The Solution Structures of Two Human IgG1 Antibodies Show Conformational Stability and Accommodate Their C1q and FcγR Ligands*†

Received for publication, December 7, 2014, and in revised form, January 28, 2015. Published, JBC Papers in Press, February 6, 2015, DOI 10.1074/jbc.M114.631002

Lucy E. Rayner‡1, Gar Kay Hui§1, Jayesh Gor‡, Richard K. Heenan‡, Paul A. Dalby‡, and Stephen J. Perkins‡2

From the §Department of Structural and Molecular Biology, Division of Biosciences, Darwin Building, University College London, Gower Street, London WC1E 6BT, United Kingdom, the ¶ISIS Facility, Science and Technology Facilities Council, Rutherford Appleton Laboratory, Harwell Oxford, Didcot OX11 0QX, United Kingdom, and the ‡Department of Biochemical Engineering, Division of Engineering, Roberts Building, University College London, Gower Street, London WC1E 7JE, United Kingdom

Background: The human IgG1 antibody subclass is the most abundant one and is widely used in therapeutic applications.

Results: Ultracentrifugation and x-ray/neutron scattering, together with atomistic modeling, revealed asymmetric concentration-independent IgG1 solution structures.

Conclusion: The complement and receptor Fc binding sites are not hindered by the Fab regions, explaining its full activity.

Significance: These solution structures clarify IgG1 activity and its therapeutic applications.

The human IgG1 antibody subclass shows distinct properties compared with the IgG2, IgG3, and IgG4 subclasses and is the most exploited subclass in therapeutic antibodies. It is the most abundant subclass, has a half-life as long as that of IgG2 and IgG4, binds the FcγR receptor, and activates complement. There is limited structural information on full-length human IgG1 because of the challenges of crystallization. To rectify this, we have studied the solution structures of two human IgG1 6a and 19a monoclonal antibodies in different buffers at different temperatures. Analytical ultracentrifugation showed that both antibodies were predominantly monomeric, with sedimentation coefficients s0.2 of 6.3–6.4 S. Only a minor dimer peak was observed, and the amount was not dependent on buffer conditions. Solution scattering showed that the x-ray radius of gyration Rg increased with salt concentration, whereas the neutron Rg values remained unchanged with temperature. The x-ray and neutron distance distribution curves P(r) revealed two peaks, M1 and M2, whose positions were unchanged in different buffers to indicate conformational stability. Constrained atomistic scattering modeling revealed predominantly asymmetric solution structures for both antibodies with extended hinge structures. Both structures were similar to the only known crystal structure of full-length human IgG1. The Fab conformations in both structures were suitably positioned to permit the Fc region to bind readily to its FcγR and C1q ligands without steric clashes, unlike human IgG4. Our molecular models for human IgG1 explain its immune activities, and we discuss its stability and function for therapeutic applications.

IgG1 is the most abundant human IgG antibody subclass (8 mg/ml) of the four found in serum. Following high specificity and affinity binding of the antigen to their Fab regions, the immune response and effector functions are mediated through the Fc region. IgG1 binds to every class of FcγR receptor (FcγR) found on immune effector cells and activates the complement cascade when C1q is recruited by several Fc regions (1). Binding to FcγRs on immune cell surfaces leads to diverse immune responses, including antibody-dependent cell-mediated cytotoxicity, to clear foreign antigen from the body. IgG1 has been extensively studied, making it the most understood and exploited human IgG subclass for the development of therapeutic antibodies. Over 30 IgG monoclonal antibodies have been approved as of June 2012 for clinical use by the Food and Drug Administration, of which 68% of marketed and late stage clinical phase therapeutic antibodies involve the human IgG1 subclass (2).

The four human IgG subclasses IgG1–IgG4 vary primarily in the hinge region, which connects the Fab and Fc regions together and contributes flexibility between these regions. The hinge length is linked with IgG functionality. The hinge is best considered as a three-part structure, in which the upper and middle hinge sections of IgG1, IgG2, IgG3, and IgG4 contain 15, 12, 62, and 12 amino acids, respectively. The order of flexibility is IgG3 > IgG1 > IgG4 > IgG2, which correlates well with the hinge length (3, 4). The upper hinge determines the arrangement between the two Fab regions and mediates flexibility and reorientations of each Fab arm; this allows IgG1 to bind to multiple antigens in different positions (5). Two cysteine residues (Cys226 and Cys229) in the middle hinge form interchain disulfide bonds between the two heavy chains to join these together (Fig. 1). The lower hinge is responsible for the flexibility and positioning of the Fc region relative to the Fab arms and affects the binding of Fc to FcγR (5, 6).

Only limited structural information is available for full-length IgG antibodies. These are difficult to crystallize because

*This work was supported by the Biotechnology and Biological Sciences Research Council. Support for this work was also provided in part by the CCP-SAS project, a joint Engineering and Physical Sciences Research Council (EP/K039121/1) and National Science Foundation (CHE-1265821) grant.

†This article contains supplemental models (4OOU, 4OQV, and 4OOW).

‡Both authors contributed equally to this work.

§To whom correspondence should be addressed. Tel.: 020-7679-7048; Fax: 020-7679-7193; E-mail: s.perkins@ucl.ac.uk.

© 2015 by The American Society for Biochemistry and Molecular Biology, Inc. Published in the U.S.A.
Solution Structure of IgG1

The human IgG1 domain structure. The heavy chains have VH, C\text{H}1, C\text{H}2, and C\text{H}3 domains, and the light chains have VL and C\text{L} domains. The hinge region between the Fab and Fc fragments is composed of 23 residues (EPKSCDKTHTCPPCPAPELLGGP) between Val215 and Ser239. The heavy chains are connected by two Cys-Cys disulfide bridges at Cys226 and Cys229. There is one N-linked oligosaccharide site at Asn297 on each of the C\text{H}2 domains. The hinge region between the Fab and Fc fragments is composed of 23 residues (EPKSCDKTHTCPPCPAPELLGGP) between Val215 and Ser239.

of the flexible domain arrangements found in IgG. Thus, hinge-deleted human IgG1 structures solved by x-ray crystallography include IgG1 Dob and Mcg (7–9). These revealed symmetric IgG structures that are not a true picture of wild-type antibody conformations. The crystal structure of a full-length human IgG1 b12 has been reported alongside full-length murine IgG1 and IgG2a (10–12). Human IgG1 b12 showed an asymmetric structure with extended hinges, although atomic coordinates for part of one of the hinge regions are missing. These crystal structures necessarily contain IgG held in a fixed position by intermolecular contacts within the crystallographic unit cell, offering only a single snapshot of the multiple conformations expected in solution (13). The advent of atomistic constrained scattering modeling has mitigated this issue. Thus, human IgG4, IgA1 and IgA2, IgD, and IgM have been studied successfully in physiological buffers, and molecular structures have been determined in Protein Data Bank coordinate formats (14–19).

Solution structures for the human IgG1 subclass are essential to understand its function and stability in the human body, especially for therapeutic applications. Joint x-ray and neutron scattering studies rectify the limitation of the single available IgG1 b12 crystal structure by enabling the study of different buffer and solution conditions on the IgG1 structure. The recent advent of high throughput x-ray measurements provides hundreds of scattering curves in a single measurement session, and these permit atomistic antibody structures to be determined for a broad range of solution conditions. Here we report solution structures for two IgG1 antibodies, IgG1 6a and IgG1 19a, with known sequences (Fig. 2). Both IgG1s were found to be predominantly monomeric in all buffer conditions tested. Both IgG1 solution structures displayed semixtended asymmetric arrangements of the Fab regions relative to the Fc region. These structures become more elongated with increase in salt concentration. By reference to the crystal structure of an FcYR-Fc complex and a docked structural model for C1q binding to Fc, it could be assessed whether the Fab regions in both IgG1 solution structures allowed enough space for the FcYR and C1q ligands to bind to the top of the Fc region in IgG1. The successful outcome of our analyses accounted for the reactivity of IgG1 for FcYR and C1q. This is in marked contrast to our recent similar analyses for human IgG4, where this binding to the Fc region was most likely sterically hindered by the Fab regions (15). Previously, conformational instabilities were found in IgG4 (15); it is therefore also crucial to identify whether or not IgG1 is also affected by the same instabilities that occur in IgG4.

EXPERIMENTAL PROCEDURES

Purification and Composition of IgG1—Both IgG1 6a and IgG1 19a were generously supplied by Dr. Bryan Smith (UCB). Immediately prior to measurements, both were further purified by gel filtration to remove nonspecific aggregates using a Superose 6 10/300 column (GE Healthcare) and then concentrated using Amicon Ultra spin concentrators (50 kDa molecular mass cut-off) and dialyzed at 4 °C against the appropriate ultracentrifugation and scattering buffer (see below). The sequence identity for the two IgG1 molecules was 100% for the C\text{H}1, C\text{H}2, C\text{H}3, and C\text{L} domains. Differences in sequences were found in the V\text{H} (65.2%) and V\text{L} (73.2%) domains. Total sequence identity between the two IgG1 forms was 88.7% (Fig. 2). The N-linked oligosaccharides at Asn297 on the C\text{H}2 domains (Fig. 2) were assumed to have a typical complex-type biantennary oligosaccharide structure with a Man\textsubscript{9}GlcNA\textsubscript{c}, core and two NeuNAc-Gal-GlcNAc antennae (20). The IgG1 6a molecular mass was calculated to be 150.1 kDa, its unhydrated volume was 193.1 nm\textsuperscript{3}, its hydrated volume was 254.4 nm\textsuperscript{3} (based on a hydration of 0.3 g of water/g of glycoprotein and an electrostricted volume of 0.0245 nm\textsuperscript{3}/bound water molecule), its partial specific volume $\bar{\nu}$ was 0.729 ml/g, and its absorption coefficient at 280 nm was 15.4 (1, 1-cm path length) (21). Likewise, IgG1 19a has a calculated molecular mass of 149.7 kDa, an unhydrated volume of 192.4 nm\textsuperscript{3}, a $\bar{\nu}$ of 0.728 ml/g, and an absorption coefficient at 280 nm of 15.6 (1, 1-cm path length).

