Molecular Immunology 92 (2017) 199–210

Contents lists available at ScienceDirect

Molecular Immunology

journal homepage: www.elsevier.com/locate/molimm

Research paper

Human myeloma IgG4 reveals relatively rigid asymmetric Y-like structure with different conformational stability of C_H2 domains

Vladimir M. Tischenko[a,1], Vladimir P. Zav'yalov[b], Sergey N. Ryazantsev[c,*]

[a] Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, Pushchino, Russia
[b] Joint Biotechnology Laboratory, Department of Chemistry, Mathematics and Natural Sciences Faculty, University of Turku, Turku, Finland
[c] California NanoSystems Institute at University of California, Los Angeles, CA 90095, USA

ARTICLE INFO

Keywords:
Human myeloma IgG4
Electron microscopy
Differential scanning calorimetry
3D structure
Single-particle 3D reconstruction

ABSTRACT

Human IgG4 (hIgG4) has weak pro-inflammatory activity. The structural basis for this is still unclear. Here a 3D model of myeloma hIgG4 was created at ~3 nm resolution using electron microscopy (EM) with negative staining and single-particle 3D reconstruction. The hIgG4 model reveals relatively rigid asymmetric Y-like structure. The model shows that one Fab subunit is closer to the upper portion of the Fc subunit (C_H2 domain) than the other Fab. This is in agreement with X-ray crystallography and X-ray/neutron scattering, recently published by others. The same hIgG4 sample was studied with differential scanning calorimetry (DSC) and fluorescence. The thermodynamics and fluorescence observations indicate that one C_H2 domain displays less conformational stability than the other. This finding is consistent with the flipping of one C_H2 domain, observed in pembrolizumab (recombinant hIgG4) by X-ray crystallography. The specific feature of hIgG4 C_H2 domains together with relatively rigid asymmetric Y-like structure, in which one Fab subunit is closer to the upper portion of the Fc subunit (C_H2 domain) than the other Fab, can explain the unique biological properties of hIgG4, such as its weak pro-inflammatory activity.

1. Introduction

The IgG molecule is composed of two identical light (L) and two identical heavy (H) chains connected by one disulfide bond between L and H chains and by different numbers of disulfide bonds between H chains depending on animal species and IgG subclasses (Burton and Woof, 1992; Edelman, 1970; Edelman et al., 1969; Fleischman et al., 1963; Gottlieb et al., 1970; Rutishauser et al., 1968). The N-terminal portions of H-chains and L-chains form two identical subunits with antigen-binding activity (Fab), while the C-terminal portions of H-chains compose Fc-subunit responsible for effector functions (Burton and Woof, 1992; Edelman, 1970; Edelman et al., 1969; Gottlieb et al., 1970; Rutishauser et al., 1968). Fab subunits are linked to the Fc subunit via a flexible hinge region (Nezlin, 1990). Human IgGs (hIgGs) are divided into four subclasses/isotypes: hIgG1, hIgG2, hIgG3 and hIgG4. Their relative concentrations in normal serum account for ~ 60, 25, 10 and 5%, respectively (Burton, 1985; Burton and Woof, 1992). The subclasses IgG1–IgG4 differ primarily at their hinges. Whereas human IgG2 and IgG4 both have hinges of 12 amino acid residues in length, human IgG1 and IgG3 have hinges of length 15 and 62 residues, respectively. The hinge is composed of upper, middle, and lower sections. The upper hinge connects the Fab region to the cysteine-rich middle hinge, while the lower hinge connects the Fc region to the middle hinge. The genetic hinge encodes to the upper and middle hinges, while the lower hinge is encoded by the C_H2 exon (Dangl et al., 1988; Kabat et al., 1987). The subclasses relate to different activities: IgG4 is unable to perform many effector functions such as antibody-dependent cellular phagocytosis, antibody-dependent cellular cytotoxicity, and complement dependent cytotoxicity; IgG2 has low activity, whereas IgG1 and IgG3 are very active (Burton, 1985; Burton and Woof, 1992). The ability of hIgG subclasses to activate effector functions can be mediated by the properties of the hinge region, and/or by the structure of the active sites located in C_H2 domains. In particular, the length and structure of the hinge region may have an effect on reciprocal

