L-FUCOSE IN CRYSTAL STRUCTURES OF IgG-Fc: REINTERPRETATION OF EXPERIMENTAL DATA

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Glycosylation of IgG-Fc plays an important role in the activation of the immune system response. Effector functions are modulated by different degrees of deglycosylation of IgG-Fc. However, the geometry of oligosaccharides covalently bound to IgG-Fc does not seem to be in good agreement with electron density in most of the structures deposited in the Protein Data Bank. Our study of correlation between the oligosaccharide geometry, connectivity, and electron density shows several discrepancies, mainly for L-fucose. Revision of refinement of two structures containing the Fc-fragment solved at the highest resolution brings clear evidence for α-L-fucosylation instead of β-L-fucosylation as it was claimed in most of the deposited structures in the Protein Data Bank containing the Fc-fragment, and also in the original structures selected for re-refinement. Our revision refinement results in a decrease in *R* factors, better agreement with electron density, meaningful contacts, and acceptable geometry of L-fucose.

Keywords: Immunoglobulin; Fc-fragment; Fucose; Geometry.

Antibodies (Ig), adaptive molecules recognizing pathogens or antigens by specific bonds in the hypervariable region, are serum glycoproteins. The most plentiful antibody isotypes circulating in bloodstream are immunoglobulins G (IgG). They are composed of three independent globular domains with specific functions. Two of the fragments contain hypervariable regions; they are responsible for antigen binding (Fab). The third fragment, fragment crystallizable (Fc), has a highly constant structure even for antibodies recognizing different antigens. Formation of complexes of IgG-Fc with Fc-receptors (FcR) on surface of the cells of the immune system is one of the first steps leading to initiation of the immune response of the body[1].

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In the native state, antibodies are a mixture of glycoproteins with different glycoforms[1–3]. The Protein Data Bank (PDB)[4] contains 23 structures comprising natively glycosylated IgG-Fc (including structures of the whole antibody and structures of Fc in complexes with FcR or other ligands), and six structures of partially deglycosylated IgG-Fc [1]. A glycoform-dependent conformational alteration of the C_H2 domains of IgG-Fc was reported based on structural comparison of X-ray structures of partially deglycosylated IgG-Fc and also on NMR spectroscopy[5].

Glycosylation is essential for binding of the Fc-receptor (FcR)[1,6]. A significant reduction of the ability to bind the C1q complement component or FcR has been observed for partially deglycosylated IgG-Fc [5]. On the other hand, fucose ablation leads to a significant increase in the affinity between Fc and FcR [7]. However, no direct interaction was found between L-fucose and FcR in the crystal structures of complexes of Fc with FcR [8,9].

Inspection of the structures containing IgG-Fc deposited in the PDB with structure factors shows that fucose localization is not supported by electron density maps for most of the models in the PDB (structure distortion, meaningless *B* factors of hydrogen bonding partners – water oxygens, etc.) that were automatically downloaded from the Electron Density Server at the Uppsala University[10], even in the two structures of IgG-Fc with the highest resolution – 1L6X [11], and 2DTQ [12]. These were selected for further refinement and correction of L-fucose geometry.

EXPERIMENTAL

The coordinate files and structure factor files of the structures 1L6X and 2DTQ were downloaded from the PDB. The structure factor files were converted from mmCIF to MTZ file format with the program CIF2MTZ from the CCP4 program package[13]. The structure refinement was done with the program REFMAC5 [14]; manual corrections were performed using the programs COOT [15] and XFIT from the XtalView program package[16].

At first, several manual corrections were made to the protein structures and water molecules with meaningful contacts were added to the structure models based on observation in electron density. After standard refinement without fucose, α-L-fucose was clearly localized in the omit difference electron density.

The structure 2DTQ was re-refined with the TLS option (the TLS groups defined according to the original paper). A high positive maximum near His268(A) and His285(A) corresponding most probably to a metal ion (very likely Mn^{2+} according to the protocol in the original paper) remained unmodeled in agreement with the original structure.

The refinement with α-L-fucose resulted in a better agreement with electron density than in the original structures; the fucose moiety forms meaningful contacts to water molecules of solvent. The decrease in the *R* factors is also caused by manual corrections applied to side chains of the proteins, and by addition of water molecules to the structures. Therefore, not

the decrease in the *R* factor, but the correct geometry and agreement with electron density is the argument for the relevance of the presented results.

The final models were validated by PROCHECK [17], SFCHECK [18], and ADIT (http://deposit.rcsb.org/adit). The summary of the refinement statistics is given in Table I.

