are a consequence of the presence of Arg409, such as a widening of the separation between residues Asn390 in one domain and Ser 400 in the other, which opens up a groove at the edge of the interface in IgG4 compared with IgG1. The effect of all these differences on the C_H3 interface, doubled as a result of the interface's two-fold symmetry, is weakening of the inter-domain interaction in IgG4 compared with IgG1. This suggests a mechanism by which Arg409 weakens the C_H3 interface in IgG4, predisposing this human antibody subclass to Fab-arm exchange.

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1. Introduction

The four sub-classes of human IgG antibodies all comprise two heavy and two light chains connected by inter-chain disulphide bonds, but a unique feature of IgG4 is its ability to exist as a “half molecule”, composed of one heavy and one light chain (Aalberse and Schuurman, 2002). IgG4 half molecules can combine with one another in a process known as “Fab-arm exchange” (FAE) to form bi-specific antibodies, which are functionally monovalent (Aalberse and Schuurman, 2002; Schuurman et al., 1999). This phenomenon occurs *in vivo* and is of interest not only because of its impact on the humoral immune response, but due to the ability of therapeutic IgG4 antibodies to exchange with endogenous IgG4 (Labrijn et al., 2009; van der Neut Kolfshoten et al., 2007).

The ability of IgG4, but not IgG1 (or the other subclasses), to undergo FAE has been attributed to the core hinge region sequence (residues 226–CPPCP–230 in IgG1 and 226–CPSCP–230 in IgG4) and the C_H3 domains (Angal et al., 1993; van der Neut Kolfshoten et al., 2007). The serine residue, rather than proline, at position 228 in the IgG4 core hinge is believed to render it more flexible and favour the formation of intra- rather than inter-heavy chain disulphide bonds (Angal et al., 1993; Bloom et al., 1997; Schuurman et al., 2001). The C_H3 domain, whose sequence in IgG1 (G1m1, 17 allotype) and IgG4 differs at only six positions, clearly plays a crucial role in FAE, as hybrid IgG4 antibodies with an IgG1 C_H3 domain do not undergo exchange (van der Neut Kolfshoten et al., 2007). Recently, it was shown that C_H3 dimer dissociation is a rate-limiting step in the exchange mechanism, especially in the absence of an inter-chain disulphide bond (Rispens et al., 2011).

The ability to undergo FAE depends upon the strength of the C_H3 dimer interaction (Labrijn et al., 2011; Rose et al., 2011). Of the six sequence differences between IgG1 (G1m1, 17 allotype) and IgG4 in C_H3 (R355Q, D356E, L358M, K409R, Q419E and P445L in IgG1 and IgG4 respectively), only residue 409 is located at the dimer interface. Mutation of Lys409 in IgG1 confirmed that this residue plays

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an important role in C_H3 dimer stability (Dall'Acqua et al., 1998) and recent work has established Arg409 as the key C_H3 domain residue in IgG4 affecting FAE (Labrijn et al., 2011). The apparent K_d of the IgG4 C_H3 domain (determined as 3.8×10^{-9} M by FRET (Rispeens et al., 2011) and 4.8×10^{-8} M by mass spectrometry (Labrijn et al., 2011; Rose et al., 2011)) is reduced when Arg409 is mutated to Lys409 (K_d of 8×10^{-9} M (Labrijn et al., 2011)), as found in IgG1.

While the structure of IgG1 has been studied extensively, the only crystal structure available for IgG4 is that of the Fc fragment at low resolution (3.15 Å) (Corper et al., 1997). Higher resolution is required to establish the role of Arg409 in FAE and we therefore determined the crystal structure of the isolated IgG4 C_H3 domain at 1.8 Å resolution, which now provides a detailed view of Arg409 and its environment at the C_H3 interface. Arg409 is found to disrupt a conserved network of water molecules that mediate inter-domain hydrogen bonds, in addition to negatively affecting contact between other interface residues, thus revealing how it exerts its effect upon FAE in IgG4.

2. Materials and methods

2.1. Cloning, expression and purification

Cloning, expression and purification of the C_H3 protein has already been described (Rispeens et al., 2010). The protein was dialysed into 20 mM Tris–HCl pH 8.5 before commencing crystallisation trials.

2.2. Mass spectrometry

Protein samples were dialysed into a solution of H₂O, 10% acetonitrile and 0.1% formic acid prior to MS analysis. Samples at a concentration of 1 µg/mL were directly infused at 40 µL/min into a Bruker Maxis ultrahigh-resolution electrospray ionisation quadrupole time-of-flight mass spectrometer. The multiple charge states were deconvoluted into a most probable single mass using the manufacturer's maximum entropy algorithm (Bruker Daltonics). The observed mass of 11,922.1 is consistent with the predicted C_H3 construct with an N-terminal pyroglutamate modification and lacking the C-terminal lysine residue (a common modification for recombinantly produced antibodies (Harris, 1995)). A mass of 11,865.1 was also observed, which likely has the two C-terminal residues (glycine and lysine) cleaved and the N-terminal pyroglutamate modification.