Abbreviations: C1q, first factor of complement; C_H1, heavy chain constant domain 1; C_H2, heavy-chain constant domain 2; C_L, light-chain constant domain; DSC, differential scanning calorimetry; EM, electron microscopy; EMAN, electron micrograph analysis software; Fab, antigen-binding subunit; FAE, Fab-arm exchange; Fc, crystallized subunit; Fcγ, Fc receptor gamma; FITC, fluoresceinyl 5-isothiocyanate; FSC, Fourier shell correlation; ΔH_{cal}, calorimetric enthalpy; ΔH_{eff}, effective enthalpy; hIgG, human immunoglobulin G; IgG, immunoglobulin G

* Corresponding author.
E-mail addresses: sryazant@ucla.edu, vlazav@utu.fi (S.N. Ryazantsev).
[1] Deceased December 2016.

http://dx.doi.org/10.1016/j.molimm.2017.10.014
Received 19 August 2017; Received in revised form 10 October 2017; Accepted 17 October 2017

orientation of subunits within the IgG molecule and/or flexibility of the molecule which may favor or suppress a particular function due to sterical constraints (Burton, 1985; Burton and Woof, 1992).

Recently we published the first complete 3D model of the myeloma hIgG2 produced by single-particle 3D reconstruction (Ryazantsev et al., 2013). The model reveals that the studied hIgG2 was asymmetric; this observation was supported by thermodynamics and fluorescence analysis (Ryazantsev et al., 2013). In addition, the long axis of Fc was tilted nearly 90° in relation to Fab plane. Thus, Fab subunits are attached to Fc at an angle, creating a tripod-like shape.

The data on the 3D structure of hIgG4 have been contradictory for many years. Some investigators believed that the profound lack of effector functions must be reflected in a profoundly different shape/structure of the hIgG4 molecule, such as T-shape (Gregory et al., 1987). Rayner et al., (2014) studied the wild-type recombinant human-mouse IgG4 (Ser^{222}) chimera with constrained X-ray and neutron scattering modeling and revealed asymmetric solution structures, in which the Fab subunits were located close enough to the Fc subunit to prevent C1q binding. Tian et al., (2015) used the in-depth analysis of small-angle X-ray scattering in solution and found that recombinant humanized IgG4 adapts to a preferred Y/T-shape, which was observed in ~53% of all cases under the experimental conditions used; of those, the Y-shape was observed in 36% and the T-shape was observed in 9%. Scapin et al., (2015) reported the structure of the full-length recombinant human IgG4 antibody pembrolizumab, solved at 2.3-Å resolution; they found that the hIgG4 molecule had an asymmetric Y-like shape. Comparison of the pembrolizumab Fc (Scapin et al., 2015) with the high resolution cryogenic structure of an isolated hIgG4-Fc (Davies et al., 2014a) revealed that the conformation of one C_H2 domain was different: in the full-length antibody, this C_H2 domain showed a rigid-body rotation of ~120° relative to the position observed in the isolated Fc. On the other hand, Davies et al., (2014a) found that in the cryogenic structure of isolated hIgG4-Fc the FG loop in C_H2 domain has a different conformation from the one observed in the hIgG1-Fc (Deisenhofer, 1981). However, in the pembrolizumab structure the FG loop in both C_H2 domains shows the same conformation observed in the IgG1-Fc, thus ruling out the conclusion of Davies et al. (2014a) that this difference in conformation of the FG loop is the main cause for the reduced Fc-receptor affinity. Recently Davies et al. (2017) solved the structure of recombinant hIgG4-Fc at room temperature (RT) at 2.7 Å. The RT hIgG4-Fc structure shows conformational diversity in the C_H2 FG loop. In contrast to the cryogenic structure, the FG loop adopts the conserved conformation found in IgG1-Fc in one C_H2 domain; however, this conformation participates in extensive crystal packing interactions. On the other hand, at RT, and free from any crystal packing interactions, the FG loop in the other C_H2 domain adopts the conformation previously observed in the cryogenic hIgG4-Fc structure (Davies et al., 2014a). Thus, despite advances in the study of hIgG4, a structural basis for understanding hIgG4 specific functions is still unclear; additional research by independent methods is needed.

