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Recent Advances in the Study of Glycoproteins*

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The study of glycoproteins, their chemical and physicochemical structures, metabolism, and biological roles has witnessed an extraordinary progress during the past two decades[1]. Outstanding hallmarks in the investigation of these complex macromolecules are the complete elucidation of the amino acid sequence of the immunoglobulins which are known to possess two carbohydrate units per molecule (35–37), porcine ribonuclease with three carbohydrate units (38) and α_1-acid glycoprotein with five such units (39–41), and the recognition that glycoproteins are involved in a vast number of essential, biological functions. These and other important achievements in this area of inquiry have been made possible, to a large extent, by newly developed and extremely sensitive methods for the preparation and purification of proteins and for the elucidation of the three-dimensional structure of these conjugated macromolecules (42). Because of the fundamental importance of the glycoproteins, investigators of many disciplines and diverse training have taken an intense interest in the study of this class of proteins.

Over the past few years the emphasis in the study of glycoproteins has shifted from structural investigations, particularly of those present in nature in the soluble form, to studies of glycoproteins of the cell, its surface membrane and organelles. This shift is largely due to the recent findings that glycoproteins are involved in many vital functions of the cell, such as cellular recognition, cellular antigens (e.g. transplantation antigens), cell-bound receptors (membrane-mediated information), conduction of nerve impulses and informational potentials.

Definition, Classification and Occurrence

Glycoproteins are probably best defined as proteins that carry covalently bound sugars. Nucleoproteins are excluded from, while (glyco) lipoproteins are included in this class of conjugated proteins. The predominant monosaccharides of these macromolecules are listed in Table I. Uronic acids are not present in glycoproteins except in the glycosaminoglycan (mucopolysaccharide) protein complexes and in certain invertebrate collagens recently discovered by SPIRO and VISHNU (43). Certain plasma glycoproteins may be free of fucose (fetuin, fibrinogen), sialic acid (Gc-components) or galactosamine (α_1-acid glycoprotein). However, they may contain sulfate or phosphate residues (fibrinogen). The polypeptide moiety of glycoproteins is composed of the expected amino acids. The collagens contain, in addition, hydroxyproline and hydroxylysine. Further, the polypeptide moiety must account for a high enough percentage of the molecule (30 to 99%) so that the protein-nature is maintained.

Table I. Predominant Carbohydrate Constituents of Certain Macromolecules

Glycoproteins	Mucopolysaccharides
Sialic acids (N, O)	Uronic acid
L-Fucose	
D-Galactose	
D-Mannose	
D-Glucose	
D-Xylose	
L-Arabinose	D-Galactose
N-Acetyl-D-glucosamine	N-Acetyl-D-glucosamine
N-Acetyl-D-galactosamine	N-Acetyl-D-galactosamine
Sulfate, Phosphate	Sulfate

It may be appropriate to add at this point that the molecular weights of glycoproteins vary from about 14,000 (ribonuclease B) to several millions (acid mucopolysaccharide-protein complexes). Further, the solubility of this group of conjugated proteins in low ionic strength, aqueous solutions ranges from an extremely high value (e.g. globular blood plasma glycoproteins)

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[1] A list of over 30 reviews on the various aspects of the study of glycoproteins was published by the applicant (1). Recent advances in this area of investigation have been reviewed by SPIRO (2, 3), SIMONS (4), GINSBERG and NEUFELD (5), MARSHALL and NEUBERGER (6), NEUBERGER and MARSHALL (7), ROBERT and PLONOVSKI (8), and MONTGOMERY (9). The treatises by JEANLOZ and BALAZS (10), GOTTSCHALK (11), and ROSSI and STOLL (12) contain further valuable information. A very large number of reviews have been written on the structure and function of the immunoglobulins. In 1970 seven such articles appeared (13–19), while in 1969 there were at least eleven (20–30). The two latest comprehensive reviews on this subject were published by KOCHWA and KUNKEL (31) and SMITH, HOOD and FITCH (32) in 1971. The very recent advances in the investigation of glycoproteins were summarized in the symposium on "Glycoproteins of Blood Cells and Plasma" (33), the symposium on "Biological and Chemical Properties of Animal Cell Surfaces" (34) and the "Colloquium on Glycoproteins" (34a).