All data were recorded in phosphate-buffered saline with different NaCl concentrations. That termed PBS-137 has a composition of 137 mm NaCl, 8.1 mm Na\text{2}HPO\text{4}, 2.7 mm KCl, and 1.5 mm KH\text{2}PO\text{4} (pH 7.4). When 137 mm NaCl was replaced by 50 mm NaCl or 250 mm NaCl, these were termed PBS-50 or PBS-250, respectively. The buffer densities were measured using an Anton Paar DMA 5000 density meter and compared with the theoretical values calculated by SEDNTERP (22). This resulted in densities of 1.00530 g/ml for PBS-137 at 20 °C (theoretical, 1.00534 g/ml), 1.00189 g/ml for PBS-50 at 20 °C (theoretical, 1.00175 g/ml), 1.01003 g/ml for PBS-137 at 20 °C (theoretical, 1.00534 g/ml), 1.11238 g/ml for PBS-250 at 20 °C (theoretical, 1.00998 g/ml), and 1.11238 g/ml for PBS-137 at 20 °C in 100% H\text{2}O.

Sedimentation Velocity Data for IgG1—Analytical ultracentrifugation data for IgG1 6a were obtained on two Beckman XL-1 instruments equipped with AnTi50 rotors. Sedimentation velocity data were acquired for IgG1 samples in PBS-50, PBS-137, and PBS-250 at 20 °C (H\text{2}O) and in PBS-137 with 100% H\text{2}O. Sedimentation velocity data were acquired for IgG1 19a only in PBS-137 (H\text{2}O) at 20 °C. Data were collected at rotor speeds of 40,000 rpm and 50,000 rpm in two-sector cells with...
Solution Structure of IgG1

column heights of 12 mm. Sedimentation analysis was performed using direct boundary Lamm fits of up to 745 scans using SEDFIT (version 14.1) (23, 24). SEDFIT resulted in size distribution analyses $c(s)$ that assume all species to have the same frictional ratio $f/f_0$. The final SEDFIT analyses used a fixed resolution of 200 and optimized the $c(s)$ fit by floating $f/f_0$ and the baseline until the overall root mean square deviations and visual appearance of the fits were satisfactory. The percentage of oligomers in the total loading concentration was derived using the $c(s)$ integration function.

X-ray and Neutron Scattering Data for IgG1—X-ray scattering data were obtained during a beam session in 16-bunch mode on Instrument ID02 at the European Synchrotron Radiation Facility (Grenoble, France), operating with a ring energy of 6.0 GeV on Beamline ID02 (25). Data were acquired using a fast readout low noise camera (FreLoN) with a resolution of 512 × 512 pixels. A sample-to-detector distance of 3.0 m was used. Both IgG1 6a and IgG1 19a were studied at four concentrations between 0.5 and 2.0 mg/ml for each condition and also at 4 mg/ml in PBS-137. IgG1 19a was studied at six concentrations between 0.5 and 2.0 mg/ml in PBS-50, PBS-137, and PBS-250 at 20 °C. IgG1 6a was studied at six concentrations between 0.22 and 1.35 mg/ml in PBS-50, between 0.30 and 1.89 mg/ml in PBS-137, and between 0.26 and 1.62 mg/ml in PBS-250. Sample volumes of 100 μl were measured in a polycarbonate capillary with a diameter of 2 mm, which avoids protein deposits during exposures, with the sample being moved continuously during beam exposure to reduce radiation damage. Sets of 10 time frames, with a frame exposure time of 0.1 or 0.2 s each, were acquired in quadruplicate as a control of reproducibility. Online checks during data acquisition confirmed the absence of radiation damage, after which the 10 frames were averaged.

Neutron scattering data were obtained on Instrument SANS2D at the ISIS pulsed neutron source at the Rutherford Appleton Laboratory (Didcot, UK) (26). A pulsed neutron beam was derived from proton beam currents of ~40 μA. SANS2D data were recorded with 4 m of collimation, a 4-m sample-to-detector distance, a 12-mm beam diameter, and a wavelength range of 0.175–1.65 nm made available by time of flight. Samples were measured in 2-mm path length circular banjo cells for 1–2 h in a thermostated rack at 6, 20, and 37 °C. Data were only collected for IgG1 6a at three concentrations between 2.0 and 4.0 mg/ml in PBS-137 in 100% 2H2O.

In a given solute-solvent contrast, the radius of gyration $R_g$ is a measure of structural elongation if the internal inhomogeneity of scattering densities within the protein has no effect. Guinier analysis at low $Q$ ($Q = 4\pi \sin \theta/\lambda$, where $2\theta$ is the scattering angle and $\lambda$ is the wavelength) gives the $R_g$ and the forward scattering at zero angle $l(0)$ (27).

$$\ln l(Q) = \ln l(0) - \frac{R_g^2 Q^2}{2} \quad (\text{Eq. 1})$$

This expression is valid in a $Q, R_g$ range up to 1.5. If the structure is elongated, the mean radius of gyration of cross-sectional structure $R_{cs}$ and the mean cross-sectional intensity at zero angle $(|l(Q)|Q)_{Q \to 0}$ are obtained from the following.

$$\text{ln}(l(Q)|Q) = (l(Q)|Q)_{Q \to 0} - \frac{R_{cs}^2 Q^2}{2} \quad (\text{Eq. 2})$$

The cross-sectional plot for immunoglobulins exhibits two distinct regions, a steeper innermost one and a flatter outermost one (28), identified by $R_{cs-1}$ and $R_{cs-2}$, respectively. The $R_{cs}$ and $R_{cs-2}$ analyses were performed using an interactive PERL script program SCTPL7 (J. T. Eaton and S. J. Perkins) on Silicon Graphics OCTANE Workstations. Indirect Fourier transformation of the scattering data $l(Q)$ in reciprocal space into real space to give the distance distribution function $P(r)$ was carried out using the program GNOM (29),

$$P(r) = \frac{1}{2\pi^2} \int l(Q)Qr \sin(Qr)dQ \quad (\text{Eq. 3})$$

where $P(r)$ corresponds to the distribution of distances $r$ between volume elements. This provides the maximum dimension of the antibody $L$ and its most commonly occurring distance vector $M$ in real space. For this, the $x$-ray $l(Q)$ curve utilized up to 365 data points in the $Q$ range between 0.09 and 1.70 nm$^{-1}$. The neutron $l(Q)$ curve utilized up to 45 data points in the $Q$ range between 0.18 and 1.5 nm$^{-1}$.

Debye Scattering and Sedimentation Coefficient Modeling of IgG1—A total of 20,000 conformationally randomized human IgG1 models were created by joining the IgG1 Fab and Fc structures with conformationally randomized hinge peptides. The crystal structure of human IgG1 b12 (Protein Data Bank code 1HZH) was used for this (10). This IgG1 structure has complete heavy chains (H and K) and light chains (L and M), with the exception of 13 missing K chain residues, namely the Fab CH1 residues 132SKSTSGG138, the core hinge residues 223THT225, and the Fc Cγ1 C terminus445PGK447 (10). IgG1 b12 has high sequence identity to IgG1 6a and IgG1 19a (Fig. 2). Most of the sequence differences occur in the $V_{H4}$ and $V_L$ domains, where antigen binding occurs. Additionally, small sequence differences in the $C\alpha1$ and $C\alpha3$ domains result from allelotype differences. Human IgG1 has four alleotypes (G1m1, G1m2, G1m3, and G1m17), which may be expressed in IgG1 as G1m1, G1m17,1 or G1m17,1,2 heavy chains (30). IgG1 b12 is the G1m17,1 alleotype with Lys214 in the $C\alpha1$ domain (Fig. 2D) and Asp356 and Leu358 in the $C\alpha3$ domain (Fig. 2G). Additionally, IgG1 b12 has Ala215 in place of the wild type Val215; this is not an allelotype difference and may have been engineered during the antibody production. IgG1 6a and 19a are both Gm3 alleotypes with Arg214 in the $C\alpha1$ domain (Fig. 2D) and Glu356 and Met358 in the $C\alpha3$ domain (Fig. 2G). The light chain subclass can be either $\kappa$ or $\lambda$. Whereas the $\kappa$ subclass has only one gene copy, there can be 7–11 gene copies of $\lambda$, depending on the haplotype (30). The $\kappa$ light chain subclass has three alleotypes (Km1, Km2, and Km3), whereas the $\lambda$ light chain subclasses have no serologically defined alleotypes. The $\kappa$ light chain alleotypes may be expressed as Km1, Km1,2, or Km3. IgG1 b12, IgG1 6a, and IgG1 19a all have the Km3 alleotype with Ala159 and Val197 in the $C\alpha1$ domain (Fig. 2B). IgG1 b12 shows a sequence difference of Arg208 in the $C\alpha1$ domain (Fig. 2B), which is not an
allotypic difference and may have been engineered. The unhydrated volumes of IgG1 b12, IgG1 6a, and IgG1 19a were calculated as 194.3, 193.1, and 192.4 nm³, respectively. The volume similarity was within acceptable limits to allow the use of IgG1 b12 as a model for the IgG1 6a and IgG1 19a modeling searches.

