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Global structure of HIV-1 neutralizing antibody IgG1 b12 is asymmetric

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ABSTRACT

Human antibody IgG1 b12 is one of the four antibodies known to neutralize a broad range of human immunodeficiency virus-1. The crystal structure of this antibody displayed an asymmetric disposition of the Fab arms relative to its Fc portion. Comparison of structures solved for other IgG1 antibodies led to a notion that crystal packing forces entrapped a "snap-shot" of different conformations accessible to this antibody. To elucidate global structure of this unique antibody, we acquired small-angle X-ray scattering data from its dilute solution. Data analysis indicated that b12 adopts a bilobal globular structure in solution with a radius of gyration and a maximum linear dimension of ~54 and ~180 Å, respectively. Extreme similarity between its solution and crystal structure concludes that non-flexible, asymmetric shape is an inherent property of this rare antibody.

Introduction

Human antibody IgG1 b12 belongs to a unique class of antibodies which can neutralize a diverse array of primary HIV-1 isolates and protect against pathogenic SHIV [1–3]. Last few decades of research has identified only four monoclonal antibodies (mAbs): b12, 2F5, 2G12 and 4E10 which can effectively neutralize HIV-1 both *in vivo* as well as *in vitro* [2,4]. The framework regions of these antibodies bind different key elements of the HIV-1 entry machinery, thereby effectively blocking the infusion of viral replication material inside host T-cells. While 2F5 and 4E10 react with the transmembrane protein gp41 [4,5], 2G12 recognizes hybrid/mannose moieties in the glycosylated portion of gp120 [6], and b12 binds to an epitope which overlaps with the region recognized by the CD4 receptor [1,7]. Crystal structures of the Fab domains of these four antibodies bound to molecules representing their ligands do provide some clues about key residues which stabilize the antigen–antibody interaction. However, to understand the structural features of a complete antibody capable of neutralizing HIV-1, only one crystal structure is available and that is of IgG1 b12 (PDB ID 1HZH) [2].

Besides being the only full-length structure of an HIV-1 neutralizing mAb, the crystal structure of the unliganded IgG1 b12 is distinctive in one particular aspect: extreme asymmetry in the positioning of the two Fab arms relative to the central Fc stem [2,8,9]. As resolved, one Fab arm in the structure extended significantly away in space, while the other remained nestled close to the central Fc region. A comparison of crystal structures solved for five other IgGs: Mcg, a human IgG1 with a deletion in the hinge region; Kol, a human IgG1 with disordered structure for Fc portion; mAb 231, an intact murine IgG2a and mAb 61.1.3, an intact murine IgG1, implied that both Fab arms of IgG molecules can occupy a wide range of symmetric and asymmetric dispositions by virtue of the flexibility encoded in their hinge regions [9]. The differences in these structures gave basis to the notion that the observed asymmetry in the crystal structure of full-length b12 was resultant of crystal packing forces and the structure is merely a "snap-shot" of the variety of conformations accessible to this antibody [2,8,9]. Considering the rare ability of this antibody to neutralize a broad spectrum of HIV-1 strains, we carried out this work to answer a specific question: Does the characteristic asymmetric shape dominate the solution structure of IgG1 b12? Small-angle X-ray scattering (SAXS) data analysis and *ab initio* structure reconstruction of unliganded full-length IgG1 b12 confirmed that the above described asymmetry is retained in the predominant solution conformation of this antibody.

Materials and methods

Source of protein samples for experiments. Purified protein samples of monoclonal IgG1 b12 at a concentration of ~1 mg/ml and >95% purity was obtained from the National Institutes of Health

Abbreviations: HIV-1, human immunodeficiency virus-1; SAXS, small-angle X-ray scattering; R_G, radius of gyration; R_C, radius of cross-section; D_{max}, maximum linear dimension

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AIDS Research and Reference Reagent Program [10,11]. To estimate the beam intensity at zero angles, hen egg white lysozyme purchased from Acros Organics (Morris Plains, NJ) was dissolved, dialyzed and purified by gel filtration in 40 mM sodium acetate buffer pH 3.8 containing 150 mM NaCl.