2.3. Crystallisation

Crystallisation was performed using the sitting drop vapour diffusion method in SWISSCI MRC 2 well crystallisation microplates and a Mosquito[®] robot. Crystals were grown at 291 K using a reservoir of 100 µL 22% (w/v) PEG 3350 and 150 mM calcium acetate, a protein concentration of 1.5 mg/mL, and a drop size of 100 nL protein and 100 nL reservoir. Crystals typically appeared after 1–2 days and were briefly cryoprotected in a solution of 20% (w/v) PEG 3350, 200 mM calcium acetate and 18% ethylene glycol before cooling in liquid nitrogen.

2.4. Data processing, structure determination and refinement

Data were collected on beamline I04 at the Diamond Light Source, UK. Integration was performed with XDS as implemented in the xia2 package (Kabsch, 2010; Winter, 2010) and additional processing was carried out using the CCP4 suite (Winn et al., 2011). Calculation of the Matthews coefficient suggested the presence of two C_H3 domains in the asymmetric unit, with an estimated solvent content of 46%. The structure was solved by molecular replacement

Table 1
Data processing and refinement statistics.

<i>Data processing</i>	
Space group	P4 ₃ 22
Unit cell dimensions	
<i>a</i> , <i>b</i> , <i>c</i> (Å)	57.09, 57.09, 138.44
Resolution (Å)	27.96 – 1.80 (1.85 – 1.80) ^a
No. of unique reflections	22 102 (1 609) ^a
Completeness (%)	99.9 (99.9) ^a
Redundancy	27.1 (26.3) ^a
Mean (<i>I</i>)/σ(<i>I</i>)	25.8 (4.7) ^a
<i>R</i> _{merge} (%)	8.3 (76.4) ^a
<i>Refinement</i>	
<i>R</i> _{work} / <i>R</i> _{free} (%)	18.26/23.68
RMSD	
Bond lengths (Å)	0.006
Bond angles (°)	1.083
Coordinate error (Å)	0.24
No. of TLS groups	
Protein chain A/B	15/15
No. of atoms	
Protein	1667
Solvent	195
Other ^b	79
Ave. <i>B</i> factor (Å ²)	
Protein	29.3
Solvent	41.2
Other ^b	47.0
Ramachandran plot ^c	
Favoured (%)	99.5
Allowed (%)	100

^a Numbers in parentheses are for the highest resolution shell.

^b Ethylene glycol, polyethylene glycol, tris buffer (2-amino-2-hydroxymethylpropane-1,3-diol), acetate ion.

^c Ramachandran plot generated by MolProbity (Chen et al., 2010).

with MOLREP (Vagin and Teplyakov, 1997) using IgG4 C_H3 domain protein atoms from PDB entry 1ADQ as a search model (Corper et al., 1997). Refinement was performed with PHENIX (Adams et al., 2010), and manual model building with Coot (Emsley et al., 2010). 5% of reflections were assigned to the *R*_{free} reflection set using PHENIX. TLS groups were assigned using the TLSMD server (Painter and Merritt, 2006). Overall structure quality was assessed with MolProbity (Chen et al., 2010) and SFCHECK (Vaguine et al., 1999). Data processing and refinement statistics are summarised in Table 1. Coordinates and structure factors have been deposited at the Protein Data Bank (PDB) with the codes 4B53 and 4B53SF respectively.

2.5. Structure analysis

The classical C_H3 homodimer observed for immunoglobulin structures is present in the crystal. The dimer was used to perform a DALI structural similarity search (Holm and Rosenström, 2010) and results were filtered to exclude structures that were not human IgG1 or IgG4. Buried surface area calculations were performed with CNS (Brünger et al., 1998) for IgG1 C_H3 dimers (solved at 2.7 Å resolution or higher) and the IgG4 C_H3 dimer (Corper et al., 1997) in which two Lys/Arg409 interfaces are present; additionally, to account for potential variation between the two Lys/Arg409 sites and to assess the contribution of a single Lys/Arg409 site, buried surface areas were also calculated for “single interface” dimers by removing half of the C_H3 interface. The Lys/Arg409 environment was analysed in detail for human IgG1 structures solved to 2.7 Å resolution or higher (Table S1). Where possible, structures were analysed in conjunction with 2Fo-Fc and Fo-Fc electron density maps automatically calculated by the Electron Density Server (Kleywegt et al., 2004). As the Lys409 (IgG1)/Arg409 (IgG4) interfaces comprise residues from both domains of the dimer, residues from the opposite domain are denoted by a prime symbol.