In order to generate conformationally randomized trial IgG1 models for scattering fits, four sets of 5000 models were created, each using different hinges sampled independently at random. Conformational randomization of the hinges was achieved using molecular dynamics in the Discovery module of the molecular modeling software Insight II (Accelrys) on Silicon Graphics OCTANE workstations. To create the first two sets of asymmetric models, a hinge peptide 220CDKTHTC226 was constrained to be of minimum lengths either between 1.72 and 2.33 nm or between 2.33 and 2.45 nm (where the latter is almost fully extended in length). Because residue Cys220 is located asymmetrically in relation to the Fc structure, all of the created models were asymmetric. Cys220 and Cys226 were used as anchor points because they connect the Fab heavy and light chains in a disulfide bridge. To create two more sets of asymmetric and symmetric models, a 19-residue hinge peptide 220CDKTHTCPPCPAPELLGGP238 was constrained with minimum lengths either between 4.66 and 6.32 nm or between 6.32 and 6.65 nm (where the latter is almost fully extended in length) in order to avoid abnormally short hinge structures. Because residue Pro238 was located asymmetrically in the Fc structure, the resulting models contained Fab arms in both symmetric and asymmetric orientations about the Fc region. The outermost two residues were anchor points for the superimposition of each hinge conformation onto the Fab and Fc structures in order to create the full IgG1 model.

The x-ray or neutron scattering curve was calculated from each IgG1 model using sphere models for comparison with the experimental IgG1 curves. A cube side of 0.541 nm in combination with a cut-off of four non-hydrogen atoms was used to convert the atomic coordinates into 1220 spheres that corresponded to the unhydrated structure seen by neutron scattering in 2H₂O. Because hydration shells are visible by x-rays, a hydration shell corresponding to 0.3 g of water/g of protein was created using HYPRO (31), giving an optimal total of 1607 spheres. The x-ray scattering curve \( I(Q) \) was calculated using the Debye equation adapted to spheres (16, 32). Steric overlap between the Fab and Fc regions was assessed using the number of spheres \( n \) in each model, where models showing less than 95% of the required total of 1607 spheres (x-ray) or 1220 spheres (neutrons) were discarded. Of the 20,000 models, 86% showed no steric overlap.

Next, the x-ray \( R_g \), \( R_{xs}^{-1} \) and \( R_{xs}^{-2} \) values were calculated from the modeled curves in the same Q ranges used for the experimental Guinier fits. Models that passed \( R_g \) and \( R_{xs}^{-1} \) filters of \( 5\% \) of the experimental value were then ranked using a goodness of fit \( R \)-factor (defined by analogy with protein crystallography) calculated in the Q range extending to 1.7 nm⁻¹. For the neutron modeling of IgG1 6a, the unhydrated sphere models were used to calculate the scattering curves. Of the 20,000 models, 91% showed no steric overlap. The models created from neutron scattering were assessed as for the x-ray scattering models above, following corrections for wavelength spread and beam divergence, but
Solution Structure of IgG1

no correction was required for a flat background caused from incoherent scattering.

Sedimentation coefficients \( s_{20,w} \) were calculated directly from the hydrated Debye sphere models using the program HYDRO (33). They were also calculated from the atomic coordinates in the HYDROPRO shell modeling program using the default value of 0.31 nm for the atomic element radius for all atoms to represent the hydration shell (34). Previous applications of these calculations to antibodies are reviewed elsewhere (35).

To assess the fit searches, the distances \( d_1, d_2, \) and \( d_3 \) were determined from the centers of mass of the Fab and Fc regions (excluding hydrogen atoms) using a Python script. The three angles between the Fab and Fc regions were defined in a Python script as the angle of intersection from the dot product between vectors. Each vector was the long axis through each Fab or Fc region as the angle of intersection from the dot product between vectors. Each vector was the long axis through each Fab or Fc region each defined as the line passing through the centers of gravity between each cluster of four cysteine \( \alpha \)-carbon atoms at the two ends of the Fab and Fc regions (one cluster at each end of each Fab or Fc region, corresponding to the conserved disulfide bridge in each immunoglobulin fold domain). Artwork was prepared using PyMOL (Schrödinger, LLC). Superimpositions of the Fc region were performed using the align function within PyMOL. To dock the Fc region with the C1q head, the Web server algorithm PatchDock (version beta 1.3) (36) was used in order to take advantage of its ability to include specified residues as potential binding sites. Its output was refined using FireDock from the same Web site (37).

Protein Data Bank Accession Numbers—The three sets of 10 best fit models are currently available as supplemental material. They have been deposited in the Protein Data Bank under accession codes 4QOU (IgG1 6a by X-rays in PBS-137), 4QOV (IgG1 19a by X-rays in PBS-137), and 4QOW (IgG1 6a by neutrons in PBS-137).

RESULTS

Purification of IgG1—Both IgG1 6a and IgG1 19a were subjected to gel filtration to ensure that the protein was monodisperse immediately prior to ultracentrifugation or scattering experiments. Both molecules eluted as a symmetric main peak at \(-15.5 \text{ ml}\) (Fig. 3) and showed a single band between 200 and 116 kDa in non-reducing SDS-PAGE that corresponds to the expected masses of 150.1 and 149.7 kDa for intact IgG1 6a and IgG1 19a, respectively. Under reducing conditions, the heavy chains for both IgG1 molecules were observed at an apparent molecular mass of 55 kDa, and the light chains were observed between 31 and 21.5 kDa, both as expected (Fig. 3).

Analytical Ultracentrifugation of IgG1—Sedimentation velocity experiments examined the size and shape of IgG1 6a at concentrations between 0.2 and 4 mg/ml, and examined IgG1 19a between 0.5 and 2.24 mg/ml. The SEDFIT analyses involved fits of as many as 745 scans, and the good agreement between the experimental boundary scans and fitted lines is clear (Fig. 4). A major monomer peak was observed at \( s_{20,w} \) values of 6.4 S for IgG1 6a and 6.3 S for IgG1 19a. These \( s_{20,w} \) values are consistent with the range of values of 6.3–6.8 S previously reported for human IgG1 (38, 39, 40). Both IgG1 6a and IgG1 19a were predominantly monomeric in solution and were accompanied by a minor dimer peak.

From the \( c(s) \) analyses, the molecular masses of the monomer peak for IgG1 6a were reported as 153 kDa (PBS-50), 146 kDa (PBS-137), and 149 kDa (PBS-250) in light water and 164 kDa (PBS-137 at 20 °C) in heavy water. These agree well with the composition–calculated mass of 150 kDa. The molecular mass of the IgG1 19a monomer peak was measured as 161 kDa (PBS-137) in light water, also in agreement with the composition–calculated mass of 150 kDa.

The apparent sedimentation rates of the IgG1 monomer were independent of sample concentration or rotor speed (Fig. 5A). Extrapolation of the corrected \( s_{20,w} \) values to zero concentration gave monomer \( s_{20,w} \) values of 6.42 S for IgG1 6a for 40,000 rpm, which is similar to that of 6.44 S for 50,000 rpm (PBS-137 at 20 °C). For IgG1 19a, the monomer \( s_{20,w} \) value was 6.34 S for both rotor speeds of 40,000 and 50,000 rpm (PBS-137 at 20 °C). All other data reported in this study are for 40,000 rpm. No change in \( s_{20,w} \) value was observed at different buffer conditions, with IgG1 6a giving \( s_{20,w} \) values of 6.42, 6.42, and 6.35 S for PBS-50, PBS-137, and PBS-250, respectively, in light water (Fig. 5A). IgG1 6a measured in PBS-137 in heavy water gave an apparent sedimentation of 3.92 S (Fig. 4B). When corrected for the buffer density and viscosity of heavy water, a \( s_{20,w} \) value of 7.01 S was obtained. Given that the partial specific volume \( \bar{v} \) for proteins is affected by the hydration shell (21, 33) and that the hydration shell for heavy water has a higher mass than that for light water, the \( \bar{v} \) values will be reduced in 100% 

2H2O. When the \( \bar{v} \) value of 0.715 ml/g was used for 20 °C in place of 0.728 ml/g, this gave \( s_{20,w} \) values similar to that of PBS-137 in light water of 6.47 S (Fig. 5A). For IgG1 19a in light water,

![Figure 3. Purification of human IgG1. A, IgG1 6a; B, IgG1 19a. For each antibody, the elution peak from a Superose 6 10/300 gel filtration column is shown on the left (milliabsorbance units (mAU) with molecular weight markers (kDa). The non-reduced and reduced SDS-PAGE analyses are shown on the right.](image-url)
buffers are shifted to lower value of 300 kDa for the IgG1 dimer. The IgG1 6a dimer heavy water). These masses are comparable with the expected 137), 257

Solution Structure of IgG1

FIGURE 4. Sedimentation velocity analyses of IgG1. The experimentally observed sedimentation boundaries for IgG1 6a in PBS-50, PBS-137, and PBS-250 in H₂O (A) and PBS-137 in ²H₂O (B) buffers were recorded at a rotor speed of 40,000 rpm and 20 °C, C. IgG1 19a in PBS-137 at 20 °C was also measured at 40,000 r.p.m. Forty boundaries (black outlines) are shown from up to 745 scans at intervals of, for example, every 15th scan for clarity, which were fitted using SEDFIT as shown (white lines). The right panel shows the observed s values in the corresponding size distribution analyses (c(s)), revealing a monomer (M) peak at s²₀,ₚ values of ~6.4 S for IgG1 6a and 6.3 S for IgG1 19a in H₂O buffers, with a minor dimer peak (D) at about 9 S. The observed s values in ²H₂O buffers are shifted to lower s values.

the s²₀,ₚ value of 6.34 S for PBS-137 at 20 °C was similar to that of IgG1 6a. This outcome indicates their similar overall shapes. The c(s) analyses for IgG1 6a revealed a minor dimer peak at s²₀,ₚ values between 9 and 10 S in the size distribution analyses c(s) (Figs. 4 and 5). The molecular masses of the dimer peak in light water were 263 ± 4 kDa (PBS-50), 260 ± 10 kDa (PBS-137), 257 ± 5 kDa (PBS-250), and 286 ± 9 kDa (PBS-137 in heavy water). These masses are comparable with the expected value of 300 kDa for the IgG1 dimer. The IgG1 6a dimer s²₀,ₚ values in light water were 9.21 ± 0.1 S (PBS-50), 9.69 ± 0.38 S (PBS-137), and 9.12 ± 0.07 S (PBS-250) at 20 °C. That in heavy water was similar at 9.35 ± 0.09 S for PBS-137. Similarly, IgG1 19a showed a small dimer peak with a s²₀,ₚ value of 8.8 ± 0.3 S in PBS-137 at 20 °C and a molecular mass of 266 ± 9 kDa in light water. This also agreed well with the predicted mass of 300 kDa for its dimer. Integration of the monomer and dimer c(s) peaks showed that the amount of dimer did not alter with sample concentration or buffer composition, with the majority of samples showing less than 5% dimer for both IgG1 6a and IgG1 19a (Fig. 5B).

