Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Int. J. Biochem. Vol. 16, No. 3, pp. 253–256, 1984
Printed in Great Britain.

0020-711X/84 $3.00 + 0.00

MINIREVIEW

A PROPOSAL: THAT THE HETEROGENEITY OF GLYCOPROTEIN *N*-GLYCANS IS A FEATURE OF THEIR BIOSYNTHESIS AND MAY BE OF FUNCTIONAL SIGNIFICANCE

L. MÄRZ
Institut für Chemie, Universität für Bodenkultur, Gregor-Mendelstrasse 33, A-1180 Vienna, Austria

and

M. W. C. HATTON
Department of Pathology, 4 N 67, McMaster University, 1200 Main Street West, Hamilton, Ontario L8N 3Z5, Canada

(*Received* 7 *June* 1983)

INTRODUCTION

Glycoconjugate research has been greatly stimulated by observations which suggest that protein-bound carbohydrate may be required to effect certain biochemical interactions, particularly those involving cell surfaces. These observations have led to numerous speculations and hypotheses on the biological significance of protein glycosylation. Among them, the concept that the carbohydrate moiety, in addition to its conformation-stabilizing and protective role towards the polypeptide, may serve as a carrier of biological information in terms of a recognition signal, appears the most attractive. Our increasing knowledge of the biosynthesis and structures of asparagine-linked glycans ("*N*-glycans"), which are found ubiquitously in secreted and membrane-inserted glycoproteins of eukaryotes, has led to the discovery that the entire range of their structures is derived from a common precursor oligosaccharide (Berger *et al.*, 1982; Hemming, 1982; Montreuil, 1980). These facts and the many reports on the involvement of protein-bound carbohydrate in specific molecular events, have stimulated us to re-evaluate the phenomenon of *N*-glycan heterogeneity in relation to its origin and to discuss its biological relevance.

N-GLYCAN HETEROGENEITY

The term "microheterogeneity" signifies a structural property of glycoproteins which possess *N*-glycans of the "complex" type (Fig. 1A). Upon electrophoresis, isoelectric focussing or ion exchange chromatography, multiple forms of a glycoprotein are observed, which are generally due to variations in the sialic acid content. They were believed initially to be caused by "incomplete sialylation" or "partial degradation" of the glycoprotein (Spiro, 1973). However, *N*-glycan heterogeneity is not restricted to the sialic acid content of the very periphery, but reflects another structural property of "complex" type structures, the variable number of "antennae" (see legend to Fig. 1A). Human transferrin, whose fractionation presents an example of sialic acid-dependent separation of molecular variants, possesses two sites of glycosylation (Spik *et al.*, 1975); at one or both of these sites, bi-, tri- and possibly, tetraantennary "complex" glycans are located (Hatton *et al.*, 1979; Kerckaert and Bayard, 1982; März *et al.*, 1982; Spik, 1982; see Fig. 1A). Other glycoproteins show similar or even more complicated arrangements, e.g. α_1-acid glycoprotein (Fournet *et al.*, 1978), rat α-fetoprotein (Bayard and Kerckaert, 1981), α_1-protease inhibitor (Bayard *et al.*, 1982).

BIOSYNTHESIS—THE BASIS OF *N*-GLYCAN HETEROGENEITY

Our present knowledge of the biosynthetic mechanism which yields the "complex" (Fig. 1A), high-mannosyl (Fig. 1B), and "hybrid" structures (Fig. 1C), provides insight into the genesis of heterogeneity. *N*-Glycan biosynthesis commences, independent from the protein, with the assembly of a large precursor oligosaccharide (Fig. 2) linked to a lipid carrier, diphosphodolichol (Hemming, 1982). Incorporation of this precursor into the protein takes place in the rough endoplasmic reticulum (RER) during polypeptide synthesis, at particular asparaginyl sites which are determined by the primary structure and the conformation of the polypeptide (Marshall, 1972; Montreuil, 1980). The initial glycoprotein passes to the *cis*-Golgi, where the precursor glycan is trimmed by a series of glycosidic enzymes to finally derive at a GN_2-MAN_5 structure. It is this section of the process which yields high-mannosyl structures. Further monosaccharide additions by specific glycosyl

Abbreviations: GN, *N*-acetyl-D-glucosamine; MAN, D-mannose; SA, sialic acid; FUC, L-fucose; GAL, D-galactose; GLC, D-glucose; ASN, L-asparagine.