Synchrotron SAXS data acquisition and processing. The SAXS data were collected at beam line X21 at the National Synchrotron Light Source (Brookhaven National Laboratory). The wavelength of the beam was 1.17 Å, and the ratio of the sample to detector distance to the diameter of charge-coupled detector was 9.38. b12 sample (15 μl) and its matched buffer were exposed for 30 s in quartz flow cell at 10 °C with a flow rate of 27 μl/min. SAXS on a lysozyme concentration series was also collected under identical conditions/setup. The images recorded on a charge-coupled detector (CCD) from protein solutions were circularly averaged, buffer subtracted, and scaled to obtain relative scattering intensity (I) as a function of momentum transfer vector, Q ($Q = [4\pi \sin\theta]/\lambda$), where λ is the beam wavelength and θ is the scattering angle. All the SAXS experiments were carried out in duplicate. No protein appeared to have suffered degradation during exposure to X-rays as characterized by the migration pattern in SDS–PAGE.

SAXS data analysis. Globular nature of the b12 molecules was confirmed by interpreting the profile Kratky plot ($I(Q)Q^2$ vs. Q) of the SAXS data [12]. For a monodisperse sample of globular protein or its complex, a plot of $\ln(I(Q))$ vs. Q^2, where $Q \cdot R_G \leqslant 1.3$, should be linear and fits into the following Eq. (1):

$$\ln[I(Q)] = \ln[I_0] - \left(R_G^2/3\right)Q^2 \quad (1)$$

Such approximation, known as Guinier analysis, for globular proteins was used to obtain an estimation of the scattering particle radius of gyration (R_G) and forward or zero-angle scatter (I_0) [12]. R_G is defined as the root-mean-square of all elemental volumes from the center-of-mass of the particle, weighted by their scattering densities and is characteristic of the overall shape of the molecule. I_0 is directly proportional to the molar particle concentration multiplied by the square of the scattering particle molecular weight for particles with comparable mean scattering density. In this work, Guinier analysis was performed using the Primus software package [13]. Indirect Fourier transformation of the scattering data over the measured Q-range gave a pair-wise distribution function of interatomic vectors, $P(r)$ (Eq. (2)). The inverse Fourier transformation of $I(Q)$ yields $P(r)$, which is the frequency of vector lengths connecting small volume elements within the entire volume of the scattering particle.

$$P(r) = (1/2\pi)\int I(Q)Q \cdot r \sin(Q \cdot r)dQ \quad (2)$$

$P(r)$ was considered to be zero at $r = 0$ Å and approaches zero at the maximum linear dimension of the particle, D_{max}. Along with Guinier approximation, the radius of gyration, R_G, and forward scatter, I_0, were estimated from the second moment and first moment of the $P(r)$, respectively. The GNOM45 software package was used for all $P(r)$ analyses [14].

Ab initio structure restoration. Employing dummy residues and constraints provided from the SAXS profile, the three-dimensional shape of the unliganded antibody was restored using the DAMMINIQ program [15]. Ten models were generated without any predefined shape or symmetry bias. An average model which best-represented all the individual solutions was generated using the DAMAVER suite of programs [16]. SUPCOMB software was used to superimpose the inertial axes of our SAXS-data based model and the crystal structure of b12 antibody in an automated manner [17].

Graphical analysis and representations. Open-Source PyMOL 0.99rc6 was used for graphical analysis and figure generation.

Results

SAXS data analysis

The SAXS data from the sample of IgG1 b12 and Kratky plot are presented in Fig. 1A and B. The peak profile of the Kratky plot confirmed that b12 molecules adopt a globular monodisperse nature