X-ray and Neutron Scattering of Human IgG1—The solution structure of IgG1 was jointly analyzed by both x-ray and neutron scattering for reason of reproducibility. X-rays in light water buffers monitor the hydration shell as well as the protein structure, whereas neutrons in heavy water buffers do not see this hydration shell.

X-rays were most effective for looking at IgG1 at 20 °C in three different NaCl concentrations. Data collection of IgG1 6a was carried out between 0.5 and 4 mg/ml, using time frame analyses to ensure the absence of radiation damage effects. Guinier analyses resulted in high quality linear plots and revealed three distinct regions of the I(Q) curves, as expected for antibodies, from which the Rg, Rs_-1, and Rs_-2 values were obtained within satisfactory Q,Rg, and Q,Rs_-2 limits (Fig. 6A and Table 1). The x-ray Rg values for IgG1 6a in PBS-50, PBS-137, and PBS-250 showed no concentration dependence, with mean values of 5.17, 5.19, and 5.32 nm, respectively (Fig. 7A). There was a slight increase in Rg with salt concentration, most notably with PBS-250. The I(0)/c values for IgG1 6a also showed no concentration dependence (Fig. 7A). Each of the Rg_-1 and Rs_-2 values were unchanged between PBS-50, PBS-137, and PBS-250, with a mean Rs_-1 value of 2.62, 2.64, and 2.65 nm, respectively, and a mean Rs_-2 value of 1.43, 1.43, and 1.42 nm, respectively. IgG1 19a was studied between 0.22 and 1.89 mg/ml in the samples showing less than 5% dimer for both IgG1 6a and IgG1 19a (Fig. 5B).

MARCH 27, 2015 • VOLUME 290 • NUMBER 13

JOURNAL OF BIOLOGICAL CHEMISTRY 8425
Solution Structure of IgG1
TABLE 1

Modeling searches of the x-ray and neutron scattering and sedimentation coefficient data for human IgG1

| Filter        | Models | Spheres | R_x,s-1 | R_x,s-2 | D_{max} | R-factor | s_{20,w} |
|---------------|--------|---------|---------|---------|---------|----------|----------|
| X-ray data IgG1 6a |        |         |         |         |         |          |          |
| IgG1 b12 ([PDB ID: 1HZH]) | None | 20,000 | 1056-1690 | 2.91-7.47 | 0.04-3.47 | 0.11-2.81 | NA | NA |
| X-ray fit, 2 mg/ml, PBS-50 | R_x,s, spheres | 10 | 1599-1634 | 5.09-5.26 | 2.56-2.66 | 1.29-1.42 | NA | NA |
| Experimental data | NA | NA | 5.21 ± 0.03; 5.25 ± 0.05 | 2.57 ± 0.04 | 1.42 ± 0.06 | 16 | NA |
| X-ray fit, 4 mg/ml, PBS-137 | R_x,s, spheres | 10 | 1600-1635 | 5.14-5.26 | 2.58-2.68 | 1.37-1.46 | NA | NA |
| Experimental data | NA | NA | 5.20 ± 0.06; 5.23 ± 0.03 | 2.61 ± 0.02 | 1.42 ± 0.01 | 16 | NA |
| X-ray fit, 2 mg/ml, PBS-250 | R_x,s, spheres | 10 | 1594-1624 | 5.00-5.18 | 2.61-2.71 | 1.38-1.48 | NA | NA |
| Experimental data | NA | NA | 5.28 ± 0.08; 5.31 ± 0.06 | 2.57 ± 0.05 | 1.47 ± 0.04 | 16 | NA |

Neutron data IgG1 6a

| Filter        | Models | Spheres | R_x,s-1 | R_x,s-2 | D_{max} | R-factor | s_{20,w} |
|---------------|--------|---------|---------|---------|---------|----------|----------|
| IgG1 b12 ([PDB ID: 1HZH]) | None | 20,000 | 862-1284 | 2.77-6.27 | 0.05-3.00 | 0.22-2.49 | NA | NA |
| Neutron fit, 4 mg/ml, PBS-137, 20 °C | R_x,s, spheres | 10 | 1213-1242 | 4.93-4.98 | 2.48-2.56 | 1.17-1.24 | NA | NA |
| Experimental data | NA | NA | 5.18; 5.16 | 2.45 ± 0.01 | 1.21 ± 0.01 | 16 | NA |

X-ray data IgG1 19a

| Filter        | Models | Spheres | R_x,s-1 | R_x,s-2 | D_{max} | R-factor | s_{20,w} |
|---------------|--------|---------|---------|---------|---------|----------|----------|
| IgG1 b12 ([PDB ID: 1HZH]) | None | 100,000 | 1060-1690 | 2.91-7.47 | 0.04-3.47 | 0.11-2.81 | NA | NA |
| X-ray fit, 2 mg/ml, PBS-50 | R_x,s, spheres | 10 | 1599-1634 | 5.09-5.26 | 2.56-2.66 | 1.29-1.42 | NA | NA |
| Experimental data | NA | NA | 5.21 ± 0.03; 5.25 ± 0.05 | 2.57 ± 0.04 | 1.42 ± 0.06 | 16 | NA |
| X-ray fit, 4 mg/ml, PBS-137 | R_x,s, spheres | 10 | 1600-1635 | 5.14-5.26 | 2.58-2.68 | 1.37-1.46 | NA | NA |
| Experimental data | NA | NA | 5.20 ± 0.06; 5.23 ± 0.03 | 2.61 ± 0.02 | 1.42 ± 0.01 | 16 | NA |
| X-ray fit, 2 mg/ml, PBS-250 | R_x,s, spheres | 10 | 1594-1624 | 5.00-5.18 | 2.61-2.71 | 1.38-1.48 | NA | NA |
| Experimental data | NA | NA | 5.28 ± 0.08; 5.31 ± 0.06 | 2.57 ± 0.05 | 1.47 ± 0.04 | 16 | NA |

---

*The optimum number of hydrated (x-ray) and unhydrated (neutron) spheres predicted from the sequence was 1607 and 1220, respectively.

*The first experimental value is from the Guinier analysis (Fig. 6), and the second one is from the GNOM R(r) analysis (Fig. 8).

*The first modeled value corresponds to that from HYDROPRO, and the second one is that from HYDROPRO.

*NA, not applicable.

*NM, not measured.

increasing NaCl concentration. Similarly, the I(0)/c values for IgG1 19a showed no concentration dependence (Fig. 7C). Each of the R_x,s-1 and R_x,s-2 values was unchanged between PBS-50, PBS-137, and PBS-250, with a mean R_x,s-1 of 2.63, 2.60, and 2.65 nm, respectively, and a mean R_x,s-2 value of 1.48, 1.42, and 1.50 nm, respectively (Fig. 7C). The x-ray R_g, I(0)/c, R_x,s-1, and R_x,s-2 values for IgG1 19a were in agreement with IgG1 6a.

Neutron scattering viewed the unhydrated protein structure in which the hydration shell is almost invisible in heavy water (33). Neutrons were most useful for temperature studies in PBS-137 because temperature-dependent conditions were less accessible by x-ray scattering. IgG1 6a in 100% 2H_2O buffer was analyzed between 2.0 and 4.0 mg/ml. The Guinier analyses revealed high quality linear fits for the same three concentration dependence between 5, 10, and 15 °C with mean R_x,s-1 values of 1.25, 1.25, and 1.20 nm, respectively (Fig. 7B). The neutron R_g, R_x,s-1, and R_x,s-2 values were slightly smaller than the corresponding x-ray values, in particular for the two R_x,s values, and this reduction is attributed primarily to the near invisibility of the surface hydration shell in heavy water, as well as the high negative solute-solvent contrast difference, which will also reduce these values (33). The distance distribution function P(r) provides structural information on IgG1 in real space, namely its overall length and the separation between its Fab and Fc regions. The x-ray P(r) analyses gave R_g values for IgG1 that were similar to those from the x-ray Guinier analyses, showing that the two analyses were self-consistent (filled and open symbols in Fig. 7A). The maximum length L of IgG1 6a was determined from the value of r when the P(r) curve intersects zero to be 16 nm for PBS-50, PBS-137, and PBS-250 (Fig. 8A). The maxima in the P(r) curves correspond to the most frequently occurring interatomic distances within the structure. For IgG1 6a, two peaks, M1 and M2, were identified in all the P(r) curves at ~4 and 7.5 nm, respectively. The M1 peak corresponds mostly to distances within each Fab and Fc region, whereas the M2 peak corresponds mostly to distances between pairs of Fab and Fc regions. No buffer dependence in the positions of peaks M1 and M2 was observed (Fig. 9A). Because M2 is unchanged, the averaged separation between the Fab and Fc regions within IgG1 remains unchanged in 50–250 mM NaCl. This finding differs from that