Table II. Carbohydrate-Protein Linkages

#	Constituents Forming the Linkage, Monosaccharide and Amino Acid	Type of Linkage (Example)	Representative Macromolecule(s)
1	N-Acetyl-glucosaminyl $\xrightarrow{\beta}$ Asparagine	N-glycosidic, stable toward weak alkali	Plasma glycoproteins
2	N-Acetyl-galactosaminyl $\xrightarrow{\alpha}$ Serine (Thr)	O-glycosidic, β-elimination	Submaxillary glycoproteins
3	Xylosyl $\xrightarrow{\beta}$ Serine	O-glycosidic, β-elimination	Proteoglycans (chondroitin sulfate-protein complex)
4	Galactosyl $\xrightarrow{\beta}$ Hydroxylysine	O-glycosidic, alkali stable	Collagens
5	Arabinosyl $\longrightarrow$ Hydroxyproline	O-glycosidic, alkali stable	Plant cell wall glycoproteins
6	N-Acetyl-galactosaminyl $\longrightarrow$ Phosphate	Phosphodiester bond	Bacterial cell wall glycoproteins

to zero (insoluble structural glycoproteins of connective tissue).

Until very recently glycoproteins were grouped into various classes according to their origin, biological function, chemical composition or physicochemical properties depending on the inclination of the investigators. It now appears reasonable to base the classification of these macromolecules on chemical and structural considerations. The various types of carbohydrate-protein linkages (Table II) seem an excellent choice for this purpose. Subdivisions may then be based on size and structure of the carbohydrate units.

In nature (microorganisms, plants, invertebrates and vertebrates) glycoproteins are found very widely. All organs, tissues and cells of the human body contain an extremely large number of diverse glycoproteins. It is of considerable interest to note further that actually only a few of the human proteins are devoid of sugars. Albumin, insulin, pepsin, chymotrypsin, trypsin carbonic anhydrases and certain basic proteins (44) are the best known examples of the "simple" proteins.

Biological Function

The largest number of biological functions are probably fulfilled by glycoproteins. They act as hormones (e.g. LH, FSH), enzymes (blood clotting, vertase, amylases) and antibodies. In addition they form, for example, the antigenic and attachment sites on the cell surface (for certain viruses) including those of the white cells and platelets and the binding sites of the intestinal mucosa for the vitamin B_{12}-protein complex. They represent some of the structural proteins of the body (collagens) and provide for the viscosity of saliva (submaxillary mucoproteins) and joint fluids (hyaluronic-acid and its protein complexes). They are involved in the active transport across cellular membranes and in the selective permeability. These examples again point to the fundamental importance of the glycoproteins.

The biological functions of glycoproteins have been reviewed by JEANLOZ and BALAZS (10), GOTTSCHALK (11), WINZLER (45) and recently by SPIRO (3) and MONTGOMERY (9). Yet, it should be realized that the function of the majority of these proteins is still unknown. The significance of their carbohydrate moieties will be discussed below.

Isolation and Characterization

The methods employed for the isolation and purification of glycoproteins and proteins have been critically reviewed by several investigators (1, 2, 11, 42, 46–49). Of the more recently introduced preparative techniques, affinity chromatography (50), disc electrophoresis (51) and electrofocusing (52–54) should be mentioned. Experience has shown that the procedures used for the isolation and purification of proteins in general are also successfully utilized for the preparation of glycoproteins. The only exception is the density gradient centrifugation (55) which takes advantage of the difference in the average partial specific volume of the amino acid ($\bar{v}$ = 0.7) and of the monosaccharide ($\bar{v}$ = 0.6) residues. While this procedure has as yet not been fully developed, it permits separation, for example, of certain mucopolysaccharide-protein complexes from each other based on differences in their carbohydrate content (56, 57a). But it should also be noted that certain mucoproteins may undergo large and irreversible changes in their tertiary structure (57b).

The principles underlying the isolation and purification techniques have been discussed in detail by the author (1). It should be noted that a procedure, when found to be very effective, can be successfully repeated only once. It is the experience of the author that a combination of techniques based on different physicochemical principles has proven to be most effective. The most promising procedures for the isolation and purification of proteins to be considered are: column chromatography employing ion exchange resins such as DEAE-, TEAE-, SE- and CM-celluloses and Sephadex ion exchange media, gel filtration by means of various types of Sephadexes and Biogels, and adsorption chromatography using hydroxylapatite. In addition, isoelectric precipitation, electrofocusing, and preparative polyacrylamide gel electrophoresis, and appropriate combinations of these me-

thods have been found very useful. It should also be pointed out that all procedures should be carried out under conditions which are known not to alter the natural state of proteins.