Therefore, we performed a structural analysis of myeloma hIgG4 in a similar fashion as for the myeloma hIgG2 (Ryazantsev et al., 2013), combining electron microscopy (EM), differential scanning calorimetry (DSC) and fluorescence. A 3D model of myeloma hIgG4 was created at ~3 nm resolution. From the EM data, the hIgG4 model reveals a relatively rigid asymmetric Y-like structure. The model shows that one Fab subunit is closer to the upper portion of the Fc subunit (C_H2 domain) than the other Fab. This is in good agreement with recently published X-ray crystallography for the recombinant hIgG4 antibody pembrolizumab (Scapin et al., 2015) and with X-ray/neutron scattering in solution for recombinant humanized IgG4 (Tian et al., 2015) and recombinant wild-type human-mouse IgG4 (Ser^{222}) chimera (Rayner et al., 2014). The thermodynamics and fluorescence observations indicate that one C_H2 domain displays less conformational stability than the other. This finding is consistent with the flipping of one C_H2 domain, observed in pembrolizumab (recombinant hIgG4) by X-ray crystallography (Scapin et al., 2015). The specific feature of hIgG4 C_H2 domains together with relatively rigid asymmetric Y-like structure, in which one Fab subunit is closer to the upper portion of the Fc subunit (C_H2 domain) than the other Fab, can explain the unique biological properties of hIgG4, such as its weak pro-inflammatory activity.

2. Material and methods

2.1. Preparation of purified myeloma hIgG4

Myeloma hIgG4 SAV was provided by the Institute of Hematology and Intensive Care, Moscow, Russia in accordance with the Ministry of Public Health instruction for handling human samples. The authors of this paper did not directly participate in sample isolation from a human donor. The hIgG4 was purified to 95% homogeneity by precipitation with $(NH_4)_2SO_4$ and ion-exchange chromatography on DEAE-Sephadex, followed by gel filtration on Ultragel AcA-34 (Denesyuk et al., 1983; Tischenko and Zav'yalov, 2003).

2.2. Preparation of purified Fab and Fc fragments

The Fab and Fc fragments were prepared by papain digestion of hIgG4 SAV according to Sanderson and Lanning (1970). Digestion was allowed to proceed for 18 h. Incubation was followed by dialysis against 10 mM sodium phosphate, pH 7.6 at 4 °C for 6 h. The Fab and Fc fragments were separated on DEAE-cellulose (Frangione et al., 1966). Then Fab and Fc fragments were separated from the uncleaved material by sequential chromatography on Superdex and on Mono-Q columns (Tischenko, 2015).

2.3. Preparation of purified C_H2 domains

The C_H2 domains were obtained from hIgG4 SAV Fc fragments by trypsin hydrolysis, as described previously (Ellerson et al., 1972), with some modifications as described below. First, the incubation before proteolysis was carried out at pH 2.8 instead of pH 2.5. Second, to stabilize the labile structure of C_H2 domains, hydrolysis was carried out in the presence of specific anti-C_H2 Fab derived from rabbit antibodies against C_H2. After completion of the reaction, the C_H2–Fab complex was dissociated in 10 mM glycine buffer, pH 4.0, and the two proteins were separated by gel filtration under the above-mentioned conditions.

2.4. Testing of purified myeloma hIgG4 and its fragments for homogeneity

The purified myeloma hIgG4 and its fragments were tested for homogeneity by several methods such as SDS and native (pH 7.3) gel-electrophoresis, immunoprecipitation, and two-dimensional immune electrophoresis using commercial mono- and polyspecific antisera by the Laurell method (Laurell, 1965), and N-terminal amino-acid analysis. The purified myeloma hIgG4 and its fragments were 95% homogenous by these criteria.

2.5. Fluorescence labeling

For fluorescence studies the C_H2 domains of hIgG4 SAV were labeled with fluoresceinyl 5-isothiocyanate (FITC). The procedure of labeling is described in detail in Tischenko et al. (1998). Briefly, the whole hIgG4 was treated with FITC in borate-buffered saline (200 mM $Na_3BO_3/NaBO_2$, 150 mM NaCl, pH 8.9 or 9.1) for 12 h at 4 °C; the FITC-protein ratio was 1:2-1:3. As a result of the reaction, 40–50% of hIgG4 was labeled. The label was in the Fc fragment, i.e., Fc fragments were preferentially labeled over Fab fragments. Moreover, within the Fc fragment the C_H2 domains (60–70%) were preferentially labeled. The labeled C_H2 domains were isolated from the whole hIgG4 SAV using the same procedure as unlabeled C_H2 domains (see above). The degree of labeling with FITC was determined from the A_{495}/A_{470} ratio as