FIGURE 6. X-ray and neutron Guinier R_g and R_x,s-1 analyses for IgG1. A, the x-ray scattering curves of IgG1 6a are shown for concentrations of 0.5, 1, 1.5, and 2 mg/ml from bottom to top in three buffers, PBS-50, PBS-137, and PBS-250, at 20 °C. In PBS-137, an additional scattering curve for 4.0 mg/ml is displayed. The filled circles represent the Q R_g and R_x,s-1 ranges used to determine the R_g and R_x,s-1 values. The Q range used for the R_g values in PBS-50 and PBS-137 was 0.09–0.28 nm^-1 with the exception of 1 mg/ml IgG1 6a in PBS-50, which was 0.15–0.28 nm^-1. The Q range used for the R_g values in PBS-250 was 0.15–0.28 nm^-1. The R_g and R_x,s-1 Q ranges were 0.31–0.47 and 0.65–1.04 nm^-1, respectively. B, the neutron scattering curves of IgG1 6a are shown for concentrations of 2, 3, and 4 mg/ml from bottom to top for IgG1 in PBS-137 (2H_2O) at 6, 20, and 37 °C. The Q range used for the R_g values was 0.18–0.28 nm^-1, and those for the R_x,s-1 and R_x,s-2 values were 0.21–0.32 and 0.67–1.20 nm^-1, respectively. C, x-ray scattering curves of IgG1 19a for concentrations of 0.22, 0.34, 0.45, 0.68, and 0.90, and 1.35 mg/ml in PBS-50 buffer; 0.30, 0.47, 0.62, 0.95, 1.27, and 1.89 mg/ml in PBS-137 buffer; and 0.26, 0.41, 0.81, and 1.62 mg/ml in PBS-250 buffer at 20 °C from bottom to top. The Q range used for the R_g values was 0.09–0.28 nm^-1 for PBS-50 and PBS-137 buffers, whereas a Q range of 0.15–0.28 was used for PBS-250 buffer. The R_x,s-1 and R_x,s-2 Q ranges were 0.31–0.47 and 0.65–1.04 nm^-1, respectively.
for IgG4, which showed a concentration dependence of $M_2$ below 2 mg/ml (14, 15). IgG1 19a showed two $M_1$ and $M_2$ peaks at similar values of $\sim 4$ and $\sim 8$ nm, respectively (Fig. 9C). IgG1 19a exhibits the same $L$ value of 16 nm as IgG1 6a in PBS-137 and PBS-250. However, the length of IgG1 19a in PBS-50 is slightly reduced at 15 nm (Figs. 8C and 9C).

The neutron $P(r)$ analyses of IgG1 6a in heavy water showed that the $R_g$ values for IgG1 6a at 6, 20, and 37 °C did not change with increasing concentration or temperature (Fig. 7B). The neutron $L$ values were 16 nm at 6, 20, and 37 °C (Fig. 8B). The two peaks $M_1$ and $M_2$ were again identified at $\sim 4$ and 7 nm, respectively, in the neutron $P(r)$ curves (Fig. 8B). The positions of $M_1$ and $M_2$ were unchanged with concentration, in agreement with the x-ray $P(r)$ data.

Starting Model for the Human IgG1 Scattering Fits—The starting model for scattering fits of IgG1 was the crystal structure of human IgG1 b12 (10). The full hinge is formally defined by the 23 residues $^{216}$EPKSCDKTHTCPPCPAPELLGGP$^{238}$ (3, 5), in which the Fab region formally ends at Val$^{215}$ and the Fc region starts at Ser$^{239}$ (Fig. 1). The IgG1 hinge contains six Pro

FIGURE 7. Concentration and temperature dependence of the x-ray and neutron Guinier analyses. The Guinier analyses are shown in Fig. 6. The open symbols show the values from the Guinier analyses, and the filled symbols show those from the $P(r)$ analyses. A and C, the x-ray values for IgG1 6a and IgG1 19a, respectively, were each measured in quadruplicate and averaged, showing the mean $\pm$ S.D. The x-ray $R_g$ values are shown for PBS-50 ($\square$ and $\blacksquare$), PBS-137 ($\bigcirc$ and $\triangle$), and PBS-250 ($\bigtriangledown$ and $\blacktriangle$). The corresponding x-ray $(I(0)/c, R_{xs-1}$, and $R_{xs-2}$ values are likewise shown. The fitted lines show the mean values in PBS-50 (dotted line), PBS-137 (dashed line), and PBS-250 (solid line) buffers. For IgG1 6a, the $(I(0)/c$ values were similar at 0.0187, 0.0158, and 0.0173 for PBS-50, PBS-137, and PBS-250, respectively. For IgG1 19a, the $(I(0)/c$ values were also similar at 0.0178, 0.0194, and 0.0188, respectively. B, the neutron values for IgG1 6a correspond to single measurements in PBS-137 (2H2O). Shown are the $R_g$ values at 6 °C ($\bigtriangleup$ and $\blacktriangle$), 20 °C ($\bigcirc$ and $\triangle$), and 37 °C ($\bigtriangledown$ and $\blacktriangle$). The fitted lines show the mean values at each temperature: 6 °C (dotted line), 20 °C (dashed line), and 37 °C (solid line).
residues and two interchain disulfide bridges at Cys\(^{226}\) and Cys\(^{229}\). The asymmetric modeling considered only the upper hinge 220CDKTHTC\(^{226}\), with Cys220 and Cys226 acting as tethers. Because this hinge is located asymmetrically relative to the Fc region, these 10,000 models do not have 2-fold symmetry. Only one of the interchain disulfide bonds is intact at Cys226. The symmetric modeling considered the upper, middle, and lower hinge, and this resulted in a 19-residue peptide, 220CDKTHTCPPCPAPELLGGP\(^{238}\). Because the two Pro\(^{238}\) residues were located in the middle of the Fc region, this approach generated both symmetric and asymmetric models. For these 10,000 further models, the Cys\(^{226}\) and Cys\(^{229}\) interchain disulfide bonds were not explicitly intact.

Conformational Searches for the Human IgG1 Solution Structure—In order to model both the IgG1 6a and IgG1 19a solution structures, 20,000 conformationally randomized IgG1 structures were created by connecting the Fab and Fc structures to one of four libraries of conformationally randomized hinge peptides of lengths 1.72–2.33 and 2.33–2.45 nm (asymmetric) and 4.66–6.32 and 6.32–6.65 nm (symmetric) (see “Experimental Procedures”). Each modeled scattering curve was compared with the experimental x-ray and neutron scattering curves. To test a broad range of solution conditions, the six modeled x-ray curves were IgG1 6a at the highest available concentrations of 2, 4, and 2 mg/ml in PBS-50, PBS-137, and PBS-250, respectively, plus IgG1 19a at the highest available concentrations of 1.4, 1.9, and 1.6 mg/ml in PBS-50, PBS-137, and PBS-250, respectively. As previously (15), the occurrence of 4% dimer was assumed to have little effect on the scattering modeling. The modeled neutron curve for IgG1 6a was the highest concentration of 4 mg/ml in PBS-137 at 20 °C in heavy water. The seven fit analyses were assessed in R-factor versus \(R_g\) graphs (Fig. 10, A–C). In all seven analyses, the occurrence of a single clear minimum in the R-factor values identified a single conformational family of solution structures for IgG1 starting from a wide range of trial orientations and translations of the two Fab and Fc regions. The lowest R-factors in the 20,000 curve fits corresponded to modeled \(R_g\) values that were close to the experimental \(R_g\) values as desired.

Filters based on the experimental scattering data were used for all 20,000 models to reject unsatisfactory models and identify the 10 best fit models for each search. (i) A \(\pm 5\%\) filter for steric overlap eliminated models in which the Fab and Fc regions sterically overlapped with each other due to inappropriate hinge conformations used in modeling. In order to match the composition-calculated volume of IgG1, sphere models needed a minimum number \(n\) of 1607 spheres for the hydrated x-ray models and 1220 spheres for the unhydrated neutron models. (ii) A \(\pm 5\%\) filter for the modeled \(R_g\) values (calculated from the same Guinier Q ranges used for the experimental analyses) identified the models that agreed best with the experimental x-ray or neutron \(R_g\) values. (iii) The models that passed the \(n\) and \(R_g\) filters were arranged in order of their lowest R-factors. The resulting 10 best fit models for IgG1 occurred as a single cluster at the R-factor minimum in each of the seven searches (green in Fig. 10, A–C), indicating a single best fit solution structure.

Only one of the interchain disulfide bonds at Cys\(^{226}\) was conserved in the asymmetric models. For the symmetric models, the pairs of two Cys\(^{226}\) and two Cys\(^{229}\) residues may not be proximate in the best fit models, because the disulfide bridges were not preserved in the libraries. In the 10 best fit models, the

![Figure 8](image-url)
Solution Structure of IgG1

Figure 9. Summary of the x-ray and neutron P(r) analyses. A and C, the concentration dependence of the peak maxima M1 and M2 for IgG1 6a and IgG1 19a, respectively, are shown for PBS-50 (solid line), PBS-137 (dashed line), and PBS-250 (dotted line). The fitted lines are the mean values in PBS-50 (dotted line), PBS-137 (dashed line), and PBS-250 (solid line) buffers. B, the neutron M1 and M2 values for IgG1 6a are shown for 6 °C (V), 20 °C (o), and 37 °C (X) in PBS-137 (H2O). The fitted lines are the mean values at each temperature: 6 °C (dotted line), 20 °C (dashed line), and 37 °C (solid line).

α-carbon separations were 0.56–1.49 nm for Cys226 and 1.14–1.55 nm for Cys229 in IgG1 6a by x-rays, 0.56–3.63 nm for Cys226 and 1.55–3.52 nm for Cys229 in IgG1 19a by x-rays, and 0.56 nm for Cys226 and 1.55 nm for Cys229 for IgG1 6a by neutrons. These α-carbon separations were comparable with an expected separation of 0.4–0.75 nm between two bridged Cys residues (41), showing that the best fit IgG1 models were compatible with disulfide-bridged hinges.

