The three-dimensional structure of the carbohydrate within the Fc fragment of immunoglobulin G

B. J. SUTTON and D. C. PHILLIPS
Laboratory of Molecular Biophysics, Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, U.K.

The IgG molecule, like all antibodies, is a glycoprotein, and with the discovery in 1959 that the rabbit IgG molecule could be split by the action of papain into two Fab (antigen-binding) and one Fc (crystallizable, complement- and cell-binding) fragment, the major glycosylation site was found to lie within Fc (Porter, 1959). (The Fc fragment consists of the C-terminal half of each of the two 'heavy' polypeptide chains of IgG.) Other minor sites have since been identified in both the Fab 'arms', and the 'hinge' which connects them to the Fc 'stem' of the Y-shaped IgG molecule (Fanger & Smyth, 1972), but all molecules of rabbit IgG contain carbohydrate N-linked to the asparagine residue 297 (human IgGl protein Eu numbering) in each of the two identical heavy chains in the Fc fragment. In this paper we report the results of an X-ray crystallographic study of rabbit Fc fragment, which has revealed the conformation of these two carbohydrate chains in relation to the three-dimensional structure of the protein.

The Fc fragment was prepared by papain digestion of IgG from pooled rabbit serum, without reduction of the single disulphide bridge between the two heavy chains at residue 229 near the N-terminus of the fragment (Aschaffenburg et al., 1979). The crystals grew in the space group P2₁ with one molecular fragment in the asymmetric unit, so that the two identical halves of the Fc were not related by an exact crystallographic two-fold rotation axis. A further consequence is that each of the two chains has a different environment within the crystal and is subject to different packing interactions with adjacent molecules.

The first electron density map was determined at a resolution of 0.45 nm by m.i.r., and an initial model was based upon that of human Fc (Deisenhofer et al., 1976) by fitting each of the four domains independently to its corresponding electron density, and rebuilding the rabbit Fc sequence to a map calculated with combined m.i.r. and model phases. Preliminary crystallographic refinement of the model has been performed at 0.27nm resolution, and the electron density map described here is calculated with combined (m.i.r. and partially refined model) phases at this resolution. No carbohydrate structure has yet been included in the model, and the map is therefore completely unbiased towards any particular interpretation.

Each of the two heavy chains in Fc folds into two domains (designated Cₙ2 and Cₙ3), and each domain consists of a 'sandwich' of two antiparallel β-sheets. Asparagine residue 297 is to be found on a sharp bend between two adjacent strands of β-sheet at the end of the Cₙ2 domain distant from the Cₙ3 domain (see Fig. 1), in the sequence Gln-Gln-Phe-Asn-Ser-Thr-Ile. At this point electron density continuous with that of the polypeptide chain extends away from each of the two Cₙ2

Fig. 1. Carbohydrate structure at the Asn-297 residues (indicated CA 297 and CA 597) with the Cα backbone of polypeptide chains 1 and 2, in the Cₙ2 domains

Abbreviations used: IgG, immunoglobulin G; m.i.r., multiple isomorphous replacement.
domains into the region between them; in contrast to the close \( C_{H} \)-\( C_{H} \) domain association, the \( C_{H} \)-\( C_{H} \) domains are well separated from each other (see Fig. 1).

The first observation regarding the electron density of the carbohydrate is that it is asymmetric with respect to the two halves of the molecule. Although there is no exact crystallographic two-fold axis relating the two polypeptide chains, the two \( C_{H} \)-\( C_{H} \) domains are related by an approximate two-fold axis (183° rotation and a small translational component). Clearly even this relationship breaks down in the carbohydrate region, and the two carbohydrate chains have therefore been built independently to their respective electron densities.

The following structure was fitted to the electron density on each half of the Fc:

\[
\begin{align*}
(6') & \rightarrow 4 \text{GlcNAc} \beta 1 \rightarrow 2 \text{Man} \\
(5') & \rightarrow 4 \text{GlcNAc} \beta 1 \rightarrow 2 \text{Man} \\
(4') & \rightarrow 4 \text{GlcNAc} \beta 1 \rightarrow 2 \text{Man}
\end{align*}
\]

This structure resembles those reported from early studies of IgG myeloma proteins [for reviews see Kornfeld & Kornfeld (1976) and Monfreuil (1980)] and is identical to that reported for bovine IgG (Tadashi et al., 1975). Recent studies of the N-linked carbohydrate from rabbit IgG (see the following paper, Rademacher et al., 1983) show that there is also heterogeneity resulting from the presence or absence of Fuc(7), and GlcNAc linked \( \beta 1 \rightarrow 4 \) to Man(3). The electron density levels for GlcNAc(1), GlcNAc(2), Man(3), Man(4) and \( \beta \text{Man(4') in both carbohydrate chains are all comparable and indicative of a single conformation, lying close to the surface of the } \text{CH2 domain (residues Phe-241, Val-264, Asp-265). The density for Fuc(7), while indicative of a single conformation, is slightly weaker, suggesting either that it is not present in all chains, or that it is more mobile than the other sugar residues. The branch at Man(3) in both chains is approximately T-shaped, and between them, again in both chains, there is an extremely weak extension of electron density at the O-4 atom of Man(3) which may indicate that a very small fraction of molecules contains GlcNAc linked \( \beta 1 \rightarrow 4 \) to Man(3).