3. Results and discussion

3.1. Overall structure

The overall structure of the IgG4 C_H3 domain is a typical immunoglobulin fold, essentially identical to that described for IgG1 (e.g. RMSD of 0.29 Å with PDB entry 1L6X for C α atoms) (Idusogie et al., 2000), and the low resolution IgG4 structure (RMSD of 0.39 Å with PDB entry 1ADQ for C α atoms) (Corper et al., 1997). Superposition of the C_H3 dimer on the low resolution IgG4 structure, and various IgG1 structures (e.g. PDB entries 1L6X, 1H3X (Krapp et al., 2003), 1OQO (<http://dx.doi.org/10.2210/pdb1oqo/pdb>; Raju et al., unpublished), 2J6E (Duquerroy et al., 2007), 3AGV (Nomura et al., 2010)), revealed that the positions of C α backbone atoms were essentially identical, indicating that no structural alteration had occurred at the interface as a result of absence of the CH₂ domains. Residues 342–445 and 342–443 were built for chains A and B of the C_H3 dimer respectively. No electron density was present for residues Gly446 and Lys447. However, analysis by mass spectrometry indicated that some modification of the sample had occurred prior to crystallisation. Electron density at the N-terminus suggested conversion of residue Gln342 to pyroglutamate in both molecules (Fig. 1A), confirmed by mass spectrometry (Fig. 2). Conversion of glutamine to pyroglutamate may be considered fortuitous, as in both molecules its O ϵ atom forms a crystal contact in the form of a water-mediated hydrogen bond with a symmetry-related molecule. The structure contained five ethylene glycol molecules, four polyethylene glycol molecules, one molecule of tris buffer, and one acetate ion, but none of these species were located at the C_H3 interface in the vicinity of Arg409.

3.2. Lys409 environment in IgG1

High resolution IgG1 structures provide a detailed view of the Lys409 environment and its involvement in the C_H3 interface. In a typical high resolution IgG1 structure (e.g. PDB code 3AVE) (Matsumiya et al., 2011), the Lys409 side chain N ζ atom points towards interfacing residues, oriented through a van der Waals interaction with Phe405' from the other C_H3 domain. Glu357, Ser364, Asn390 and Thr411 from the same C_H3 domain as Lys409 contribute to the C_H3 interface, as do Lys370', Asp399' and Ser400' from the other. The C_H3 interface in the immediate vicinity of Lys409 is predominantly hydrophilic in nature, although there are only two inter-domain hydrogen bonds between residue side chains, namely Lys409 and Asp399', and Asn390 and Ser400'.

However, a series of water molecules at the C_H3 interface, located in two conserved and two semi-conserved positions, mediate a network of inter-domain hydrogen bonds (Fig. 3A). Water1, semi-conserved and present in 46% of IgG1 structures analysed, mediates an inter-domain hydrogen bond between Glu357 and Lys370'. Water2, present in 74% of structures lies in a position to mediate inter-domain hydrogen bonds between Ser364, Thr411, Lys409 and Lys370'. It is noteworthy that Water2 is tetrahedrally coordinated by these protein side chains, as this particular type of coordination geometry has been found to “stiffen” proteins (Mao et al., 2000).

Likewise Water4, present in 46% of structures, can form a hydrogen bond with Water3, and mediate another between Asn390 and Asp399'. In contrast Water3, present in 82% of structures, does not mediate inter-domain hydrogen bonds but forms hydrogen bonds with Lys409 (side and main chain), Thr411 and Water4. Water 3 thus plays an indirect role in the inter-domain hydrogen bond network, and may also be important for orienting the Lys409 side chain.