The best fit modeled curves showed good visual fits in all seven cases with the experimental curves (Fig. 11, A–C). In most cases, the Rv values for the 10 best fit models were within error of the experimental values (Table 1). The seven sets of models (Fig. 12, A–C) generally displayed asymmetric arrangements of the two Fab regions compared with the Fc region. Both IgG1 6a and IgG1 19a showed mostly asymmetric structures, although a few symmetric structures were observed for IgG1 6a in PBS-137 and IgG1 19a in PBS-250. In summary, both IgG1 6a and IgG1 19a appeared to exhibit a T-shaped arrangement in PBS-50 and a Y-shaped arrangement in PBS-250 with intermediate T- and Y-shaped structures in PBS-137 (Fig. 12, A and C). This shape difference would account for the slightly increased Rv values seen in high salt. Surveys of the distances between the centers of the Fab and Fc regions in the best fit IgG1 6a x-ray and neutron models and IgG1 19a x-ray models showed similar distributions (Fig. 13). The x-ray R-factor values for the best fit IgG1 models (pink in Fig. 10, A and C) were acceptable at 3.0–3.1% for IgG1 6a and at 2.8–3.7% for IgG1 19a (Table 1). The neutron R-factor values were acceptable at 2.6–2.7% (pink in Fig. 10B). These R-factor values compare well with those from other similar modeling fits (35).

Sedimentation Coefficient Modeling of Human IgG1—The $s_{20,w}^{0}$ values of the best fit x-ray hydrated IgG1 models were calculated for comparison with the average experimental values of 6.42 S for IgG1 6a and 6.34 S for IgG1 19a (Fig. 5). For the best fit hydrated sphere models, the $s_{20,w}^{0}$ values were 6.67–6.82 and 6.63–6.90 S for IgG1 6a and IgG1 19a, respectively, using HYDRO (Table 1). The corresponding $s_{20,w}^{0}$ values using HYDROPRO were 6.37–6.73 and 6.37–6.68 S for IgG1 6a and IgG1 19a, respectively (Table 1). Given that the calculations should be accurate to within ±0.21 S (35), the modeled $s_{20,w}^{0}$ values agree well with the experimental values.

**DISCUSSION**

The availability of abundant x-ray scattering data for two IgG1 molecules in three buffers permitted a detailed appraisal of the solution structure of human IgG1 and its comparison with the less stable IgG4 solution structure. These experiments were supported by complementary neutron scattering and ultracentrifugation experiments. The data sets enabled atomistic conformational analyses that resulted in seven independent determinations of an asymmetric IgG1 solution structure (Fig. 12, A–C). The combination of these IgG1 solution structures with a docking model for the interaction between human IgG1 Fc and the crystal structure of the C1q globular head (42, 43) and the crystal structure of the human Fc-FcyR receptor (44) shows that the Fc region of human IgG1 is exposed and enables this to react readily with its two major effector ligands, unlike human IgG4 (15).

IgG1 has the highest IgG serum concentration of the four IgG subclasses IgG1–IgG4 at an average level of 8 mg/ml (in a range of 5–12 mg/ml), comprising ~60–70% of the total IgG in normal adult serum (1). IgG1–IgG4 have different heavy chain isotypes, which primarily differ in the hinge region (Fig. 2H). IgG1 activates complement-mediated lysis via C1q binding in the complement classical pathway and binds to all three classes of human Fcy receptors FcyRI, FcyRII, and FcyRIII. IgG1 has different affinities for the FcyRI, FcyRII, and FcyRIII, and its binding to different FcyRs on different immune cells results in different immune responses, including antibody-dependent cell-mediated cytotoxicity, proinflammatory cytokine production, and phagocytosis (45). The precise role of the four IgG subclasses in the immune response is unclear. A recent temporal model suggests that the different properties of the IgG subclass, their concentrations, and their emergence at different stages facilitate a more cohesive immune response (46).
understanding of the distinct properties of the IgG subclasses is desired; we now have complete scattering analyses for human IgG1 and IgG4.

Solution Structure of Human IgG1 6a and IgG1 19a—The two monoclonal IgG1 antibodies studied here have 88.7% total sequence identity, with identical hinge regions, and differ primarily in their VH and VL domains (Fig. 2). Our x-ray data collection involved the measurement of 52 and 70 curves in two beam sessions (or 520 and 700 curves if time frames are included) (Fig. 7). This abundant data collection enabled the use of different buffers with two different human IgG1s. The use of three NaCl concentrations examined potential electrostatic effects on the IgG1 structure, whereas heavy water is a known promoter of protein self-association. By comparison, earlier scattering studies on human IgG1 reported few scattering and ultracentrifugation runs or were performed in non-physiological buffer conditions (28, 38, 39, 47–49). Both IgG1 6a and IgG1 19a showed similar experimental \( R_g \) and \( R_{xs} \) values and the same overall length of 16 nm (Table 1). The two IgG1 molecules also displayed similar experimental \( s_{20,w}^0 \) values of

---

**FIGURE 10. Constrained modeling analysis for IgG1.** The 20,000 goodness of fit R-factors are compared with the calculated x-ray and neutron \( R_g \) values for the IgG1 6a and IgG1 19a models. The 20,000 asymmetric and symmetric models are shown in yellow. The 10 best fit models with the lowest R-factors are shown in green, with the best fit model shown in pink. The experimentally observed \( R_g \) values are shown by vertical solid lines with error ranges of \( \pm 5\% \) shown by dashed lines. A, hydrated x-ray models are compared with experimental x-ray data for IgG1 6a in PBS-50, PBS-137, and PBS-250 at 20 °C. B, unhydrated neutron models are compared with the experimental neutron curve for IgG1 6a in PBS-137 in \( ^2\text{H}_2\text{O} \) at 20 °C. C, hydrated x-ray models are compared with experimental x-ray data for IgG1 19a in PBS-50, PBS-137, and PBS-250 at 20 °C.
6.3–6.4 S, which were indistinguishable within error. The only change with buffer conditions was a small increase in the \( R_g \) values in 250 mM NaCl. The 30 best fit structures for IgG1 6a and IgG1 19a were predominantly asymmetric, with only one symmetric model for IgG1 6a in PBS-137 and two symmetric models for IgG1 19a (PBS-250) (Fig. 12). Little difference was seen between the two IgG1 antibodies. The hinge length is measured by the \( \beta \)-carbon positions of the flanking residues Cys220 and Pro238, with a maximum possible length of 6.65 nm. The best fit structures gave similar hinge lengths of 2.4–5.0 ± 0.6 nm for IgG1 6a (x-rays), 1.6–5.0 ± 0.7 nm for IgG1 19a (x-rays), and 3.2–4.9 ± 0.5 nm for IgG1 6a (neutrons). These lengths show that this hinge is semiextended. The slight \( R_g \) increase in 250 mM NaCl was best explained by a shift from T-shaped structures in low salt to Y-shaped structures in high salt.

The only crystal structure for an intact human IgG is currently that for IgG1 b12 (10), and this structure was used to model the solution scattering data in this study. Two full-length murine IgG crystal structures have also been solved for IgG1 61.1.3 (11) and IgG2a Mab231 (12). These crystal structures only offer a single view of the antibody immobilized in the crystal lattice, in contrast to the expectation that antibodies may
display a large range of conformations in solution. Atomistic scattering modeling of the solution structure of IgG1 enhances our understanding of the IgG1 b12 crystal structure and yields the averaged arrangement of the Fab and Fc fragments. IgG1 b12 was crystallized in 800 mM ammonium sulfate and 100 mM sodium cacodylate, pH 6.5. This IgG1 crystal structure showed no symmetry and an asymmetric arrangement of the Fab regions, with one Fab closely packed on top of the Fc region and the other Fab extended outward. The two Cys<sup>220</sup>-Pro<sup>238</sup> hinge lengths were 3.8 and 3.9 nm. Both agree with the modeled hinge lengths for IgG1 6a and IgG1 19a above. The IgG1 b12 <i>R</i><sub>g</sub>, <i>R</i><sub>xs</sub>-1, and <i>R</i><sub>xs</sub>-2 values were calculated as 5.12, 2.60, and 1.56 nm, in agreement with the values for IgG1 6a and IgG1 19a (Table 1). The IgG1 b12 <i>σ</i><sup>20,ω</sup> value was 6.84 and 6.57 S from HYDRO and HYDROPRO, in agreement with the experiment (Table 1). The <i>d</i><sub>1</sub>, <i>d</i><sub>2</sub>, and <i>d</i><sub>3</sub> values between the Fab and Fc regions were also similar to those for IgG1 6a and IgG1 19a (Fig. 13). It is concluded that the solution structures of IgG1 6a and IgG1 19a are similar to the IgG1 b12 crystal structure.

The comparison of IgG1 6a and IgG1 19a with human IgG4 (15) displayed some differences. Despite similar molecular weights, the <i>R</i><sub>g</sub> values of human IgG1 are 0.1–0.2 nm larger.
than those for human IgG4, which has \( R_g \), \( R_{xs-1} \), and \( R_{xs-2} \) values of 4.92, 2.56, and 1.37 nm, respectively. The \( s_{20,w} \) values of IgG1 6a and IgG1 19a are ~0.4 S smaller than IgG4, whose \( s_{20,w} \) value is 6.8 S (15). Both data sets indicate that IgG1 is more elongated than IgG4. This is attributable to the longer IgG1 hinge sequence, in which the upper hinge contains three extra residues compared with IgG4 (Fig. 2).