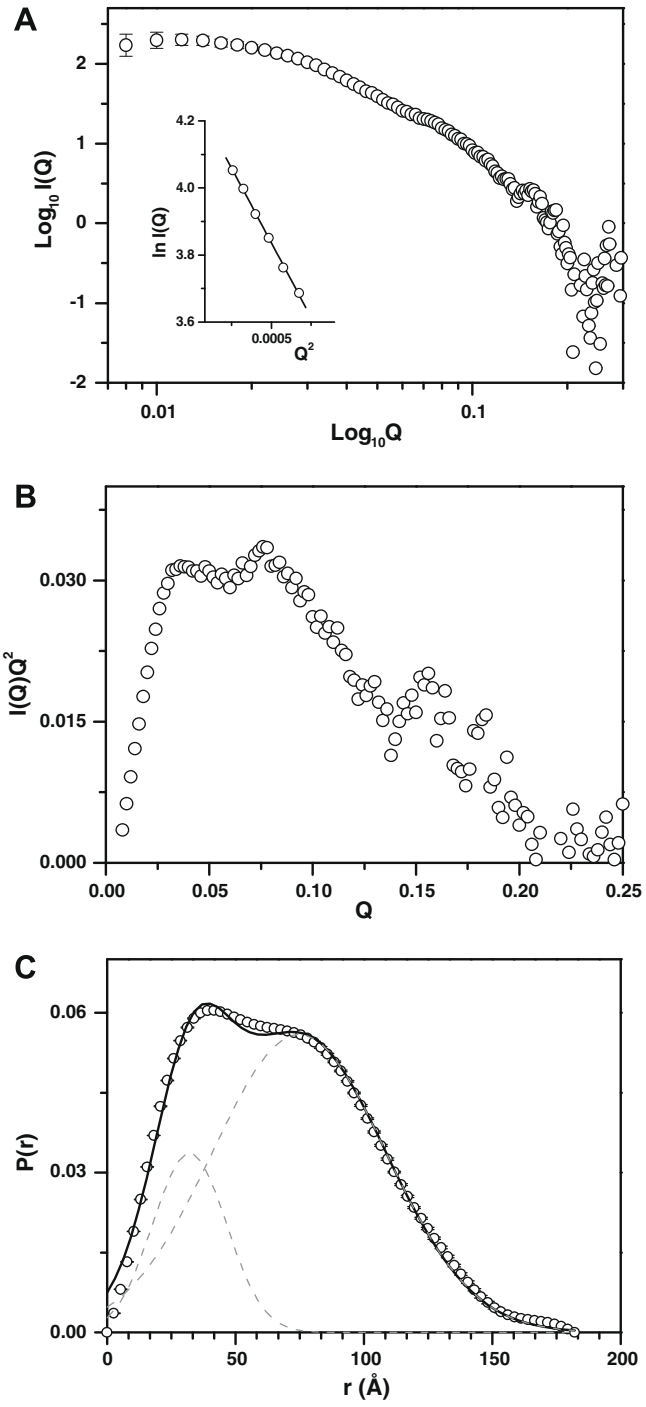

Fig. 1. Small-angle scattering data and $P(r)$ analysis. (A) The SAXS intensity data acquired from solutions containing unliganded IgG1 b12 is shown as function of Q. Guinier analysis of the low Q data is shown as inset. (B) Kratky plot (○) of IgG1 b12 calculated from its SAXS data is presented. (C) The calculated $P(r)$ curve solved for the unliganded IgG1 b12 (○) is presented as a function of r. Deconvulation of the curve highlights the two peaks with maxima at 32 and 75 Å (gray) and their summation (black line) is compared with the original $P(r)$ profile.

Table 1
Structural parameters deduced for the IgG1 b12 molecules from Guinier and $P(r)$ analysis are tabulated below.

Sample	Guinier analysis		$P(r)$ analysis				
	R_G (Å)	I_0	D_{max} (Å)	R_G (Å)	I_0	Mass (KDa)	Actual Conc. (mg/ml)
Standard (lysozyme)	14.3 ± 0.1	7.3[a]	44 ± 1	14.4 ± 0.1	7.3[a]	14.2	1[a]
IgG1 b12	52.5 ± 0.3	73.1 ± 0.3	182 ± 10	53.8 ± 0.1	73.6 ± 0.2	150	0.9

[a] The values for lysozyme were estimated for a 1 mg/ml by plotting an I_0 vs. c plot from SAXS data of samples ranging from 6.5 to 0.5 mg/ml.