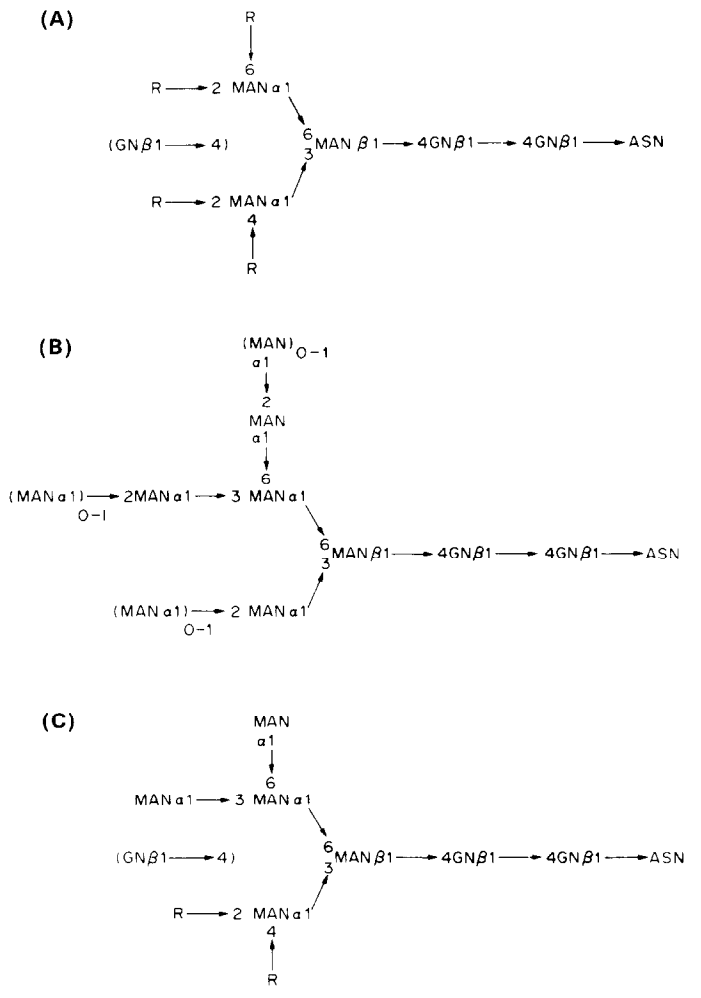

Fig. 1. Schematic presentation of bi-, tri- and tetraantennary "complex" (Fig. 1A), high-mannosyl (Fig. 1B) and "hybrid" (Fig. 1C) type *N*-glycan structures. R symbolizes an "antenna" of the sequence sialic acid–galactose–*N*-acetylglucosamine.

transferases, probably in the *trans*-Golgi region, results in a glycoprotein bearing "complex" *N*-glycans.

In contrast to polypeptide synthesis, the assembly of *N*-glycans is not a template-based process, directed by a master code. Rather, it is controlled by the availability of nucleotide-sugars, metabolism-dependent levels of the lipid carrier (Hemming, 1982), the specificities of the glycosidases and the numerous glycosyl transferases. The number of antennae and degree of sialylation not only depend upon the presence of specific glycosyl transferases but also upon limitations imposed by the developing glycoprotein.

The important consequence of the complicated design of this system is the creation of a variety of structures, basing on the activity of enzymes which partly require, partly exclude each other's action, and partly compete for an intermediary product.