**Interaction of Human IgG1 with C1q**—Our atomistic models for intact IgG1 enable the binding of C1q to IgG1 to be assessed. As before, molecular docking of the IgG1 Fc and C1q crystal structures was performed to evaluate this interaction. This structural approach had previously shown that the rabbit IgG interaction with C1q was sterically allowed, but that of human IgG4 with C1q was restricted (15, 50). This C1q binding site occurs at the top of the \( C_{\beta2} \) domain in the Fc region near the hinge. Functionally, the reactivity of C1q with the four human IgG subclasses correlates with upper hinge length in the order of IgG3 > IgG1 > IgG2 > IgG4, with IgG4 not activating complement (3). A hingeless IgG1 antibody cannot bind or activate C1q (51). Mutagenesis studies of the hinge modulate C1q binding. These studies include disruption of the inter-heavy chain disulfide covalent bridges in the core hinge that removed the C1q interaction, whereas substitutions in the upper hinge increased C1q binding (52). The mutation of Leu\(^{234}\) and Leu\(^{235}\) to Ala residues in the lower hinge of human IgG1 b12 also removed C1q binding, suggesting that the lower hinge is also important (53). A human IgG1 mutant with Thr\(^{223}\) and His\(^{224}\) deleted in the upper hinge and Pro\(^{227}\) and Pro\(^{228}\) deleted in the core hinge cannot bind or activate C1q (52). The isolated IgG4-Fc region binds C1q, although intact human IgG4 does not, suggesting that the Fab regions are also important for the C1q interaction (54). In other experiments, the mutation of human IgG1 to mimic the disulfide bonding of IgG4 removed its antibody-dependent cell-mediated cytotoxicity activity (55). Reduction of the inter-heavy chain disulfide bridges showed that these are important for C1q binding (54). Thus the upper, core, and lower hinge contribute to C1q binding and activation, as do the hinge disulfide bridges.

Docking studies were performed using a shape complementarity method based on the PatchDock server (36) with the best
fit IgG 6a and IgG 19a models (Fig. 14, A and B). Human IgG residues involved in C1q binding include Asp270, Lys322, Pro329, and Pro331 in the Fc CH2 region (56, 57). Docking and molecular dynamic simulations identified 19 C1q and 12 Fc contact residues in the IgG1-C1q complex (see Table 2 of Ref. 43). Using these residues to guide the docking, both Fab regions in both IgG 6a and IgG 19a were seen to be positioned away from the C1q binding site, hence enabling C1q to bind. Steric clashes between the docked IgG1-C1q complexes were evaluated and compared with those for docked IgG4-C1q models (Fig. 15). The globular C1q head has a molecular mass of 44.1 kDa and an unhydrated volume of 57.1 nm³ (21). Residues making main-chain clashes were identified using Swiss-PdbViewer (58), and their amino acid volumes were summed to estimate a notional C1q volume obstructed by the Fab arms. Based on all of the best fit structures, the mean obstructed volume for IgG 6a-C1q was 6.9 nm³, that for IgG 19a-C1q was 2.1 nm³, and that for IgG4-C1q was 19.1 nm³. This comparison showed that the IgG 4 Fab regions hindered C1q binding by about 3–9 times the obstructed volume of the IgG 1 Fab regions. The binding of therapeutic antibodies to native FcγR in vivo is sometimes exploited to produce drug action. The affinities of the four human IgG subclasses for specific FcγRs vary due to the presence of different contact residues in the Fc fragment and the FcγRs. Human IgG1 and IgG3 bind to all of the Fcγ receptors (FcγRI, FcγRIIA, FcγRIIB/C, FcγRIIIA, and FcγRIIIB), whereas IgG2 and IgG4 only bind to some of them. For FcγRI, IgG1 and IgG3 bind most strongly (K₆ of 6.5 and 6.1 × 10⁷ M⁻¹ respectively), IgG4 binding is slightly weaker (3.4 × 10⁷ M⁻¹), and IgG2 displayed no measurable binding. For

Sequence differences between the IgG subclasses may also account for the reduced binding of C1q, with Pro329 and Pro331 likely to be important for this (60, 61). The strength of Fc-C1q binding is not directly correlated to complement activation, with IgG1 better able to activate complement-mediated lysis than IgG3, despite the stronger binding of IgG3 to C1q, for example (62). This suggests that binding of C1q alone is not enough to activate the complement cascade. The Fc-C1q affinity is low, with a dissociation constant Kᵯ of ~10⁻⁴ M (63, 64). Localized IgG clusters may bind a C1q hexamer through multivalent contacts to increase the strength of the C1q-Fc interaction, as exemplified by a hexamer configuration of an IgG1 mutant (65).
the remaining Fc-RII and Fc-RIII subclasses, IgG1 and IgG3 bound to all of the Fc-RII and Fc-RIII receptors with high $K_a$ values ranging between $1.2 \times 10^7$ M$^{-1}$ to $9.8 \times 10^6$ M$^{-1}$. In contrast, IgG4 showed low affinity binding, with $K_a$ values in the region of $2 \times 10^7$ M$^{-1}$ for Fc-RIIIA/B/C and no measurable binding to Fc-RIIIB. IgG2 showed mostly lower affinities than IgG1, IgG3, and IgG4 for all Fc-Rs, including no measurable Fc-RIII receptors with high $K_a$ values in Rg.$_R$ (Figs. 7 and 9). No temperature dependence was observed for IgG1 6a by neutron scattering, with no changes in $R_g$ and $R_w$, and no movement of the M1 and M2 peaks (Figs. 8 and 9). In marked contrast to IgG1, human IgG4 displays conformational instabilities in the $P(r)$ curves below 2 mg/ml, these being attributable to different diffusion-collision events at different salt concentrations (Fig. 7), indicating that more elongated structures arise in higher salt concentrations from changes in the electrostatic interactions between surface amino acid residues. However, no changes were revealed by the M1 and M2 values or $s_{20,w}$ values (Figs. 7 and 9). No temperature dependence was observed for IgG1 6a by neutron scattering, with no changes in $R_g$ and $R_w$, and no movement of the M1 and M2 peaks (Figs. 8 and 9). In marked contrast to IgG1, human IgG4 displays conformational instabilities in the $P(r)$ curves below 2 mg/ml, these being attributable to different diffusion-collision events at different salt concentrations or to the occurrence of Fab arm exchange in dilute IgG4 concentrations (14, 15). Human IgG1 also showed no significant concentration-dependant dimerization by neutron scattering in heavy water, unlike the noticeable dimer formation seen for IgG4.

IgG1 aggregation and self-association are relevant also to the immune response in vivo as well as in treatments with therapeutic antibodies. Human serum naturally contains a total of 1% dimeric IgG1, with less than 0.03% of this being covalent therapeutic drugs, such as epratuzumab (73), which is currently

---

**FIGURE 15. Comparison of the human IgG1 and IgG4 complexes with their ligands.** A, best fit model of IgG1 6a. B, best fit model of IgG1 19a. C, best fit model of IgG4 (Ser222). C1q is shown in yellow, and FcγRIII is shown in cyan.
REFERENCES

1. Hamilton, R. G. (2001) The Human IgG Subclasses, Calbiochem Corp., La Jolla, CA

2. Strohl, W. R., and Strohl, L. M. (2012) Therapeutic Antibody Engineering: Current and Future Advances Driving the Strongest Growth Area in the Pharmaceutical Industry. Woodland Publishing Ltd., Cambridge, UK

3. Dangl, J. L., Wensel, T. G., Morrison, S. L., Stryer, L., Herzenberg, L. A., and Oi, V. T. (1988) Segmental flexibility and complement fixation of genetically engineered chimeric human, rabbit and mouse antibodies. EMBO J. 7, 1989–1994

4. Roux, K. H., Strelets, L., and Michaelis, T. E. (1997) Flexibility of human IgG subclasses. J. Immunol. 159, 3372–3382

5. Brekke, O. H., Michaelis, T. E., and Sandlie, I. (1995) The structural requirements for complement activation by IgG: does it hinge on the hinge? Immunol. Today 16, 85–90

6. Lund, J., Winter, G., Jones, P. T., Pound, J. D., Tanaka, T., Walker, M. R., Artymiuk, P. J., Arata, Y., Burton, D. R., and Jeffers, R. (1991) Human FcγRI and FcγRII interact with distinct but overlapping sites on human IgG. J. Immunol. 147, 2657–2662

7. Sarma, R., and Laudin, A. G. (1982) The three-dimensional structure of a human IgG1 immunoglobulin at 4 Å resolution: a computer fit of various structural domains on the electron density map. J. Appl. Cryst. 15, 476–481, 10.1107/S002188988202124X

8. Marquart, M., Deisenhofer, J., and Huber, R. (1980) Crystallographic refinement and atomic models of the intact immunoglobulin molecule Kol and its antigen-binding fragment at 3.0 Å and 1.9 Å resolution. J. Mol. Biol. 141, 369–391

9. Guddat, L. W., Herron, J. N., and Edmundson, A. B. (1993) Three-dimensional structure of a human immunoglobulin with a hinge deletion. Proc. Natl. Acad. Sci. U.S.A. 90, 4271–4275

10. Saphire, E. O., Parren, P. W., Pantophlet, R., Zwick, M. B., Morris, G. M., Rudd, P. M., Dwek, R. A., Stanfield, R. L., Burton, D. R., and Wilson, I. A. (2001) Crystal structure of a neutralizing human IgG against HIV-1: a template for vaccine design. Science 293, 1155–1159

11. Harris, L. J., Skaltsky, E., and McPherson, A. (1998) Crystallographic structure of an intact IgG1 monoclonal antibody. J. Mol. Biol. 275, 861–872

12. Harris, L. J., Larson, S. B., Hasel, K. W., and McPherson, A. (1997) Refined structure of an intact IgG2a monoclonal antibody. Biochemistry 36, 1581–1597

13. Saphire, E. O., Stanfield, R. L., Crispin, M. D. M., Parren, P. W. H. I., Rudd, P. M., Dwek, R. A., Burton, D. R., and Wilson, I. A. (2002) Contrasting IgG structures reveal extreme asymmetry and flexibility. J. Mol. Biol. 319, 9–18

14. Abe, Y., Gor, J., Bracewell, D. G., Perkins, S. J., and Dalby, P. A. (2010) Masking of the Fc region is human IgG4 by constrained x-ray scattering modelling: implications for antibody function and therapy. Biochem. J. 432, 101–111

15. Rayner, L. E., Hui, G. K., Gor, J., Heenan, R. K., Dalby, P. A., and Perkins, S. J. (2014) The Fab conformations in the solution structure of human IgG4 restrict access to its Fc region: implications for functional activity. J. Biol. Chem. 289, 20740–20756