in solution. Applying Guinier approximation for globular particles, the slope of the linear fit to the data over the low Q-range (0.01–0.024 Å^{-1}) provided an R_G of 52.5 ± 0.3 Å (Table 1). Besides analyzing low q data, $P(r)$ analysis for monodisperse globular particles was performed using a wider Q-range (0.01–0.25 Å) to obtain a more complete estimation of the structural parameters specific to the predominant shape of this antibody in solution (Fig. 1C). The calculated $P(r)$ for b12 molecules displayed a two-peak profile of the distribution curve of possible interatomic vectors and suggested a bilobal shape of the b12 molecules characterized by a D_{max} of 182 Å and an R_G of 53.8 ± 0.1 Å (Table 1).

Structure reconstruction

To visualize the three-dimensional structure of the b12 molecule in solution, ten independent dummy atom models were generated using DAMMIN software [15]. For individual runs, the software fixed about 1350 dummy residues for modeling. The final χ of their calculated intensity against raw data for the ten models ranged from 0.90 to 0.92. Comparison of the calculated $I(Q)$ profiles of the models with the measured SAXS data is presented in Fig. 2A. To distill out the common shape computed in the ten individual structures, DAMAVER suite of programs [16] were used to superimpose and average the individual solutions (Fig. 2B). Using CRYSOL program [18], the calculated SAXS profile from the crystal structure of b12 molecule and our measured SAXS data were compared and it resulted in a χ^2 value of only 1.5 over a Q-range of 0.01–0.25 Å^{-1}. This suggested that our SAXS-data based model and crystal structure can be reliably superimposed by aligning their inertial axes. Finally, SUPCOMB software [17] was employed to superimpose the inertial axes of our SAXS-based model and the known crystal structure of the b12 antibody (Fig. 2C and D).

Discussion

Global structure of IgG1 b12 from SAXS data analysis

For compact, globular particles, $I(Q)$ decreases as a function of Q^{-4}, so the Kratky plot ($I(Q)Q^2$ vs. Q) is bell-shaped. However, in the case of disordered particles, coherently scattering entities are single chains, whose scattering intensity decreases as a function of Q^{-1} [12]. In such cases, the Kratky plot can be like a hyperbola to straight line. Such analyses have been used in the past to confirm/distinguish globular compact shapes preferred by proteins from their disordered or denatured forms [19–21]. Bell-shaped curved calculated for the b12 molecules confirmed their globular nature in solution (Fig. 1B). Considering their globular nature, both Guinier approximation and calculated $P(r)$ curve suggested an R_G close to 53 Å and the D_{max} was estimated to be around 182 Å for b12 molecules (Table 1). Stephen J. Perkins and coworkers have previously studied solution structural properties of IgA and IgG antibodies from SAXS and SANS (neutron scattering) experiments in conjunction with analytical ultracentrifugation and constrained molecular dynamics simulations. They studied different IgG and IgA antibodies, and their mutants in concentration range of ~0.4 to ~3 mg/ml by both X-ray and neutrons, and no significant change in the structural parameters was observed for any antibody as a function of its concentration [22–24]. Importantly, no report could be found which suggests any inter-particulate or aggregation effect in antibodies at lower concentrations. Thus, we did not have any reason to suspect such effect in case of IgG1 b12 and without further diluting the stock sample provided to us by NIH AIDS reagent program, we carried out SAXS experiments only at one concentration (~1 mg/ml). In fact, comparison of the I_0 values from b12 with those extrapolated for 1 mg/ml sample of lysozyme indicated that the actual concentration of our sample was 0.9 mg/ml (Table 1). The R_G and D_{max} values computed for human IgG1 b12 molecule were comparable to the radius of gyration and cross-section estimated from SAXS and SANS experiments by earlier groups for IgG antibodies [22–24]. Particularly, Mayans et al. reported R_G values from 5.3 to 5.7 nm for Bovine IgG1/2, Porcine IgG and Rabbit IgG [22]. It must be mentioned here that the Boehm et al. found the R_G and D_{max} values to be significantly higher for the IgA1 than IgG antibodies, and they concluded that Fab arms are more stretched out in space in IgA1 antibodies [23].