While earlier, purification of proteins was considered to be almost an art, certain guidelines for this purpose have now evolved. Prior to fractionation, it appears best to analyze the protein preparation to be fractionated by ultracentrifugation and paper electrophoresis. The appearance of more than one compound on ultracentrifugation suggests, in terms of gross separation, fractionation by gel filtration through an appropriate Sephadex or Biogel (58). Fractionation based on solubility such as by ammonium sulfate (59, 60) should also be considered at this point. Electrophoretic heterogeneity suggests the use of ion exchange chromatography, isoelectric precipitation, and, if still further resolution is required, electrofocusing and/or preparative disc electrophoresis. It should be noted that, when electrophoretic homogeneity near neutrality has been ascertained, analysis at a slightly acidic *p*H value should also be carried out because certain protein preparations which are known to appear monodisperse near neutrality, can often be resolved into two or more components when examined at other *p*H values (61). Attention should also be paid to the distinction between heterogeneity as a result of the presence of different protein entities and the occurrence of heterogeneity due to variants of a protein or due to instability of a protein as revealed by several bands or gradients (62). Although judging from these general guidelines, it might appear that the purification of a protein is simple and straightforward, it nevertheless should be pointed out that the purification of each protein has and will present certain new problems to be resolved.

The procedures utilized for the complete chemical and physicochemical characterization of proteins and glycoproteins are described in detail in "Structure of Proteins" (42) and "Complex Carbohydrates" (5) and by several other authors (1, 63–67). The molecular weight, sedimentation constant, diffusion coefficient, partial specific volume, and chemical composition of a large number of highly purified glycoproteins, primarily those of human plasma, have been compiled (1, 9, 11, 47, 68–73). The following new techniques are now successfully used for this purpose. Gas-liquid chromatography of appropriate derivatives of the monosaccharides obtained after methanolysis of glycoproteins seems to afford the most correct compositional values (74, 75). Under the experimental conditions employed, only a minute percentage of destruction of the sugars takes place in contrast to the considerable losses due to hydrolysis with mineral acids. In an intermediate procedure, the hydrolysis is carried out with the aid of sulfonated resins (76, 77). Excellent progress has also been made in the gas-liquid chromatographic analysis of all common amino acids (78, 79).

Structural Studies

The present review deals with the structures of the globular glycoproteins only. The chemistry of the various glycosaminoglycans was discussed earlier (80, 81).

The structural investigations of glycoproteins may be divided into three parts, one pertaining to the primary (amino acid and monosaccharide sequences), the other to the secondary (conformations), and the third to the tertiary structure of these macromolecules.

At this point it may be added that proteins and glycoproteins, like other high-molecular weight natural polymers, are characterized by their stable structures which result from covalent linkages (primary structures), hydrogen bondings (secondary structures or conformations and interactions (electrostatic interaction, hydrogen bonding, interaction of non-polar side chains, VAN DER WAALS interaction) among the amino acid side chains (tertiary structures). These stable structures, considered to be those of the native state, may be altered under certain conditions. Such alterations, which are a consequence of changes in or destruction of the secondary and tertiary structures, lead, in the extreme, to irreversible denaturation of the protein with the formation, in general, of insoluble aggregates and are often accompanied by loss of biological activity. Hence, the protein chemist is concerned not only with the preparation of proteins in homogeneous form but also with the preservation of their native states and biological activities. It should be noted that recent studies on polysaccharides revealed that those macromolecules indeed possess distinct secondary and tertiary structures (82).

The methods for the determination of the amino acid sequences of proteins first developed by SANGER and his co-workers have since been refined significantly (42, 83). The primary structure of many "simple" proteins with much higher molecular weights has been elucidated during the past decade (84). As demonstrated by the studies on the 7S-globulins (35–37), porcine ribonuclease (38), avidin (85), and α_1-acid glycoprotein (39–41) the techniques used for establishing the structure of unconjugated proteins are also applicable for the elucidation of the amino acid sequences of glycoproteins. Each glycoprotein will, of course, present special problems as did each non-conjugated protein. The general approach for the elucidation of the amino acid sequence of a protein (42, 83) involves (a) chemical methods for the cleavage of well defined linkages (86, 87), primarily that of methionine by CNBr, and (b) enzymatic methods (42, 83) using trypsin and chymotrypsin that effect limited hydrolysis of the protein cleaving specific peptide bonds. The separation, purification and determination of the structure of the resulting peptides and glycopeptides have been described in detail (42,88). The use for the elucidation of the amino acid sequences of newly introduced enzymes, such as thermolysin, carboxypeptidase C (88a) and aminopeptidase M should also be mentioned.