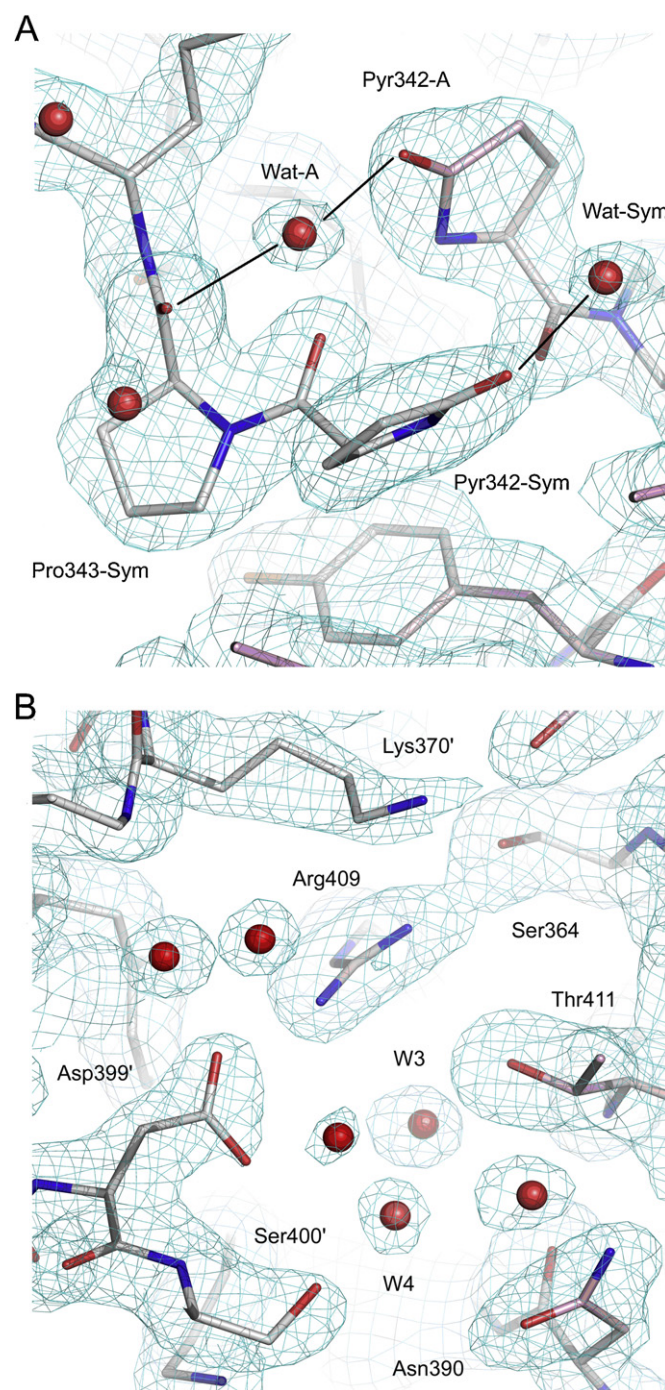


Fig. 1. (A) N-terminal residue Gln342 is modified to pyroglutamate. Electron density for pyroglutamate at the N-terminus is shown using a 2Fo-Fc electron density map contoured at 1 σ . Wat-A forms hydrogen bonds with pyroglutamate at the N-terminus of molecule A (Pyr342-A), and the main chain carbonyl of Pro343 from a symmetry related molecule (Pro343-Sym), thus creating a crystal contact. Likewise, Wat-Sym forms hydrogen bonds with pyroglutamate from the N-terminus of the symmetry related molecule (Pyr342-Sym) and the main chain carbonyl of Pro343 from molecule A (not shown). Carbon atoms from C_H3 molecule A are coloured in pink, while those of the symmetry related molecule are coloured in grey. Water molecules are depicted as red spheres, and hydrogen bonds as black lines. (B) The Arg409 environment. Electron density for Arg409 and surrounding residues is shown using a 2Fo-Fc electron density map contoured at 1 σ . Carbon atoms from the two C_H3 domains are coloured in pink and grey, and water molecules are depicted as spheres. Conserved water molecules Water3 (W3) and Water4 (W4) are labelled. (For interpretation of the references to colour in the artwork, the reader is referred to the web version of the article.)

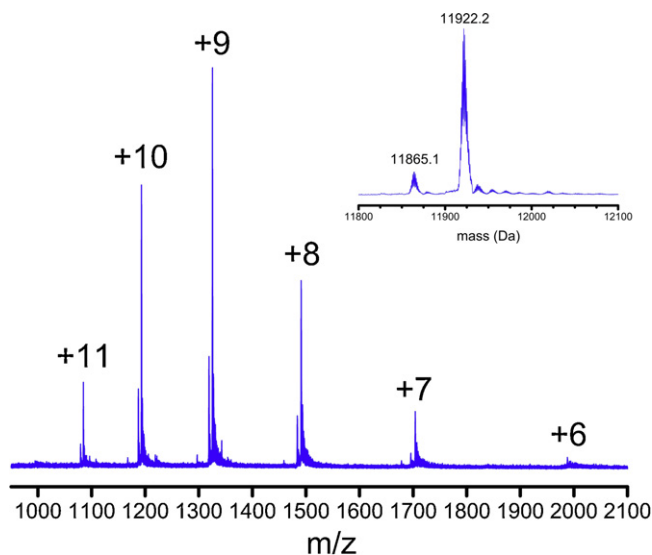


Fig. 2. Unprocessed raw and deconvoluted (inset) mass spectrometry data for the IgG4 C_H3 domain construct. The sample was dialysed into H₂O/acetonitrile/formic acid (90:10:0.1 by volume) and protein was directly infused at a concentration of 1 µg/mL into a Bruker MaXis electrospray tandem TOF mass spectrometer. The dominant peak of 11,922.2 Da is consistent with the predicted molecular weight of a C_H3 construct that forms the expected intramolecular disulfide bond, lacks the C-terminal lysine residue and has an N-terminal pyroglutamate modification, as observed in the structure (Fig. 1A). A lower intensity peak is observed with a mass of 11,865.1 Da; this is 57 Da smaller than the dominant protein species and has likely also had the penultimate glycine residue removed.

3.3. Arg409 disrupts a conserved water network in IgG4

In the IgG4 C_H3 domain, Phe405' orients the Arg409 side chain towards the hydrophilic interface residues, and Arg409 also forms a hydrogen bond with Asp399'. The more bulky guanidinium group occupies an equivalent position to Water2 in IgG1, and this conserved water molecule is absent in IgG4 (Figs. 1B and 3B). In the absence of Water2, Arg409 instead forms an intra-domain

hydrogen bond with Ser364. Thr411 and Lys370' are without a hydrogen bond partner, and thus three potential inter-domain hydrogen bonds that are present in IgG1 are absent in IgG4 as a direct result of this mutation.