The $P(r)$ curve computed for b12 showed a two-peak profile which implied that this antibody adopts a bilobal shape in solution with some void space between the two domains. Deconvulation of the $P(r)$ curve resulted in two peaks with maxima at $r \sim 32$ and 75 Å representing the frequently occurring interatomic distances within the predominant structure adopted by b12 molecules. In earlier reports on IgG and IgA antibodies, the peak at ~40 Å has been interpreted as the representative of most commonly occurring distances within a single Fab or Fc portion as these are ~80 Å in their maximum length [23,24]. The second peak at ~75 Å represents interatomic distances across Fab and Fc portions. Interestingly, $P(r)$ curves published earlier from SAXS/SANS data of bovine IgG1 and IgG2 antibody showed only one broad peak at 54–57 Å while IgA1 antibodies displayed two distinct peaks in their $P(r)$ curves [23]. The presence of only one peak in the former was interpreted as a sign of high level of flexibility across the hinges which allowed the Fab arms of the bovine IgG to adopt a diverse range of structures relative to central Fc. On the other hand, presence of two peaks in the $P(r)$ curve of human IgA1 correlated well with known reduced flexibility across their hinges due to high-incidence of O-glycosylation and Pro residues, which rigidly positions the Fab arms distally from the Fc [23]. The extended structures of IgA molecules are also reflected in their R_G (~6.2 nm) and D_{max} (~20 nm) values which are significantly higher than IgG antibodies. In light of previous reports, presence of two distinct peaks in the $P(r)$ calculated for the b12 molecules concluded that one or both Fab arms remain extended in this IgG1 antibody similar to IgA and/or due to some unusual conformational rigidity a diverse range of structures are not adaptable by this HIV-1 neutralizing antibody.

The dummy atom model of the solution structure of full-length b12 molecules agreed with the results from the $P(r)$ analysis and showed a bilobal shape (Fig. 2B). Superimposition of the inertial axes of our SAXS-data based model and crystal structure showed remarkable similarity (Fig. 2C and D). This was somewhat expected from the low χ^2 value calculated using the CRYSOL program. Different orientations presented in Fig. 2C and D show that apart for

