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Global structures of IgG isotypes expressing identical variable regions

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ABSTRACT

Until relatively recently the immunoglobulin molecule was viewed as composed of two independent domains comprised of the variable (V) and constant (C) regions. However, recent work has established that the C region mediates allosteric changes in the V region that can influence specificity and affinity. To further explore cross-domain interrelationship in murine IgG structure we carried out solution small angle X-ray scattering (SAXS) measurements for four V region identical IgG isotypes. SAXS analysis revealed elongated Y-shaped structures in solution with significantly different, isotype-dependent domain orientations. To further explore local C region effects on the V region, the IgG_3 Fab crystal structure from the same family was determined to 2.45 Å resolution. The IgG_3 Fab crystal structure differs from a closely related previously solved IgG_1 Fab revealing significant structural differences, which may account for isotype-related specificity differences in V region identical Abs. Among the four murine isotypes, IgG_3 was the most different in solution with regards to overall structure as well as aggregate formation in solution suggesting that the greater apparent affinity of this isotype resulted from polyvalent complexes with enhanced avidity. Our results provide additional evidence that Ig V and C domains influence each other structurally and suggest that V region structure can have significant effects on overall Ig structure.

1. Introduction

Immunoglobulin molecules are products of the adaptive humoral immune response that function as antimicrobial proteins. Igs are heterodimers in which each monomer contains two polypeptide chains, a heavy (H) and a light (L) chain. Each H and L chain has a C region that defines the class, or isotype of the antibody (Ab), as well as a V region that provides the interface that directly interacts with the Ag, also known as the paratope. The C region of an IgG molecule, which is the most prevalent form of Ig in both human and murine sera, is composed of the CH1–CH3 domains, which are located in both the Fab and Fc regions. The C region defines the isotype and confers effector properties such as interactions with Fc receptors, half-life, and complement actions (Ravetch and Kinet, 1991). In mice there are four IgG isotypes that have been found to be responsible for the identification and clearance of many peptide and polysaccharide antigens (Ags): IgG_1, IgG_{2a}, IgG_{2b} and IgG_3 (Snapper and Mond, 1993). Interactions of IgG-Ag complexes with other cells of the immune system include Ab-dependent cytotoxicity (ADCC), phagocytosis, regulation of lymphocyte proliferation and Ab secretion. These effector functions are mediated through the interactions of Fc domains of IgG Ab or immune complexes with specific receptors on immune cells known as the Fcγ receptors (FcγRs) (Ravetch and Kinet, 1991).

In recent years several laboratories have reported that the specificity and affinity of Abs can be influenced by the C regions such that these properties are not solely the purview of V region structure (Cooper et al., 1993; Janda et al., 2012; McLean et al., 2002; Pritsch et al., 2000; Torres and Casadevall, 2008; Torres et al., 2005, 2007b; Tudor et al., 2012; Xia et al., 2012). The mechanism for this effect presumably involves isotype dependent influences on V region secondary structure following antigen (Ag) binding (Janda and Casadevall, 2010). Furthermore, IgG molecules express different electronic emission spectra and proteolytic capacity (Janda et al., 2012). Much is known about the crystal structures of both Fab and Fc regions of Ig molecules, as separate domains. Each region is composed of two or three domains of anti-parallel β-sheets that form β-barrel domains (Davies et al., 1975). Although a few groups have been able to crystallize entire Ig molecules, there are very few

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complete structures from those crystals (Saphire et al., 2002). The first two were human myeloma IgG_1 molecules, Mcg (Edmundson et al., 1970) and Dob (Terry et al., 1968) whose sequences do not have hinges or intra-light chain disulfide bonds that occur in normal IgGs. Furthermore, due to the hinge deletion, Mcg and Dob are conformationally restricted and have little to no effector function, and their crystal structures were compact, T-shaped and symmetric (Saphire et al., 2002). Two other human IgG_1 structures, Kol (Huber et al., 1976) and Zie (Ely et al., 1978) with full-length hinges, failed to provide ordered electron density in their Fc domains (Saphire et al., 2002). Only three full-length IgG crystals thus far have been able to provide a complete picture involving all three IgG domains. The first is murine anti-canine lymphoma 231 IgG_{2a} (PDB: 1IGT) (Larson et al., 1991), the second is murine anti-phenobarbital 61.1.3 IgG_1 (PDB: 1IGY) (Harris et al., 1998) the third is human b12 IgG_1 anti-HIV-1 gp120 (Saphire et al., 2001, 2002) (PDB: 1RHH). There is significant variation in the orientation of the domains of these structures, suggesting that a considerable degree of flexibility exists within IgG molecules (Harris et al., 1998; Saphire et al., 2002). This flexibility, most of which occurs around the hinge region of the Ab molecule, makes IgGs difficult candidates for crystallographic studies. Hinge flexibility also raises the possibility that structures obtained by X-ray crystallography are snapshots of a single conformation (Sawaya and Kraut, 1997), such that in solution and *in vivo*, there may be considerable dynamic flexibility.