FATE OF GLYCOSYLATION PRODUCTS INSIDE AND OUTSIDE THE CELL

From the discussion so far, it is perhaps remarkable that a glycoprotein contains *N*-glycans of such relative uniformity! Indeed, variations in the structure of a forked heterosaccharide, particularly at the distal extremities should not be a surprising feature. But, of all the variants of a glycosylated protein which are produced, which ones are "tolerated" by the producing cell, i.e. found acceptable and allotted a task? Which are determined to be unacceptable, how is the decision made, and what is their fate? At present little is known of the extent of quality control which takes place within the cell prior to transport of the finished product. However, Hercz and Harpaz (1980) have reported, that people who are homozygous for the Z allele of the α_1-protease inhibitor locus synthesize a molecular variant that is not secreted. Examination of the cell contents shows the RER fraction to be rich in α_1-protease inhibitor possessing not the expected "complex", but instead high-mannosyl glycans. Presumably possession of the "wrong" *N*-glycan precluded secretion of this protein from the cell; yet the accumulation of α_1-protease inhibitor was not matched by an increased intracellular catabolism. Consideration of the plasma glycoproteins shows clearly that some heterogeneity can

be tolerated by the producing cell and by the circulation of the host. For example, partially sialylated forms of α_1-acid glycoprotein and transferrin have been isolated from plasma samples taken from various patients (Schmid *et al.*, 1964; Stibler *et al.*, 1979); on the other hand secreted proteins whose *N*-glycans are too deficient in distal glycosyl units would be rapidly removed from the circulation by the sensitive hepatic receptors (Aswell and Harford, 1982). Given, then, that a degree of glycoprotein heterogeneity is tolerated by the host, does this mean that each of the heterogeneous forms is of specific physiological benefit to the host through the design of its *N*-glycan moiety? At this time, little information is available to directly answer this question with confidence. Many experimental systems have dealt, directly or indirectly, with the investigation of the relationship between *in vivo* and *in vitro* behaviour and function of glycoconjugates on one hand and the presence and structure of carbohydrate on the other. In a number of cases, the glycans have been shown to exert a direct influence on certain biological features of their parent molecules (Carayon *et al.*, 1980; Manjunath and Sairam, 1982; Miller *et al.*, 1982; Moyle *et al.*, 1975; Stern and Sefton, 1982; Stibler *et al.*, 1979) which reaches beyond the mere change of physico–chemical properties and supports the notion that presence and design of the glycans may influence the biological properties of glycoproteins, especially their destiny in circulation. Indeed, if the sole purpose of glycosylation were to impart certain physico–chemically important features on the protein pertaining to its stability, it would surely suffice to randomly add a number of sugar residues instead of maintaining a mechanism for the construction of heterosaccharides with highly conserved structural elements via the synthesis of a precursor much larger than the final product in the mature glycoprotein. However, to our knowledge, no comparative study *in vivo* of individual, glycan-characterized sub-forms of a glycoprotein has yet been reported. Such a study may reveal any glycan-dependent physiological characteristics.

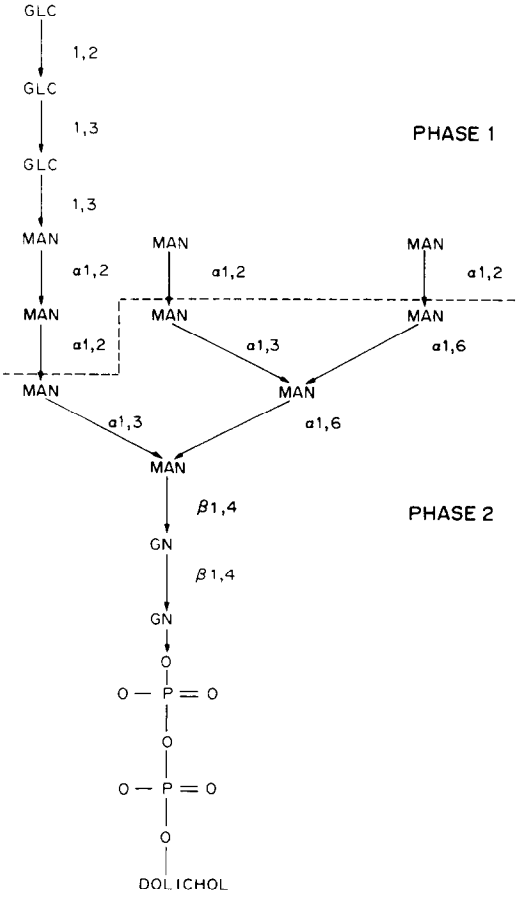

Fig. 2. Schematic presentation of the precursor oligosaccharide and the phases of processing. Cessation of this process during phase 1 yields high-mannosyl, further modifications in phase 2 "complex" and "hybrid-type" structures.

