



## Pharmacological Effects of Glycosylation

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# Pharmacological Effects of Oligosaccharides

There have been several good reviews of the role of carbohydrates in the structure and function of glycoproteins (see e.g. (1-3)). The purpose of this short article is to illustrate some of the areas that are of current interest within the fields of human physiology and pathology.

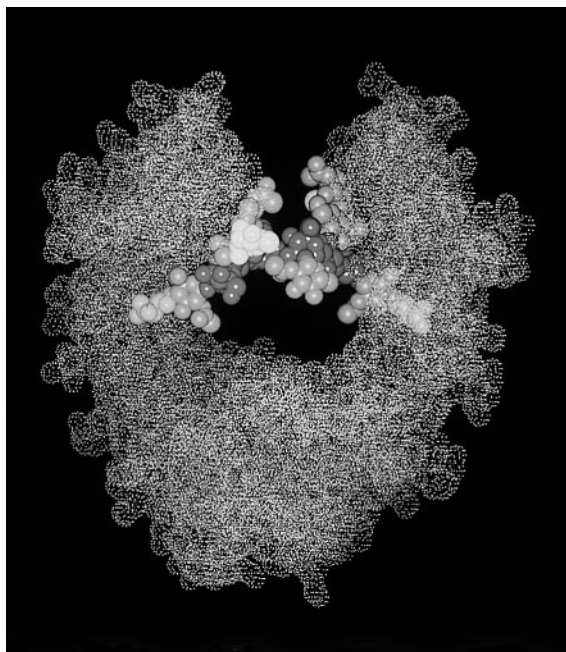
## CLEARANCE AND BIODISTRIBUTION

The numerous mammalian lectins (for review, see 4) are known to bind to the carbohydrate moieties present on glycoproteins. Such carbohydrates are thus of great importance in determining the pharmacokinetic profile of any recombinant glycoprotein. There are several glycan determinants on circulating proteins that participate in well-established clearance mechanisms; they include the hepatic Gal/GalNAc receptor, the hepatic fucose receptor, the mannose-6-phosphate receptors and the Man/GlcNAc receptor of the reticulo-endothelial system(5-7). Such determinants present on a recombinant glycoprotein will thus lead to rapid clearance from the serum. Examples of this include desialylated erythropoietin (EPO) and granulocyte-macrophage colony stimulating factor (GM-CSF) which are rapidly cleared from the serum and localized to the liver (8-10). Cebon *et al.*(11) isolated GM-CSF from human lymphocyte culture which exhibited a wide range of glycoforms, largely due to variable occupation of the two N-linked sites. An inverse relationship exists between N-glycosylation site occupancy and biological activities as measured by receptor affinity and specific activity. Further, Donahue *et al.*(10) observed that the various glycosylated forms of rhGM-CSF from CHO cells displayed distinguishable clearance rates and organ distributions, with the glycoform having the highest amount of glycosylation exhibiting the slowest clearance.

## IMMUNOGENICITY

Carbohydrates can shield as much as a 20 nm<sup>2</sup> area on the surface of a glycoprotein(12). Parts of a glycoprotein that could be immunogenic may thus be "hidden" from the immunosurveillance system. Glycoproteins obtained from recombinant sources may have altered glycosylation or no glycosylation, thus exposing novel antigenic sites. For example, Colby *et al.*(13) found immunological non-identity between CHO recombinant interferon- $\beta$  and *E. coli* recombinant interferon- $\beta$ . Masking of epitopes by oligosaccharides has been shown to occur in influenza virus(14) where an N-linked oligosaccharide prevented a monoclonal antibody from binding to the H3 viral haemagglutinin protein chain. The absence of O-glycosylation on recombinant GM-CSF obtained from yeast was shown to cause the formation of serum antibodies directed against GM-CSF in 4 out of 13 patients(15). It is not clear, however, that these antibodies were neutralizing.

Inter-species variation in glycosylation patterns means that care may be necessary in selection of the cell-type in which



Molecular model of the Fc receptor of human IgG. (Courtesy of The Glycobiology Institute, Oxford, UK.)

to express a recombinant glycoprotein. Galili *et al.*(16) estimated that ~1% of human serum IgG is directed against the carbohydrate epitope Gal $\alpha$ 1,3Gal. This epitope occurs in place of terminal sialylation as a cell-specific feature of the C127 murine cell line, often used for the expression of recombinant glycoproteins. Again, there is no evidence to date of any adverse clinical responses as a result of this epitope being present on glycoproteins(17) but it may prevent cells of nonprimate grafts being xenotransplanted(18)

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