16. Boehm, M. K., Wood, J. M., Kerr, M. A., and Perkins, S. J. (1999) The Fab and Fc fragments of IgA1 exhibit a different arrangement from that in IgG: a study by x-ray and neutron solution scattering and homology modelling. J. Mol. Biol. 286, 1421–1447

17. Marquart, M., Deisenhofer, J., and Huber, R. (1980) Crystallographic refinement and atomic models of the intact immunoglobulin molecule Kol and its antigen-binding fragment at 3.0 Å and 1.9 Å resolution. J. Mol. Biol. 141, 369–391

18. Furtado, P. B., Whitty, P. W., Robertson, A., Eaton, J. T., Almogren, A., Kerr, M. A., Wood, J. M., and Perkins, S. J. (2004) Solution structure determination of monomeric human IgA2 by x-ray and neutron scattering, analytical ultracentrifugation and constrained modelling: a comparison with monomeric human IgA1. J. Mol. Biol. 338, 921–941

19. Sun, Z., Almogren, A., Furtado, P. B., Chowdhury, B., Kerr, M. A., and Perkins, S. J. (2005) Semi-extended solution structure of human myeloma immunoglobulin D determined by constrained x-ray scattering. J. Mol. Biol. 353, 155–173

20. Deisenhofer, J. (1981) Crystallographic refinement and atomic models of a human Fc fragment and its complex with fragment B of protein A from Staphylococcus aureus at 2.9- and 2.8-Å resolution. Biochemistry 20, 2361–2370

21. Perkins, S. J. (1986) Protein volumes and hydration effects: the calculation of partial specific volumes, neutron scattering matchpoints and 280-nm absorption coefficients for proteins and glycoproteins from amino acid sequences. Eur. J. Biochem. 157, 169–180

22. Laue, T. M., Shah, B. D., Ridgeway, T. M., and Pelletier, S. L. (1992) in Analytical Ultracentrifugation in Biochemistry and Polymer Science (Har- dinc, S. E., Rowe, A. J., and Horton, J. C., eds) pp. 90–125, Royal Society of Chemistry, Cambridge, UK

23. Schuck, P. (1998) Sedimentation analysis of non-interacting and self-associating solutes using numerical solutions to the Lamm equation. Biophys. J. 75, 1503–1512

24. Schuck, P. (2000) Size-distribution analysis of macromolecules by sedimentation velocity ultracentrifugation and Lamm equation modelling. Biophys. J. 78, 1606–1619

25. Narayanan, T., Diat, O., and Bösecke, P. (2001) SAXS and USAXS on the high brilliance beamline at the ESRF. Nucl. Instrum. Methods Phys. Res. A 467, 1005–1009

26. Heenan, R. K., Rogers, S. E., Turner, D., Terry, A. E., Treadgold, J., and King, S. M. (2011) Small angle neutron scattering using Sans2d. Neutron News 22, 19–21, 10.1080/10448632.2011.569531

27. Glatter, O., and Kratky, O. (1982) Small Angle X-ray Scattering, pp. 17–51, Academic Press, Inc., New York

28. Piltz, L., Kratky, O., Licht, A., and Seela, M. (1973) Shape and volume of anti- polyclonal antibodies in the presence and absence of tetra-o- alanine as followed by small-angle x-ray scattering. Biochemistry 12, 4998–5005

29. Semenyuk, A. V., and Svergun, D. I. (1991) GNOM: a program package for small-angle scattering data-processing. J. Appl. Crystallogr. 24, 537–540, 10.1107/S002188989100081X

30. Jefferis, R., and Lefranc, M. P. (2009) Human immunoglobulin allotypes: possible implications for immunogenicity. MAbs 1, 332–338

31. Ashton, A. W., Boehm, M. K., Gallimore, I. R., Pepsy, M. B., and Perkins, S. J. (1997) Pentameric and decameric structures in solution of the serum amyloid P component by x-ray and neutron scattering and molecular modelling analyses. J. Mol. Biol. 272, 408–422

32. Perkins, S. J. (2001) X-ray and neutron scattering analyses of hydration shells: a molecular interpretation based on sequence predictions and
Solution Structure of IgG1

modelling fits. *Biophys. Chem.* 93, 129–139

33. García de la Torre, J., Navarro, S., Lopez Martinez, M. C., Diaz, F. G., and Lopez Cascales, J. (1994) HYDRO: a computer program for the prediction of hydrodynamic properties of macromolecules. *Biophys. J.* 67, 530–531

34. García De La Torre, J., Huertas, M. L., and Carrasco, B. (2000) Calculation of hydrodynamic properties of globular proteins from their atomic-level structure. *Biophys. J.* 78, 719–730

35. Perkins, S. J., Okemefuna, A. I., Nan, R., Li, K., and Bonner, A. (2009) Constrained solution scattering modelling of human antibodies and complement proteins reveals novel biological insights. *J. R. Soc. Interface* 6, S679–S696

36. Schneidman-Duhovny, D., Inbar, Y., Nussinov, R., and Wolfson, H. J. (2005) PatchDock and SymmDock: servers for rigid and symmetric docking. *Nucleic Acids Res.* 33, W363–W367

37. Andrusier, N., Nussinov, R., and Wolfson, H. J. (2007) FireDock: fast interaction refinement in molecular docking. *Proteins* 69, 139–159

38. Kilar, F., Simon, I., Lakatos, S., Vonderviszt, F., Medgyesi, G. A., and Závodszky, P. (1985) Conformation of human IgG subclasses in solution: small-angle x-ray scattering and hydrodynamic studies. *Eur. J. Biochem.* 147, 17–25

39. Gregory, L., Davis, K. G., Sheth, B., Boyd, J., Jefferis, R., Nave, C., and Burton, D. R. (1987) The solution conformations of the subclasses of human IgG deduced from sedimentation and small angle x-ray scattering studies. *Mol. Immunol.* 24, 821–829

40. Phillips, M. L., Tao, M. H., Morrison, S. L., and Schumaker, V. N. (1994) Human/mouse chimeric monoclonal antibodies with human IgG1, IgG2, IgG3 and IgG4 constant domains: electron microscopic and hydrodynamic characterization. *Mol. Immunol.* 31, 1201–1210

41. Richardson, J. S., and Richardson, D. C. (1989) *In Prediction of Protein Structure and the Principles of Protein Conformation* (Fasman, G. D., ed.) pp. 1–98, Plenum Press, New York, NY

42. Gaboriaud, C., Juanhuix, J., Gruel, A., Lacroix, M., Darnault, C., Pignon, D., Verger, D., Fontecilla-Camps, J. C., and Arlau, G. (2003) The crystal structure of globular head of complement protein C1q provides a basis for its versatile recognition properties. *J. Biol. Chem.* 278, 46974–46982

43. Schneider, S., and Zacharias, M. (2012) Atomic resolution model of the antibody Fc interaction with the complement C1q component. *Mol. Immunol.* 51, 66–72

44. Sondermann, P., Huber, R., Oosthuizen, V., and Jacob, U. (2000) The 3.2 Å crystal structure of the human IgG1 Fc fragment-FcyRIII complex. *Nature* 406, 267–273

45. Bruhns, P., Iannasci, B., England, P., Mancardi, D. A., Fernandez, N., Jorieux, S., and Daëron, M. (2009) Specificity and affinity of human Fc receptors and their polymorphic variants for human IgG subclasses. *Blood* 113, 3716–3725

46. Collins, A. M., and Jackson, K. J. L. (2013) A temporal model of human IgE and IgG antibody function. *Front. Immunol.* 4, 235

47. Calmettes, P., Cser, L., and Rajnavolgyi, E. (1991) Temperature and pH dependence of immunoglobulin G conformation. *Arch. Biochem. Biophys.* 291, 277–283

48. Ashish, Solanki, A. K., Boone, C. D., and Krueger, J. K. (2010) Global structure of HIV-1 neutralizing antibody IgG1 b12 is asymmetric. *Biochem. Biophys. Res. Commun.* 391, 947–951

49. Liljestrom, W. G., Shire, S. J., and Scherer, T. M. (2012) Influence of the cosolute environment on IgG solution structure analyzed by small-angle x-ray scattering. *J. Immunol.* 187, 2714–2723

50. Manning, M. C., Chou, D. K., Murphy, B. M., Payne, R. W., and Katayama, P. W. (2001) The structure of a human type III Fc receptor in complex with Fc. *J. Biol. Chem.* 276, 16469–16477

51. Luo, Y., Lu, Z., Raso, S. W., Entrican, G., and Tangarone, B. (2009) Dimers and multimers of monoclonal IgG1 exhibit higher in vitro binding affinities to Fc receptors. *mAbs* 1, 491–504

52. Dall’Acqua, W. F., Cook, K. E., Danschroder, M. M., Woods, R. M., and Wu, H. (2006) Modulation of the effector functions of a human IgG1 through engineering of its hinge region. *J. Immunol.* 177, 1129–1138

53. Hezareh, M., Hessel, A. J., Jensen, R. C., van de Winkel, J. G., and Parren, P. W. (2001) Effector function activities of a panel of mutants of a broadly neutralizing antibody against human immunodeficiency virus type 1. *J. Virol.* 75, 12161–12168

54. Isenman, D. E., Dorrington, K. I., and Painter, R. H. (1975) The structure and function of immunoglobulin domains. *J. Immunol.* 114, 1726–1729

55. Dorai, H., Wesolowski, J. S., and Gillies, S. D. (1992) Role of the inter-heavy and light chain disulfide bonds in the effector functions of human immunoglobulin IgG1. *Mol. Immunol.* 29, 1487–1491

56. Iwura, T., Fukuda, J., Yamazaki, K., Kanamaru, S., and Arisaka, F. (2014) Intermolecular interactions and conformations of antibody dimers present in IgG1 biopharmaceuticals. *J. Biochem.* 155, 63–71