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Meeting Report

A colloquium on the Glycobiology of Proteins organized by Colin Hughes and John Walker for the Glycobiology and Industrial Biochemistry and Biotechnology Groups of the Biochemical Society was held at the University of Kent, Canterbury, UK, on 8 September 1994. In the opening talk Ed Cole (Genzyme, Framingham) described the properties of human anti-thrombin III produced in the milk of transgenic goats. Heparin binding activity and thrombin inhibition were similar to, or rather higher than the natural plasma protein. Several differences in glycosylation were found between the natural and transgene products, notably substitution of GalNAc for Gal at the termini of complex-type N-glycans, reduced sialylation and enhanced fucosylation in the transgene protein which collectively may be related to a pattern of glycosylation dominant in mammary gland, and the appearance of *N*-glycolyl neuraminic acid which is a characteristic feature of caprine glycoproteins. Oligomannose and/or hybrid structures were only found on the transgene product, probably at one glycosylation site (Asn_{155}). Clearance of the transgene product was much faster than the natural form. Remodelling of the N-glycans by full sialylation with $\alpha 2 \rightarrow 6$ sialyl transferase, by endo H treatment or a combination of these treatments improved circulatory persistence very effectively.

Nigel Jenkins (University of Kent, Canterbury) described the effects of varying cell culture conditions on the glycosylation of human *y*-interferon expressed in CHO cells. The nature and extent of glycosylation at Asn_{25} and Asn_{97} was influenced by the lipid composition of the media including those lipids carried on BSA, and in chemostat cultures under steady-state conditions by glucose addition. Most sensitive to culture conditions was occupancy of glycosylation site Asn_{97} and hence the ratio of mono- to bi-glycosylated molecules.

Dirk van den Eijnden (Vrije Universiteit, Amsterdam) discussed in detail the GalNAc$\beta 1 \rightarrow$4GlcNAc (*N,N'*-diacetyllactosdiamine, LacDiNAc) unit found in an increasing number of examples at the termini of N- and O-glycans, including the goat transgenic anti-thrombin III. The specific *N*-acetylgalactosaminyl transferase has been most thoroughly studied in the avian schistosome *Tricholbilharzia ocellata* and its intermediate host the mollusc *Lymnaea stagnalis*. It shows many similarities to mammalian $\beta 1 \rightarrow 4$ galactosyl transferase including binding of α-lactalbumin which promotes synthesis of GalNAc$\beta 1 \rightarrow$4Glc, analogous to synthesis of lactose by galactosyl transferase in the presence of α-lactalbumin. Low stringency hybridization screening of an *L. stagnalis* genomic library with $\beta 1 \rightarrow 4$ galactosyl transferase cDNA identified two different but related cDNAs from the albumen and prostate gland respectively. Several exons show considerable homologies (up to 56%) with corresponding exons of the murine galactosyl transferase. Surprisingly, the prostate enzyme has no GalNAc transferase activity. Instead it produces a *N,N'* chitobiose structure, GlcNAc$\beta 1 \rightarrow$4GlcNAc, prompting van den Eijnden to propose yet another terminal feature of N-glycans and a new pathway, the chitobio-pathway. It will be interesting to see if such structures occur in snail glycoproteins and elsewhere and the properties of glycoproteins bearing such unusual features.

In an interesting review Frank Hemming (University of Nottingham) discussed the present and potential use of hyphal fungi, mainly *Trichoderma* and *Aspergillus* species for production of heterologous glycoproteins. Major problems include lack of information on glycosylation patterns (although hyper-mannosylation found in yeasts may not occur) and low yields, which may be solved by use of high secretor species or fusion proteins with homologous enzymes such as glucoamylase.

Bryan Winchester (Institute of Child Health, London) emphasized the importance of protein glycosylation by discussion of the Carbohydrate Deficient Glycoprotein Syndrome (CDGS), a heterogenous disease family of at least three clinical types. In the Type II CDGS, fibroblasts from patients lack N-acetylglucosaminyl transferase II activity and produce glycoproteins with truncated Man_3 hybrid N-glycans. The more common Type I CDGS, which like other CDGS states is identified by the under-sialylation of plasma proteins such as serotransferrin in electrophoresis, is characterized by reduced *N*-glycosylation site occupancy. In the case of serotransferrin, either of the two N-glycosylation sites may be unutilized, apparently at random. The defect is not known but could involve pool sizes of sugar precursors, for example dolichol-P-mannose, levels of oligosaccharyl transferase or levels of chaperones involved in glycoprotein folding.

Simon Davies (Oxford University) has used two methods to obtain minimally glycosylated forms of the cell adhesion molecule CD2 for crystallization studies: expression in lectin-resistant cells or in cells treated with N-glycan processing inhibitors followed by endo H release of high mannose oligosaccharides. The extracellular portion of CD2

consists of two Ig-like domains joined by a flexible linker exactly as predicted from the sequence data. In contrast to a recent report from another group, the antibody- and ligand- (CD58) binding properties of minimally glycosylated CD2 were found to be indistinguishable from the fully glycosylated CD2. An unglycosylated CD2 derivate obtained by peptide N-glycosidase I also bound ligand strongly although kinetic analysis indicated a tendency to aggregate.

Finally, Roy Jefferis (University of Birmingham) showed how glycosylation of chimeric human-mouse anti-NP antibodies produced in murine J558L cells varies according to culture conditions and the influence on Fc functions. The structure of terminal sequences especially differed: for example, in shallow cultures the antibodies contained less agalacto-forms of the N-glycan compared with antibodies produced in hollow fibre bioreactors or *in vivo* as ascites.

R. C. Hughes
N.I.M.R.