SAXS is a powerful technique that gives information about the size, shape, surface-to-volume ratio, distribution and oligomerization of molecules in solution (Bernado and Svergun, 2012). Previously SAXS data involving IgG molecules has been collected on a number of human IgGs (Abe et al., 2010; Ashish et al., 2010; Gregory et al., 1987; Kilar et al., 1985; Lu et al., 2006; Rathore et al., 2011), as well as one murine IgG – a family of anti-dansyl isotypes using whole IgGs (Igarashi et al., 1990). Igarashi et al. collected SAXS data on a family of murine IgG switch variants: IgG_1, IgG_{2a}, IgG_{2b} and an IgG_{2a} with a C_{H1} deletion (referred to from now on as IgG_{2a}(s)). The radii of gyration (R_g) values that they found for these mAbs ranged from 50.7 to 53.9 Å, which are within the same range as those found for human IgGs, and the hinge angles were IgG_{2a}(s), 15°; IgG_1, 180°; IgG_{2a}, 100° and IgG_{2b}, 120° (Igarashi et al., 1990). These hinge angles are not similar to those that have been identified by X-ray crystallography for the murine 231 IgG_{2a} (PDB: 1IGT) which is 172°, and for the murine 61.1.3 IgG_1, which is 115° (Saphire et al., 2002). These differences in hinge angles, obtained by different techniques, may be attributed to the flexible behavior of hinge regions. Flexible hinge regions facilitate an ensemble of conformations of Fab–Fc hinge orientations. X-ray crystallography may capture one or few of these conformations depending on how many molecules with different conformations are present in the asymmetric unit, in addition crystal contacts may also affect Fab–Fc orientations. Consequently, there is a need for studies to further delineate the structure of IgGs in solution, and on a set which has been at least in part characterized at the atomic level by crystallography.

Elucidating the mechanisms by which the C region can affect the specificity, affinity and avidity of IgGs is critical to better understand the Ig molecule, isotype switching and new possibilities in Ig engineering. To this end, we have first determined the global envelope structures of four murine IgG isotypes with solution SAXS to infer isotype-dependent global structural differences, and second, we have determined the IgG_3 Fab crystal structure to 2.45 Å resolution and compared it to a previously published IgG_1 Fab crystal structure from a related IgG_1 to elucidate structural differences imposed by C regions in atomic resolution. Furthermore, using all atom molecular dynamics simulations, we have modeled the 3E5 IgG_3 Fab domain bound to the peptide antigen P1. Hence, this paper includes two partially independent stories related to isotype structural variability – the comparison of four IgG isotype solution structures and the crystallographic comparison of two Fabs arising from the same V region genes. The results provide insight to the mechanism by which the C region affects the affinity, avidity and specificity of the V region.

2. Materials and methods

2.1. mAb preparation

The IgG switch variants of 3E5-IgG_3 have been described previously (Dam et al., 2008; Torres et al., 2007a). The murine mAbs were secreted by hybridoma cell culture supernatants and purified by protein A or G affinity chromatography (Pierce). mAbs were concentrated and buffer exchanged against 0.1 M Tris–HCl, 0.05 M NaCl pH 8.0. mAb concentration was determined by absorbance at 280 nm using an E value of 1.35. 3E5 IgG_1 and IgG_3 samples were monodisperse by Sephacryl-200 high pressure liquid chromatography, the other isotypes were not tested (data not shown).

2.2. X-ray scattering

X-ray scattering was obtained during a single beam session at the National Synchrotron Light Source (NSLS) at Brookhaven National Laboratories (BNL), New York on the X9A beamline. Details of the instrument configuration are published elsewhere (Allaire and Yang, 2011). All IgGs were 1–2 mg/ml, in samples with volumes of 15 μl each. Measurements were taken in flow cells to reduce radiation damage. Four frames of 30 s each were taken for each sample. Measurements were checked continuously for radiation damage and then averaged. The reduction of the scattering data was done by correcting the data for buffer scatter and intrinsic anomalies specific to the experimental set up, *i.e.* detector inhomogeneity and dark noise using standard data reduction software, PyXS at NSLS X9A beamline.

2.3. Analysis of reduced scattering data

The analysis was done by utilizing GNOM (Svergun, 1999), which evaluates the pair-distance distribution function, $P(r)$, and calculates the radius of gyration, R_g, from one-dimensional scattering curves for mono-disperse systems. The scattering length unit $2\pi \sin\theta/\lambda$ was used with λ in Å. The low-resolution *ab initio* envelopes of Igs were calculated from their respective scattering profiles using DAMMIN (Svergun, 1992). DAMMIN uses simulated annealing for global minimization of the discrepancy (χ), between the experimental scattering curves and those that were calculated from dummy atom models. Different scattering angle, q (Å^{-1}), and distance, R (Å), ranges were used for individual Ig runs; IgG_1 (q = 0.013–0.080, R = 1–175), IgG_{2a} (q = 0.017–0.113, R = 1–175), IgG_{2b} (q = 0.015–0.078, R = 1–175) and IgG_3 (q = 0.013–0.095, R = 1–185). The q and R pair, which gave the best χ value in DAMMIN fast mode calculations, was used for the production runs, which were done in DAMMIN slow mode. In production runs, 10 individual models were generated for each Ig; P1 symmetry (no symmetry) was imposed in all calculations and a sphere was used as initial model. The calculations gave excellent χ values for IgG_1, IgG_{2a} and IgG_{2b} with χ^2 values of 1.06, 1.45 and 0.98 respectively. IgG_3 suffered from an inherent propensity of its Fc regions to aggregate (Greenspan and Cooper, 1993), which limited the soluble mono-disperse concentrations during data collection, rendering a mediocre quality fit and a χ^2 of 29.20. Ten DAMMIN models were then subjected to envelope averaging using DAMAVER (Volkov and Svergun, 2003), where models were compared pairwise resulting in a normalized spatial discrepancy (NSD) value for each model. The model with the lowest NSD value was