In IgG1, the Nζ atoms of Lys409 and Lys370' are bridged by Water2, and the smallest inter-atomic Nζ–Nζ' distance observed is 4.15 Å (the largest is 5.97 Å, with an average distance of 4.95 Å). In IgG4, not only is Water2 absent, but if Lys370' were to adopt the same conformation as in IgG1, the closest Arg409 and Lys370' nitrogen side chain atoms would be only ~3 Å apart. Therefore, in IgG4, the Lys370' side chain moves to accommodate the presence of Arg409, and the distance between the closest nitrogen side chain atoms are 4.02 Å and 4.19 Å in the two C_H3 domains. The new position of the Lys370' Nζ atom is close to that occupied by Water1 in IgG1. Like Water2, Water1 is absent in IgG4, and the inter-domain water-mediated hydrogen bond between Glu357 and Lys370' is lost. In one C_H3 domain, the side chain of Lys370' is in a position to form an inter-domain hydrogen bond with Glu357, and in both, an intra-domain hydrogen bond with Gln347' is also possible. Incidentally, absence of Water1 in 54% of IgG1 structures is not due to an altered Lys370 conformation. Water3 is present in IgG4 and could form a hydrogen bond with the Arg409 main chain, Thr411 and Water4. Water4 forms a hydrogen bond with Water3 and Asp399', but not Asn390, as in IgG1.

In a recent *in silico* model of the IgG4 C_H3 interface, the Arg409 guanidinium group was observed to occupy the same location as a buried water molecule in IgG1 – referred to as Water2 in this work (Labrijn et al., 2011). However, the overall Arg409 side chain position, and resulting hydrogen bond network, is different from that observed in the C_H3 domain crystal structure.

3.4. The buried surface area of the IgG4 C_H3 interface is lower than that of IgG1

The buried surface area of the human IgG1 C_H3 interface ranges from 2186 Å² to 2596 Å², with an average value of 2313 Å². At 2255 Å², the C_H3 interface of the low resolution IgG4 structure (Corper et al., 1997) falls within this range, but is below the IgG1

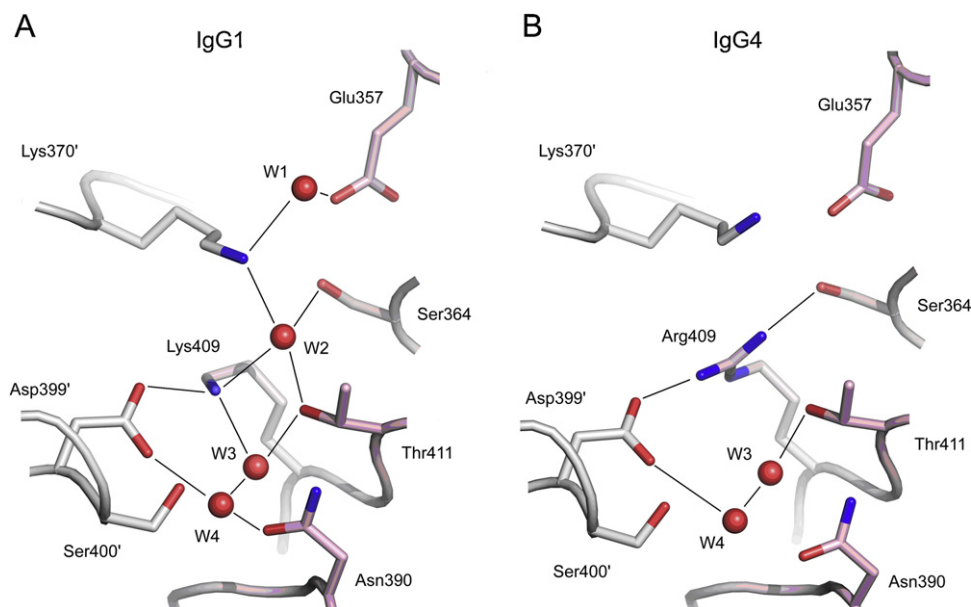


Fig. 3. The environment of residue 409. (A) Lys409 in IgG1. Residues in the vicinity of Lys409 are shown, with carbon atoms coloured in grey and pink to indicate the two C_H3 domains. The four conserved water molecules (Waters 1–4) are labelled W1–W4. Potential hydrogen bonds are depicted as black lines. For clarity, the hydrogen bond between Asn390 and Ser400' is not indicated. PDB entry 3AVE for IgG1 Fc (Matsumiya et al., 2011) is shown. (B) Arg409 in IgG4. The orientation and colour scheme is the same as for (A). Only two of the four conserved water molecules, Water3 (W3) and Water4 (W4), are present in IgG4, and the hydrogen bond network, depicted as black lines, is altered. For clarity, Phe405' is not shown. (For interpretation of the references to colour in the artwork, the reader is referred to the web version of the article.)