Sequenators which are based on the direct EDMAN degradation procedure of peptides or proteins are now gradually replacing the somewhat laborious manual techniques (89–91). Under ideal conditions up to 60 successful steps may be achieved with the automated procedure (89), although on the average this is rarely attained. For the elucidation of the structure of certain "difficult to elucidate" peptides (92, 93) and of certain peptides available only in minute quantities such as the releasing factors of the gonadotropins (94), mass-spectrometric procedures have recently been improved to such an extent that they can be applied successfully.

Studies on the primary structure of glycoproteins utilizing isolated glycopeptides have established the amino acid sequences near the carbohydrate-polypeptide linkages for many glycoproteins including α_1-acid glycoprotein, fibrinogen, 7S γ-globulin, luteinizing hormone, ovalbumin, ovomucoid, ribonuclease and transferrin (2, 3, 38, 88, 95–97).

These studies have led to the recognition of the triplet sequence

$$-\text{Asn}-\text{X}-\overset{\text{Ser}}{\underset{\text{Thr}}{}}-,$$

that probably acts as a signal for the enzyme to attach N-acetylglucosamine to certain asparagine residues of the nascent polypeptide chain of glycoproteins (2, 3, 38, 88, 95–97). It should be kept in mind that these tripeptide sections obviously must be located at the surface of the polypeptide molecule, which has already assumed its conformation, in order for the first monosaccharide residue of the carbohydrate unit to be attached. A critical analysis, permitting evaluation of the above mentioned hypothesis, would require the study of the surface of a protein devoid of carbohydrate (e.g. serum albumin) for tripeptides with the mentioned sequence (95, 98). Regarding certain carbohydrate-free proteins, it is also possible that the cells responsible for the production of these proteins do not synthesize the required sugar transferases. This concept has recently been confirmed by SPIRO and co-workers (98a) by a study on the *in-vitro* glycosylation of silk collagen which is formed devoid of sugar. Moreover, the findings that a large number of such tripeptides are free of sugars (98) and that ribonuclease B possesses a carbohydrate unit, although ribonuclease A which has the same amino acid sequence does not, suggests that probably additional factors play a role in influencing the mechanism of the attachment of the initial N-acetyl-glucosamine residues to nascent proteins (95).

The primary structure of glycoproteins requires the elucidation of the following problems in addition to those shared with "simple" proteins: (a) the linkage between the carbohydrate and polypeptide moiety, (b) the size, number and chemical composition of the carbohydrate units and (c) the sequence of the carbohydrate units, including the extent of branching and further microheterogeneity within these units. These three problems have been solved to various degrees with different glycoproteins.

(a) Four types of linkages between the polypeptide chain(s) and the carbohydrate units of animal glycoproteins have been well established (for reviews see 2, 3, 9, 93) (Table II). The first type involves an N-glycosidic bond between the β-amide nitrogen of an asparagine residue and an N-acetyl-glucosamine residue, first shown by NEUBERGER (99) in his studies on ovalbumin. This type of linkage is present in many globular glycoproteins including α_1-acid glycoprotein, ceruloplasmin, fetuin, IgG-immunoglobulin and thyroglobulin (for summary see 3, 38, 88, 95). The second and third types of these linkages, which have been elucidated by MUIR (100), MEYER and co-workers (101) and RODEN (102, 103) is typical of the mucopolysaccharide-protein complexes and submaxillary mucoproteins and involves O-glycosidic linkages between an N-acetylgalactosamine and a serine or threonine residue and between a xylose and a serine residue, respectively. Characteristically, these two linkages are relatively sensitive to alkaline hydrolysis (β-elimination). The fourth type of bond occurring in collagens (3, 104) is also O-glycosidic in nature and involves a galactose and the hydroxyl group of a hydroxylysine residue. However, this linkage is unusually stable toward alkaline hydrolysis.

An additional carbohydrate-polypeptide linkage was found in bacterial cell wall material involving phosphodiesters (105). A further bond was discovered in walls of certain plants which involves an arabinose and a hydroxyproline residue (106a). The recent studies in this area have been summarized by SPIRO (2, 3) and MONTGOMERY (9). Of further interest are the observations by SPIK (107), SPIRO and others (3) who reported that certain glycoproteins possess two types of such linkages.