In an earlier report, Regoeczi *et al.* (1980) compared the fates of two forms of desialylated human transferrin, which differed significantly in their carbohydrate contents, in the circulation of the rabbit. Whereas one transferrin type was cleared very rapidly by the liver, the other was preferentially catabolized by the cells of the bone-marrow. Despite the use of modified glycoprotein forms in a heterologous system, this study showed that the carbohydrate moiety could function as a "sorting" or "routing" signal, a role which appears to be of general relevance to cell–cell and cell–macromolecule interactions (Ashwell and Morell, 1974; Kaplan *et al.*, 1977; Prieels *et al.*, 1978; Rauvala *et al.*, 1981; Regoeczi *et al.*, 1975; Stahl *et al.*, 1978; Yen and Ballou, 1973).

CONCLUDING REMARKS

In conclusion, the heterogeneous nature of the *N*-glycan moiety of a glycoprotein is a consequence of its mode of synthesis; the cell obviously tolerates certain of the heterogeneous forms of circulating glycoproteins. Whether a similar tolerance exists for heterogeneity in membrane glycoproteins, remains to be shown. It is, at present, a reasonable guess that a singular specific duty exists for each of the forms of a glycoprotein which is based on the design(s) and arrangement(s) of the associated glycan(s). Thus, a change in the pattern of the heterogeneity of a glycoprotein could represent metabolic "fine-tning" to allow greater emphasis of one physiological function at the expense of, or in addition to, other commitments (Hatton *et al.*, 1983).

Acknowledgements—M.W.C.H. is a Research Associate of the Ontario Heart Foundation. Work discussed in this paper was supported by funds from the Medical Research Council of Canada. L.M. is a recipient of a grant from the "Fonds zur Förderung der wissenschaftlichen Forschung", Austria.

REFERENCES

Ashwell G. and Morell A. G. (1974) The role of carbohydrate in the hepatic recognition of circulating glycoproteins. *Adv. Enzym.* **41**, 99–128.

Ashwell G. and Harford J. (1982) Carbohydrate-specific receptors of the liver. *A. Rev. Biochem.* **51**, 531–554.

Bayard B. and Kerckaert J. P. (1981) Uniformity of carbo-

hydrate chains within molecular variants of rat α_1-fetoprotein with distinct affinity for Concanavalin A. *Eur. J. Biochem.* **113,** 405–414.

Bayard B., Kerckaert J. P., Laine A. and Hayem A. (1982) Uniformity of glycans within molecular variants of α_1-protease inhibitor with distinct affinity for Concanavalin A. *Eur. J. Biochem.* **124,** 371–376.

Berger E. G., Buddecke E., Kamerling J. P., Kobata A., Paulson J. C. and Vliegenthart J. F. G. (1982) Structure, biosynthesis and function of glycoprotein glycans. *Experientia* **38,** 1129–1162.

Carayon P., Amr S. and Nisula B. (1980) A competitive antagonist of thyrotropin: asialo-choriogonadotropin. *Biochem. biophys. Res. Commun.* **97,** 69–74.

Fournet B., Montreuil J., Strecker G., Dorland L., Haverkamp J., Vliegenthart J. F. G., Binette J. P. and Schmid K. (1978) Determination of the primary structures of 16 asialo-carbohydrate units derived from human plasma α_1-acid glycoprotein by 360-MHz ^{1}H NMR spectroscopy and permethylation analysis. *Biochemistry* **17,** 5206–5214.

Hatton M. W. C., März L., Berry L. R., Debanne M. T. and Regoeczi E. (1979) Bi- and triantennary human transferrin glycopeptides and their affinities for the hepatic lectin specific for asialo-glycoproteins. *Biochem. J.* **181,** 633–638.

Hatton M. W. C., März L. and Regoeczi E. (1983) On the significance of heterogeneity of plasma glycoproteins possessing *N*-glycans of the complex type: a perspective. *Trends Biochem. Sci.* **8,** 287–291.

Hemming F. W. (1982) Control and manipulation of the phosphodolichol pathway of protein *N*-glycosylation. *Biosci. Rep.* **2,** 203–221.

Hercz A. and Harpaz N. (1980) Characterization of the oligosaccharides of liver Z variant α_1-antitrypsin. *Can. J. Biochem.* **58,** 644–648.