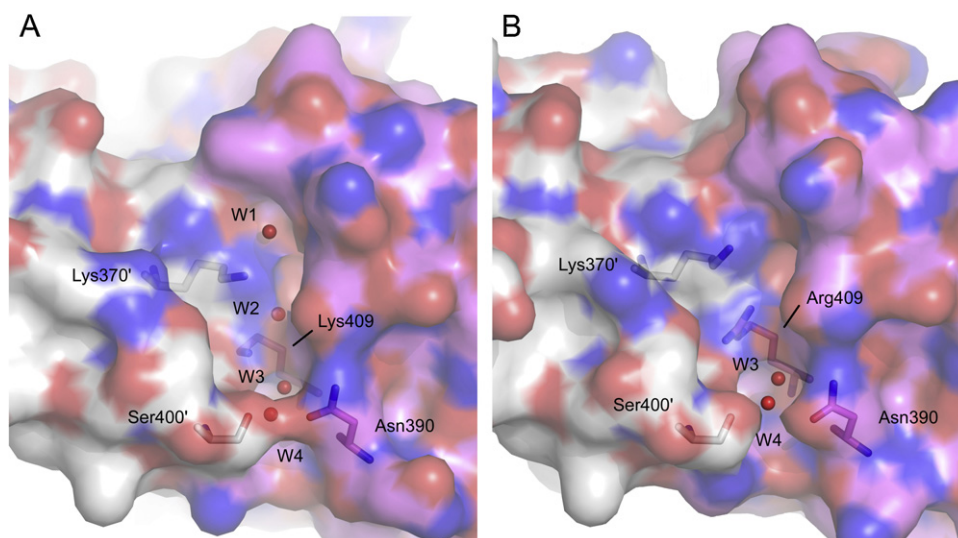


Fig. 4. The C_H3 interface creates a short, irregular groove at its edge. (A) IgG1 interface. Lys409 is located at the bottom of the groove. A hydrogen bond between Ser400' and Asn390 "closes" the groove at one end. The positions of the four conserved water molecules (Waters 1–4, labelled W1–W4) are shown. Carbon atoms from the two C_H3 domains are coloured in grey and pink, nitrogen atoms in blue, and oxygen atoms in red. PDB entry 3AVE for IgG1 Fc (Matsumiya et al., 2011) is shown. (B) IgG4 interface. Arg409 is also located at the bottom of the groove. The orientation and colour scheme is the same as for (A). Only conserved water molecules Water3 (W3) and Water4 (W4) are present. An increase in the distance between Ser400' and Asn390 precludes formation of a hydrogen bond between their side chains and the groove remains "open". (For interpretation of the references to colour in the artwork, the reader is referred to the web version of the article.)

average. On the other hand, the buried surface area between IgG4 C_H3 domains in the high resolution structure reported here is $2149 \text{ \AA}^2$, lower than that of the smallest IgG1 interface, and well below the IgG1 average.

In the modified "single interface" IgG1 C_H3 dimer, which assesses a single Lys409 site, the buried surface area ranges from $1314 \text{ \AA}^2$ to $1450 \text{ \AA}^2$ for IgG1, with an average value of $1409 \text{ \AA}^2$. The buried surface area of the low resolution IgG4 structure ($1404 \text{ \AA}^2$) is comparable with the IgG1 average. However, the buried surface areas for the high resolution IgG4 C_H3 dimer are $1323 \text{ \AA}^2$ and $1361 \text{ \AA}^2$, which are below the IgG1 average and at the lower end of the IgG1 range of values. Thus despite some variation between the contributions of the two pseudo-symmetrically related halves of the interface, it is clear that the buried surface area for the IgG4 C_H3 interface is lower than that for IgG1.

3.5. Arg409 affects inter-domain contact at the edge of the C_H3 interface

A surface representation of the edge of the C_H3 interface reveals a short irregular groove on opposite sides of the domain dimer. In IgG1, the Lys409 side chain is located at the bottom of the groove (Fig. 4A) and forms a hydrogen bond with Asp399', located on the DE loop (Fig. 5). Ser400', also from the DE loop, forms a hydrogen bond with Asn390, and in doing so, "closes" the groove at one end (Fig. 4A). IgG1 appears to be inherently flexible in this region of the C_H3 interface, as revealed by examination of inter-atomic distances. Although the Lys409–Asp399' hydrogen bond is maintained, the distance between Lys409 and Asp399' $\text{C}\alpha$ atoms varies from $9.51 \text{ \AA}$ to $10.05 \text{ \AA}$ with an average of $9.68 \text{ \AA}$, and this is accompanied by a change in that between Lys409 and Ser400' $\text{C}\alpha$ atoms, which varies from $10.86 \text{ \AA}$ to $11.91 \text{ \AA}$ with an average of $11.28 \text{ \AA}$. Increasing Lys409–Ser400' distance is correlated with movement of the DE loop away from the C_H3 interface, and an increase in the Asn390–Ser400' distance, which varies from $7.05 \text{ \AA}$ to $8.87 \text{ \AA}$ with an average of $7.97 \text{ \AA}$. The overall effect is reduced contact between Asn390 and Ser400' side chains.

In the IgG4 C_H3 dimer, the Arg409 side chain maintains hydrogen bonds with Ser364 and Asp399'. The Arg409–Asp399' $\text{C}\alpha$

distances are $10.13 \text{ \AA}$ and $10.25 \text{ \AA}$, longer than the greatest Lys409–Asp399' $\text{C}\alpha$ distance in IgG1, as are the Arg409 and Ser400' $\text{C}\alpha$ distances, at $12.06 \text{ \AA}$ and $12.18 \text{ \AA}$, both the result of DE loop movement (Figs. 4B and 5). The Asn390–Ser400' distances in IgG4 are $8.88 \text{ \AA}$ and $9.02 \text{ \AA}$ and the associated reduction in contact between Asn390 and Ser400' side chains results in a more open-ended C_H3 interface groove in IgG4 (Fig. 4B), and a smaller buried surface area.