At this point it is necessary to distinguish between the two carbohydrate chains. Fig. 1 shows the conformation of both chains in relation to the Ca atoms of the two polypeptide chains, whose designations 1 and 2 will also be used to refer to the carbohydrate.

The electron density of the 1→6 branch of chain 2 is as strong as that for the preceding sugar units, but is too extensive merely to accommodate Man(4') and GlcNAc(5') and may represent an alternative conformation to that shown in Fig. 1, or a further sugar unit linked to Man(4'). The density for Gal(6') is slightly weaker, either because it is not present in all molecules, or because it has a greater degree of mobility. However, up to Gal(6'), this branch is in close contact with residues on the surface of the \( C_{H} \)-\( C_{H} \) domain of chain 2 (principally Phe-243, Pro-246 and Thr-260), and is also close to those of the \( C_{H} \)-\( C_{H} \) domain of an adjacent molecule in the crystal (including the salt-bridged residues Asp-281 and Lys-317). Beyond Gal(6') there is no further electron density, but the intermolecular space density extends beyond it toward the \( C_{H} \)-\( C_{H} \) domain of an adjacent molecule in the crystal. (Note that this crystal environment is different from that of the 1→6 branch of chain 1.)

The conformation of the 1→3 branch is also very different from that of chain 2. The electron density is weaker even for GlcNAc(5), but extends beyond that to which Gal(6') is fitted, becoming progressively weaker. There is no contact between this branch of chain 1 and any part of chain 2, nor with the surface of the protein, nor that of any adjacent molecules in the crystal; the decreasing electron density along the chain may reflect progressively greater mobility. The more extensive density for both this and the 1→6 branch of chain 1 may indicate the presence of terminal AcNeu units (for which there was no positive evidence of chain 2).

In summary, the two N-linked carbohydrate chains attached to the two heavy chains in rabbit Fc adopt different conformations, and may differ also in composition [Gal(6) and AcNeu attachment for example] and mobility. Almost all of the carbohydrate structure shown in Fig. 1, however, has well-defined electron density and is therefore relatively immobile. GlcNAc(1), GlcNAc(2), Man(3) and the 1→6 branch of each chain make several contacts, mainly hydrophobic, with residues on the surface of the protein.

The two carbohydrate chains make contact with each other between GlcNAc(5) (chain 2), GlcNAc(2) (chain 1) and Man(3) (chain 1), and it therefore appears that they are responsible for maintaining the atypical disposition of the two \( C_{H} \)-\( C_{H} \) domains. [This contrasts with the study of human Fc (Deisenhofer, 1981) in which there appears to be little or no contact between the two carbohydrate chains.]

The present carbohydrate structure has not yet been refined crystallographically, nor included in the model used in the calculation of the electron density map. These steps will provide a more precise definition of the contacts between carbohydrate and protein, and may also improve the quality of the more diffuse regions of the electron density map.

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Structural and conformational analysis of immunoglobulin-derived N-linked oligosaccharides

T. W. RADEMACHER* S. W. HOMANS, D. L. FERNANDES and R. A. DWEK
Department of Biochemistry, University of Oxford, South Parks Road, Oxford OXI 3QU, U.K.

and T. MIZUOCCHI, T. TANIGUCHI and A. KOBATA
Department of Biochemistry, Kobe University School of Medicine, Chuo-Ku, Kobe, Japan

Introduction

Immunoglobulins are glycoproteins which are composed of two distinct types of polypeptides—light and heavy chains.

*To whom correspondence should be addressed.

Despite a number of studies extending over the last 10 years the exact number of IgG oligosaccharide chains, oligosaccharide type and sequence remains controversial (Abel et al., 1968). In addition, rigorous studies of species, allotype and sub-class related differences have not yet been performed. Oligosaccharide units have been reported to be present on the light chain (variable region) and heavy chain (Fd, hinge and C2 domain) (Spiegelberg et al., 1970). The oligosaccharides attached to the C2 domain have been conserved throughout evolution and are the focus of this report.

The multiplicity and variability of glycosylation on the Fab, despite a number of studies extending over the last 10 years the exact number of IgG oligosaccharide chains, oligosaccharide type and sequence remains controversial (Abel et al., 1968). In addition, rigorous studies of species, allotype and sub-class related differences have not yet been performed. Oligosaccharide units have been reported to be present on the light chain (variable region) and heavy chain (Fd, hinge and C2 domain) (Spiegelberg et al., 1970). The oligosaccharides attached to the C2 domain have been conserved throughout evolution and are the focus of this report.

Fig. 1. Core and outer-chain heterogeneity of rabbit IgG N-linked oligosaccharide

(a) Composite structure of N-linked oligosaccharide obtained from intact rabbit IgG. (b–e) Structures of cores found on rabbit IgG Fc fragment and relative distributions; 86% of the oligosaccharides contain no sialic acid and 14% contain a single sialic acid residue. No di-sialylated molecules were found on Fc fragments. In contrast, intact rabbit IgG contains 74% neutral, 17% mono-sialylated and 9% di-sialylated.