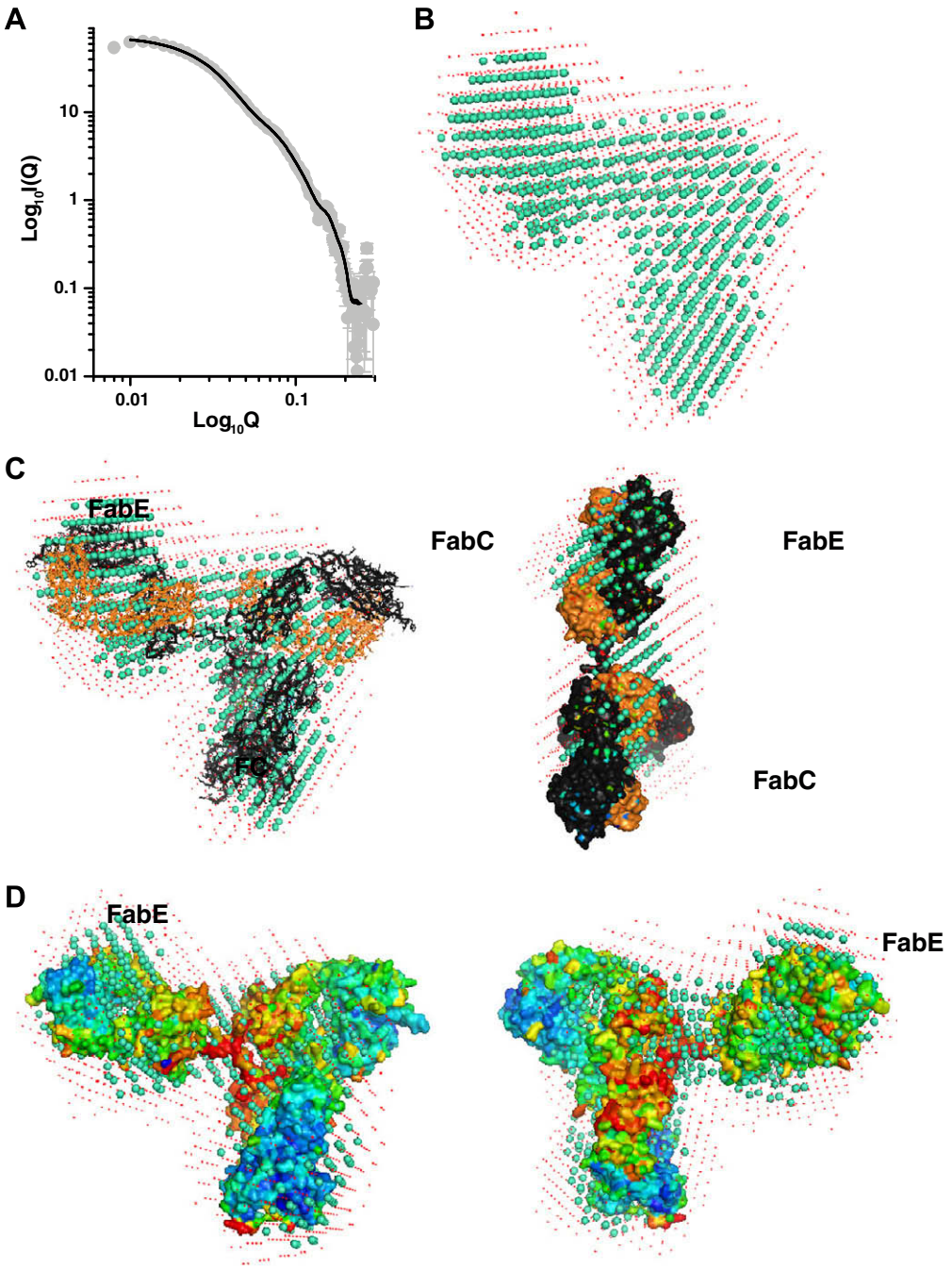

Fig. 2. Model of unliganded full-length IgG1 b12 antibody from SAXS data. (A) Calculated SAXS intensity profile (–) of ten DAMMIN models of unliganded IgG1 b12 onto the measured experimental data (∘). (B) Dummy atom model of the unliganded IgG1 b12 antibody in solution. (C) Different views of the automated superimposition of the inertial axes of the SAXS-based model (cyan; space-fill mode) and the crystal structure of IgG1 b12 (heavy chains black and light chains orange ribbon mode; PDB ID 1HZH). The extended Fab arm, the closed Fab arm and the Fc portion as seen in the crystal structure are labeled as FabE, FabC and Fc, respectively. (D) Two orthogonal views of the superimposition of the SAXS-based model and crystal structure, where the crystal structure is colored as per experimentally determined B-factors. The red regions indicate high region of motion in the structure relative to other parts. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

the apex portion of the closed Fab arm, the extended Fab arm and the Fc of the crystal structure can fit within the volume of the SAXS-data based model. We also observed that our model has extra or unoccupied volume near the hinge region of the Fab arm, a region which got resolved with relatively higher B-factor during crystal structure refinement (Fig. 2D). Overall, results discussed in this communication conclude that (1) the unliganded b12 molecules do not adopt a wide range of conformations, even in dilute solution; (2) their predominant structure involves an asymmetric disposition of the two Fab arms relative to central Fc; and (3) since crystal and solution structure are very similar, the global structure of this HIV-1 neutralizing mAb is asymmetric. While our communication concludes that b12 antibody adopts a non-flexible asymmetric structure, a very important question still remains to be answered: since majority of the human antibodies elicited in HIV-1 infection are IgG1 and the isotype of the b12 is not unique, then whether b12 is uniquely effective because its global structure is inherently rigid and possess less entropy. Currently, experiments are underway in our lab to correlate the neutralizing potency of IgG1 mAbs with engineered rigidity in their hinge region.

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