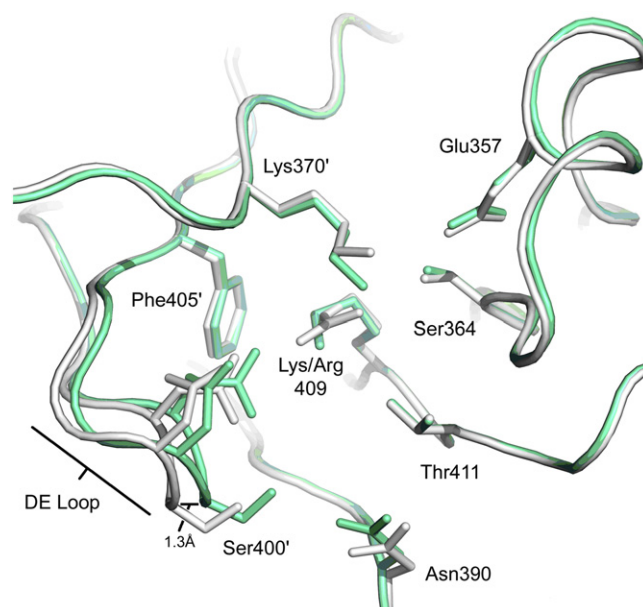


Fig. 5. Movement of the DE loop affects contact between Ser400' and Asn390. C_H3 domain $\text{C}\alpha$ atoms from PDB entry 3AVE for IgG1 Fc (Matsumiya et al., 2011) (green) were superposed onto the C_H3 dimer (white). The positions of polypeptide backbone residues are essentially identical, apart from those for the DE loop, the movement of which reduces contact between Ser400' and Asn390. After superposition, the distance between Ser400' $\text{C}\alpha$ atoms is $1.3 \text{ \AA}$. The altered position of the Lys370' $\text{N}\zeta$ atom in IgG4 is also evident. For clarity, water molecules are not shown. (For interpretation of the references to colour in the artwork, the reader is referred to the web version of the article.)

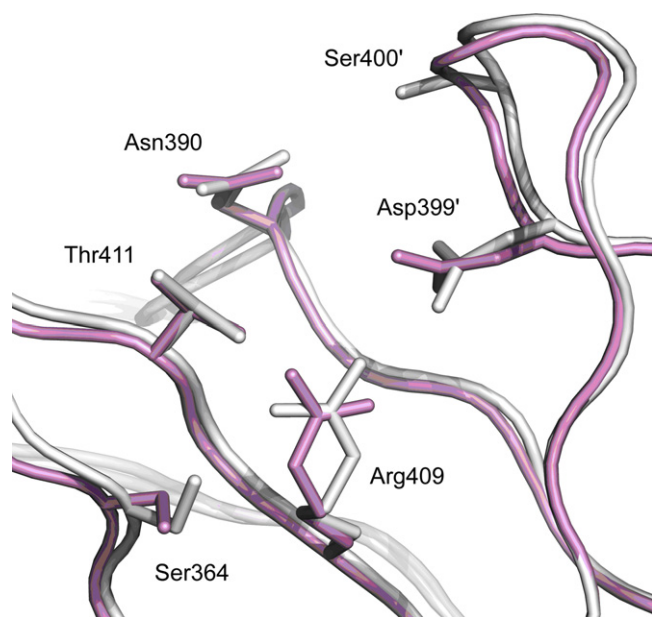


Fig. 6. The conformations of Arg409. The orientation of the Arg409 guanidinium group in the high resolution C_H3 domain crystal structure (white) differs from that in the low resolution IgG4 Fc structure (PDB entry 1ADQ; Corper et al., 1997) (pink). In the low resolution structure, the guanidinium group is oriented towards Asp399', which rotates its carboxylate group, the side chain of Ser400' side is disordered, and the conformation of Ser364 also differs. For clarity, water molecules from the high resolution C_H3 domain structure, and Lys370' from both structures, are not shown. (For interpretation of the references to colour in the artwork, the reader is referred to the web version of the article.)

These effects are principally the result of the bulky Arg409 guanidinium group adopting a conformation to maintain hydrogen bonds with both Ser364 and Asp399'. This has the consequence that in IgG4 the inter-domain distances quoted above cannot be any shorter, and although there is a spectrum of distances observed in the IgG1 structures, those for IgG4 are clearly distinct.

3.6. Arg409 and Fab-arm exchange

The high resolution C_H3 domain structure reported here reveals that the effect of Arg409 on the C_H3 interface is disruption of a conserved water-mediated hydrogen bond network, loss of contact at the end of a groove at the edge of the C_H3 interface, and a reduction in buried surface area. These observations offer a structural explanation for the recent studies that have both established Arg409 as the key residue from the C_H3 domain controlling FAE (Labrijn et al., 2011) and demonstrated that exchange is dependent on the strength of the C_H3 dimer interaction (Labrijn et al., 2011; Rose et al., 2011). As the process of FAE requires the rate-limiting step of C_H3 domain dissociation (Labrijn et al., 2011; Rispen et al., 2011) it is not hard to envisage how the presence of Arg409 may promote this first step, given a two-fold symmetrically weakened interface within which the $C\alpha$ atoms of this key residue are only ~ 14 Å apart.