RESULTS AND DISCUSSION

Problems with refinement of saccharides in all protein structures deposited in the PDB containing saccharides have been already reported. About 30% of the saccharide-containing structures in the PDB comprise at least one error within the carbohydrate moieties[19]. Interpretation of oligosaccharides in maps of electron density is difficult for their heterogeneity (always present partial deglycosylation) and also for their lower conformational stability.

TABLE I
Refinement statistics of the re-refined structures 1L6X and 2DTQ

Parameter	1L6X	2DTQ
Resolution range, Å	20.0–1.65	50.0–2.00
Space group	$C222_1$	$P2_12_12_1$
No. of reflections used for refinement	35,573	38,712
Asymmetric unit content	monomer	dimer
R factor	0.202 (0.206)[a]	0.191 (0.194)[a]
R_{free}	0.247 (0.284)[a]	0.239 (0.239)[a]
No. of refined protein atoms	1969	3353
No. of refined saccharide atoms	121	198
No. of refined water molecules	424	471
Wilson *B* value, Å^2	18.3	32.9
Average *B* values, Å^2		
Protein	19.7	39.4
Saccharides	29.8	62.3
Water molecules	33.3	45.9
Ramachandran plot		
Most favored regions, %	99.6	93.4
Additional allowed regions, %	0.4	6.6
R.m.s. deviations from ideal		
Bond lengths, Å	0.011	0.011
Bond angles, °	1.384	1.473

[a] The values in parentheses are the *R* factors of the original structures deposited in the PDB.

Twenty nine structures containing IgG-Fc deposited in the PDB were analyzed with PDBCaRe [20], a web-based tool detecting discrepancies in connectivity and in nomenclature of saccharides (http://www.glycosciences.de/tools/pdbcare/). β-L-Fucose was modelled in seven structures with the highest resolution, and in nineteen structures in total (Table II). Uneasy to interpret electron density for structures refined at resolutions lower than 2.7 Å resulted in non-homogeneously "glycosylated" crystallographic models of two proteins and presence of 6-deoxy-α-L-altrose in the actual fucose position in the three oldest structures of IgG-Fc. The saccharides in two structures were not interpreted in difference electron density in spite of the presence of high positive maxima (PDB codes 1FCC and 1ADQ). Presence of fucose in the individual structures of IgG-Fc is summarized in Table II.

TABLE II
Review of monosaccharides modelled instead of α-L-fucose in IgG-Fc structures deposited in the PDB. Detected by program PDBCaRe[a]

Monosaccharide unit modelled instead of α-L-fucose in chain A, chain B	PDB code (resolution in Å)
α-L-Fucose, α-L-fucose	1H3W [1] (2.8)
α-L-Fucose, none	1HZH [21] (2.7)
α-L-Fucose, β-L-fucose	1E4K [22] (3.2)
β-L-Fucose, β-L-fucose	**1L6X** [11] (**1.65**)[b], **2DTQ** [12] (**2.0**)[b], 1OQO [23] (2.3), 1H3T [1] (2.4), 1H3U [1] (2.4), 1H3X [1] (2.5), 1OQX [23] (2.6), 1DN2 [24] (2.7), 1I1C [25] (2.7), 1I1A [25] (2.8), 1IGT [26] (2.8), 1T83 [27] (3.0), 1H3V [1] (3.1), 1IGY [28] (3.2), 1MCO [29] (3.2), 1T89 [27] (3.5), 1H3Y [1] (4.1), 2GJ7 [30] (5.0)
Incorrectly built L-fucose	2IWG [31] (2.4)
6-Deoxy-α-L-altrose, 6-deoxy-α-L-altrose	1FC2 [32] (2.8), 1FC1 [32] (2.9), 1FRT [33] (4.5)
None, none	2DTS [12] (2.2)[c], 1ADQ [34] (3.2), 1FCC [35] (3.2), 1F6A [36] (3.5)

[a] The chemical composition does not necessarily correspond to the reality. [b] These structures were selected for re-refinement because of resolution. [c] The structure with PDB code 2DTS is the structure of defucosylated IgG-Fc.

