SUPPLEMENTARY ONLINE DATA

The solution structures of native and patient monomeric human IgA1 reveal asymmetric extended structures: implications for function and IgAN disease

RESULTS

This Supplement reports the generation of conformational libraries of two types of trial IgA1 model structures for the X-ray scattering fits. These addressed the modelling of (i) the two N-glycans at Asn263, (ii) the two hinges between the Fab and Fc regions, and (iii) the two C-terminal tailpieces.

(i) In order to construct the two “initial” T-shaped and Y-shaped PTerm455 structures, N-glycan conformations at Asn263 were positioned against the lower surface of the Fc region and in hydrogen-bond contact with this surface at residues that include Arg382 and Arg392. This conformation was seen in the crystal structure (PDB 1OW0) and denoted as NG0 (Figure S1A). Molecular dynamics were used to explore alternate conformations available to these N-glycans (Experimental). During the equilibration phase of the simulations, both N-glycans moved into the solvent and revealed two more distinct conformations (Figure S1C,D). The two clusters were identified from the distribution into two groups of the distances between the tips of the N-glycans and Pro293/Glu393 (Distance 1 and Distance 2 in Figure S1B). The two structures closest to the mean values of Distance 1 and Distance 2 for each conformation were selected to represent these, and termed NG1 and NG2. NG1 is perpendicular to and NG2 is parallel to the central axis of the Fc region (Figure S1C,D).

(ii) Secondly, the 20 backbone φ and ψ angles in the 21-residue hinge of PTerm455 were varied in randomised steps to produce 57,611 non-overlapping PTerm455 structures, starting from the two “initial” models (Experimental). After adding the NG0, NG1 and NG2 glycan conformers, the resulting 172,833 PTerm455 models were assessed in R factor vs. Rg graphs. Three distinct minima were seen (Figure S2). The lowest R factors corresponded to modelled Rg values close to the experimental neutron Rg value of 5.84 ± 0.18 nm as desired [24]. These corresponded to PTerm455 structures with the NG0 conformer (blue in Figure S2), suggesting that the NG0 conformer best represented the solution structure of PTerm455. The other two minima with higher R factors (magenta in Figure S2) correspond to the NG1 and NG2 glycan conformers, one at an Rg value of 5.8 nm close to experiment, and the other at an Rg value of 6.4 nm which is too large. The curve fits did not favour the existence of extended N-glycan conformers in PTerm455.

(iii) Trial full-length IgA1 structures were constructed from the 36,621 PTerm455 models with R factors below 7% with NG0, NG1 and NG2 glycan conformations in both T-shaped and Y-shaped structures (inset, Figure S2). This construction required the two C-terminal tailpieces with their N-glycans at Asn459. Following a molecular dynamics simulation (Experimental), principal component analysis showed that over 77% of the variability in the tailpiece structures was represented by the first two principal components PC1 and PC2 (Figure S3). Clustering of the PC1 and PC2 structures suggested four conformationally-distinct tailpieces termed TP1-TP4 (Figure S3B). The TP1-TP4 structures were extended from the Fc region (Figure S3C). Attaching the centroid structure of TP1-TP4 to each of the 36,621 PTerm455 models by superimposition at Arg450 gave 146,484 candidate IgA1 models.
FIGURE LEGENDS

Figure S1. Simulation of possible N-linked glycan conformations in the Fc region of the tailpiece-free IgA1 structure.

(A) Top-down and face-on views of the Fc region with the N-glycan conformation at Asn263 modelled directly from the Fc crystal structure (NG0). This structure was used to initialize the molecular dynamics simulations of the tailpiece-free IgA1 (PTerm455). The green and pink spheres indicate Pro293 and Glu393 (Figure 1C) that were used to monitor the glycan conformation (orange spheres at the two terminal sialic acid residues) during the simulations.

(B) Survey of Distance 1 (sialic acids-Pro293) vs. Distance 2 (sialic acids-Glu393) for all the glycan conformations observed in the simulations. The greater of the two distances for each glycan is plotted. The resulting two distinct conformations are denoted NG1 and NG2. Note the two axes are drawn to different scales for reason of clarity.

(C,D) Top view and face-on views of the Fc region to show representative structures of the two horizontal (C, NG1) and vertical (D, NG2) extended glycan conformations at Asn263 determined by the simulations. These were used in the subsequent modelling. Note the assumption of symmetry in the glycan conformations.

Figure S2. Constrained scattering modelling of the PTerm455 structure. PTerm455 is the tailpiece-free IgA1 molecule, and was modelled with both hinges present, no O-glycans present, and the NG0, NG1 or NG2 glycan conformations, denoted in blue, red and purple respectively. The resulting 172,833 goodness-of-fit R factors were compared with the corresponding calculated neutron Rg values for the conformationally-varied unhydrated structures. The experimental Rg value is shown by the vertical solid line with error ranges of ±10% shown by dashed lines. The inset shows an expanded view of the 36,621 best-fit models with R factors below 7%.

Figure S3. Principal component analysis of the IgA1 tailpiece conformations explored in molecular dynamics simulation.

(A) Percentage of the conformational variance observed over the simulation described by each principal component (PC). The first two components (PC1 and PC2) account for 77.7% of the cumulative variation following summation.

(B) Projections for each snapshot of the simulation trajectory along PC1 and PC2. Hierarchical clustering was applied to identify four clusters of conformations denoted as TP1 (black), TP2 (red), TP3 (blue) and TP4 (green).

(C) The centroid structure of each of the four clusters TP1-TP4 used to complete the full IgA1 models. Only the Fc region is shown face-on in a ribbon view, together with two N-glycosylated tailpieces. The two heavy chains are shown in grey and light blue.
FIGURE S1
FIGURE S2
FIGURE S3