3.7. Can Arg409 adopt different conformations at the C_H3 interface?

The Arg409 environment in the high resolution C_H3 domain structure reported here is different to that observed in the low resolution (3.15 Å) IgG4 Fc structure (Corper et al., 1997). Instead of forming the hydrogen bonds with Ser364 and Asp399' seen in the high resolution structure (Figs. 3B and 4), the guanidinium group of Arg409 adopts a different conformation and forms a hydrogen

bond only with Asp399', which rotates its carboxylate group (Fig. 6). In addition, Ser364 and Lys370' also adopt slightly different conformations, the side chain of Ser400' is disordered beyond its $C\alpha$ atom, and none of the four conserved water molecules is detected in the low resolution structure. These differences, in particular the apparent absence of the water molecules, may however be due to the difference in resolution of the two analyses, and the question remains whether Arg409 can adopt alternative conformations at the C_H3 interface. If indeed there is conformational flexibility involving Arg409 and surrounding residues, this might further promote domain dissociation and FAE.

4. Conclusions

We have solved the high resolution crystal structure of the IgG4 C_H3 domain dimer to investigate the effects of Arg409 on the IgG4 C_H3 interface and further explore its role in FAE. This is the second crystallographic view of the IgG4 C_H3 domains, which differ from the extensively studied IgG1 C_H3 domains by six residues, and represents a significant improvement in resolution from 3.15 Å to 1.8 Å. Comparison of the high resolution IgG4 structure with similarly high resolution IgG1 structures reveals that Arg409 in IgG4 disrupts a conserved network of water molecules that are responsible for mediating inter-domain hydrogen bonding in IgG1. The buried surface area of the IgG4 C_H3 dimer interface is generally lower than that in IgG1, and contact between two residues in particular, Asn390 and Ser400', is weakened. Following recent reports indicating that the presence of Arg409 reduces the strength of the C_H3 interaction and C_H3 dimer stability, we now provide a structural explanation for the role of this residue in determining IgG4's unique ability to undergo FAE.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.molimm.2012.10.029>.

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SUPPLEMENTARY INFORMATION

Table S1. The list of IgG1 structures included in the analysis

<i>PDB entry</i>	<i>Resolution (Å)</i>	<i>No. of IgG1 chains in the asymmetric unit</i>	<i>Reference</i>
1L6X	1.65	1	Idusogie, E.E. et al. (2000) <i>J. Immunol.</i> 164:4178-4184
3AVE	2.00	2	Matsumiya S. et al. (2011) <i>J. Mol. Biol.</i> 408:1001
3AGV	2.15	2	Nomura Y. et al. (2010) <i>Nucl. Acids Res.</i> 38:7822-7829
3AY4	2.20	2	Mizushima T. et al. (2011) <i>Genes Cells.</i> 16:1071-1080
3SGJ	2.20	2	Ferrara, C. et al. (2011) <i>Proc. Natl. Acad. Sci. USA</i> 108:12669-12674
2DTS	2.20	2	Matsumiya S. et al. (2007) <i>J. Mol. Biol.</i> 368:767-779
3D6G	2.30	2	Moiani D. et al. <i>unpublished</i> . DOI:10.2210/pdb3d6g/pdb
1OQO	2.30	2	Raju T.S. et al. <i>unpublished</i> . DOI:10.2210/pdb1oqo/pdb
3C2S	2.30	1	Oganesyan, V. et al. (2008) <i>Acta Crystallogr.</i> D64:700-704
2IWG	2.35	2	James, L.C. et al. (2007) <i>Proc. Natl. Acad. Sci. USA</i> 104:6200-6205
3SGK	2.40	2	Ferrara, C., et al. (2011) <i>Proc. Natl. Acad. Sci. USA</i> 108:12669-12674
1H3U	2.40	2	Krapp S. et al. (2003) <i>J. Mol. Biol.</i> 325:979-989
1H3T	2.40	2	Krapp S. et al. (2003) <i>J. Mol. Biol.</i> 325:979-989
1H3X	2.44	2	Krapp S. et al. (2003) <i>J. Mol. Biol.</i> 325:979-989
3FJT	2.50	2	Oganesyan, V. et al. (2009) <i>Mol. Immunol.</i> 46:1750-1755
3DO3	2.50	2	Braden, B.C. <i>unpublished</i> . DOI:10.2210/pdb3do3/pdb
2WAH	2.51	2	Crispin, M. et al. (2009) <i>J. Mol. Biol.</i> 387:1061-1066
2QL1	2.53	1	Oganesyan, V. et al. (2008) <i>Mol. Immunol.</i> 45:1872-1882
1OQX	2.60	2	Raju T.S. et al. <i>unpublished</i> . DOI:10.2210/pdb1oqx/pdb
1DN2	2.70	2	DeLano, W.L. et al. (2000) <i>Science</i> 287:1279-1283
1HZH	2.70	2	Saphire, E.O. et al. (2001) <i>Science</i> 293:1155-1159