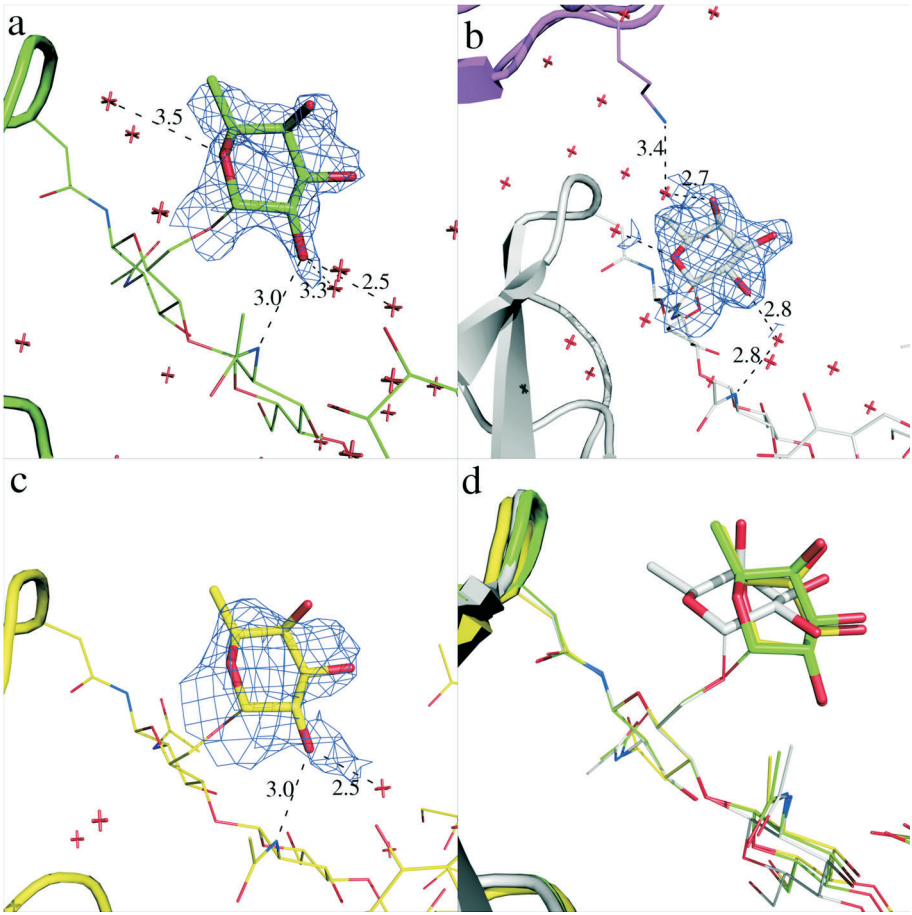

FIG. 1

α-L-Fucose in newly refined structures of human IgG1-Fc: a The newly refined structure with the original PDB code 1L6X – chain A. b The newly refined structure (originally 2DTQ) – chain A. c The newly refined structure 2DTQ – chain B. d A superposition of the first *N*-acetylglucosamines of the newly refined structures. Fucose is represented by sticks, ribbons represent the secondary structure of protein chains, side chains of Asn297, Lys326 (in b), and oligosaccharides are shown as lines. The $2|F_o| - |F_c|$ electron density (blue, contoured at 1 σ level in a and b, at 0.75 σ level in c) is displayed up to a distance of 3 Å from fucose. Contacts to hydrogen bonding partners are shown as black dashed lines with interatomic distances in Å. Figure 1d illustrates the high mobility of fucose depending on interactions with the solvent and other molecules. The figures were prepared with PYMOL [37]

Two structures with the highest resolution (1L6X and 2DTQ) were selected for further analysis, re-refinement, and re-evaluation of fucosylation of IgG-Fc. Significantly distorted geometry of β-L-fucose is observed in these original structures.

The oligosaccharides were newly modeled and refined. The major structural difference was in α-L-fucosylation, which is supported by better agreement with higher quality electron density.

A comparison of oligosaccharides shows high mobility of fucose. α-L-Fucose in the re-refined structure 1L6X is clearly localizable in electron density (Fig. 1a). Resolution 2 Å of the re-refined structure 2DTQ is sufficient for clear interpretation of fucose bound to chain A, where fucose interacts with Lys326(A) of the symmetry-related molecule in the crystal via a water molecule (Fig. 1b). Fucose bound to chain B is not stabilized by interaction with a symmetry-related molecule and, therefore, the map of electron density was hard to interpret (Fig. 1c). Conformations of α-L-fucose found in the re-refined structures are different. Superposition of the re-refined structures shows that the conformation of α-L-fucose in the re-refined structure 2DTQ bound to chain A is affected by its interaction with a symmetry-related molecule (Fig. 1d).

However, the original structural data of IgG-Fc available from the PDB often contain β-L-fucose instead of the correct α-L-fucose and twenty three structures containing IgG-Fc describe different fucose geometry to that of the presented re-refined structures. Also the original structures containing α-L-fucose exhibit significantly distorted geometry and high positive maxima of difference electron density near fucose or contain fucose only in one of the chains.

Extraction of information on oligosaccharides of IgG-Fc from structural data deposited in the PDB should be done carefully. The newly refined crystallographic models contain improved models of the oligosaccharides covalently bound to IgG-Fc.

The refined structures and supplementary data including the structure factors files, electron density maps, and reports from the validation tools are accessible at URL http://www.xray.cz/suppl/fuc.

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