Ishihara H. and Tejima S. (1983) Structure and location of asparagine-linked oligosaccharides in the Fc region of a human immunoglobulin D. *Biochem. biophys. Res. Commun.* **110,** 181–186.

Kaplan A., Achord D. T. and Sly W. S. (1977) Phosphohexosyl components of lysosomal enzyme are recognized by pinocytosis receptors of human fibroblasts. *Proc. natn. Acad. Sci. U.S.A.* **74,** 2026–2038.

Kerckaert J. P. and Bayard B. (1982) Glycan uniformity within molecular variants of transferrin with distinct affinity for Concanavalin A. *Biochem. biophys. Res. Commun.* **105,** 1023–1030.

Manjunath P. and Sairam M. R. (1982) Biochemical, biological, and immunological properties of chemically deglycosylated human choriogonadotropin. *J. biol. Chem.* **257,** 7109–7115.

Marshall R. D. (1972) Glycoproteins. *A. Rev. Biochem.* **41,** 673–702.

März L., Hatton M. W. C., Berry L. R. and Regoeczi E. (1982) The structural heterogeneity of the carbohydrate moiety of desialylated human transferrin. *Can. J. Biochem.* **60,** 624–630.

Miller M. S., Bruch R. C. and White H. B. (1982) Carbohydrate compositional effects on tissue distribution of chicken riboflavin-binding protein. *Biochim. biophys. Acta* **715,** 126–136.

Montreuil J. (1980) Primary structure of glycoprotein glycans. *Adv. Carbohydr. chem. Biochem.* **37,** 157–223.

Moyle W. R., Bahl O. P. and März L. (1975) Role of the carbohydrate of human chorionic gonadotropin in the mechanism of hormone action. *J. biol. Chem.* **250,** 9163–9169.

Prieels J.-P., Pizzo S. V., Glasgow L. R., Paulson J. C. and Hill R. L. (1978) Hepatic receptor that specifically binds oligosaccharides containing fucose α 1–3 *N*-acetylglucosamine linkages. *Proc. natn. Acad. Sci. U.S.A.* **75,** 2215–2219.

Rauvala H., Carter W. G. and Hakomori S.-I. (1981) Studies on cell adhesion and recognition. *J. Cell Biol.* **88,** 127–137; 138–148; 149–159.

Regoeczi E., Hatton M. W. C. and Charlwood P. A. (1975) Carbohydrate-mediated elimination of avian plasma glycoprotein in mammals. *Nature* **254,** 699–701.

Regoeczi E., Chindemi P. A., Hatton M. W. C. and Berry L. R. (1980) Galactose-specific elimination of human asialotransferrin by the bone marrow in the rabbit. *Archs Biochem. Biophys.* **205,** 76–84.

Schmid K., Burke J. F., Debray-Sachs M. and Tokita K. (1964) Sialic acid-deficient α_1-acid glycoprotein produced in certain pathological stages. *Nature* **204,** 75–76.

Spik G., Bayard B., Fournet B., Strecker G., Bouquelet S. and Montreuil J. (1975) Studies on glycoconjugates: LXIV. Complete structure of two carbohydrate units of human serotransferrin. *FEBS Lett.* **50,** 296–299.

Spik G. (1982) Personal communication.

Spiro R. G. (1973) Glycoproteins. *Adv. Protein Chem.* **27,** 349–467.

Stahl P., Rodman J. S., Miller J. S. and Schlesinger P. H. (1978) Evidence for receptor-mediated binding of glycoproteins, glycoconjugates and lysosomal glycosidases by alveolar macrophages. *Proc. natn. Acad. Sci. U.S.A.* **75,** 1399–1403.

Stern D. F. and Sefton B. M. (1982) Coronavirus protein: structure and function of the oligosaccharides of the avian infectious bronchitis virus glycoprotein. *J. Virol.* **44,** 804–812.

Stibler H., Borg S. and Allgullander C. (1979) Clinical significance of transferrin in relation to alcohol consumption. *Acta med. scand.* **206,** 275–282.

Yen P. H. and Ballou C. E. (1973) Composition of a specific intercellular aggregation factor. *J. biol. Chem.* **248,** 8316–8318.