(b) For the determination of the size, number and chemical composition of their carbohydrate units, the glycoproteins are completely digested with enzymes, primarily pronase, and the resulting glycopeptides isolated first by gel filtration and then resolved by ion exchange chromatography. The glycopeptides of many glycoproteins, notably α_1-acid glycoprotein, fetuin, α_2-macroglobulin, ovalbumin and submaxillary mucoproteins have been studied extensively by CUNNINGHAM, NEUBERGER, PIGMAN, SPIRO, SCHMID and many others. These investigations summarized recently by SPIRO (2, 3) and MONTGOMERY (9), have demonstrated that the number, molecular weight and the chemical composition of the carbohydrate units of many glycoproteins differ considerably from each other. It has also been established that the carbohydrate moiety of all glycoproteins is present as several units, an exception being ovalbumin (107a) and ribonuclease B which have only one such unit. The number of carbohydrate units of a glycoprotein varies from one (ovalbumin) to about 500 (ovine submaxillary mucoprotein) and their sizes from a disaccharide (ovine submaxillary mucoprotein) to a heteropolysaccharide

with a molecular weight of 7,000 (yeast invertase). Equally important, the different carbohydrate groups of a given protein also vary in size.

(c) As to the composition of the carbohydrate moiety of these macromolecules, it should be noted that with few exceptions, no integral ratios of the monosaccharide residues have been obtained. The reason for this observation is that the carbohydrate units of a single glycoprotein differ from each other with regard to their chemical composition. It is surprising that the single carbohydrate unit of the ovalbumin molecules derived even from a single egg are not constant in composition, pointing further to the complexity of the structure of the carbohydrate moiety (104). The reasons for this observation are severalfold, primarily the microheterogeneity of the carbohydrate units (7, 104, 108) and the inability to subfractionate certain heterogeneous glycopeptide preparations (see below). It also should be realized that sensitive criteria for assessing the degree of heterogeneity of glycopeptide preparations are lacking at present. It appears that chromatography on Dowex 50 at very low ionic strength is the most sensitive criterium which has been developed so far for establishing homogeneity of a carbohydrate unit preparation (104). This has been demonstrated by the separation of the three types of carbohydrate units of thyroglobulin (108a). It would then appear that a large part of the results reported so far on compositions and structures of carbohydrate units probably represents average values. The earlier proposed structures of certain carbohydrate units of macro-immunoglobulins (109) and stem bromelian (110, 111) reflect this situation. Because of these difficulties encountered particularly earlier in elucidating the structures of the carbohydrate units of glycoproteins, it is quite understandable and not surprising that two investigators who study apparently the same compound, propose different structures (110, 111)!

There are two fundamental types of microheterogeneity—central and peripheral—found in the carbohydrate moiety of glycoproteins. In the former case the heterogeneity is located in the core of the heteropolysaccharide groups. The different carbohydrate units have different numbers of branching points and, hence, different numbers of polysaccharide chains. The data derived from the investigation of such cores fit several structures probably because mixtures of carbohydrate units were studied. The terminal polysaccharides of these units have a constant structure. For example, in many globular glycoproteins including α_1-acid glycoprotein, the carbohydrate units have terminal trisaccharides of the following sequence: NANA ($2 \rightarrow 2, 3, 4, 6$) Gal β ($1 \rightarrow 4$) GlcNAc. In addition, certain polysaccharide branches terminate in GlcNAc (3). The peripheral heterogeneity is found in blood group substances. The core of these compounds has a constant sequence. However, the termini vary because of the presence or absence of certain glycosyl transferases. The formation of these enzymes is genetically determined so that, in turn, the different types of blood group substances are also genetically transmitted.

The partial sequence of the carbohydrate moiety of several globular glycoproteins has been determined to a considerable extent (for reviews see Ref. 3, 9). Sialic acid and fucose, as just mentioned, have always been found to occupy terminal positions of the polysaccharide chains. The sialyl residues are O-glycosidically linked to galactose, the penultimate monosaccharide. The different linkages between the sialyl and galactosyl residues (112, 113) of certain glycoproteins, referred to as positional isomerism (3) or primary prostetic allomerism (62), have been suggested to be the cause of the polymorphism of certain glycoproteins (1). The position of galactose was established by periodate oxidation of the desialized glycoprotein and by the use of a β-galactosidase. The third monosaccharide residue of the polysaccharide chains of many globular glycoproteins is N-acetyl-glucosamine which, in turn, is linked to mannose. The latter sugar is bound to the core which consists of further mannose and N-acetyl-glucosamine residues, and which is then attached to the polypeptide chain[2]. While each glycoprotein seems to possess its own unique structural pattern, variations in the branching of the oligosaccharide chains of a single protein frequently exist (2). In view of the different chemical compositions of the carbohydrate units, for example of α_1-acid glycoprotein (114), further progress in the elucidation of their structures can only be achieved if carbohydrate units of well defined sizes and compositions are studied. Such preliminary investigations have been reported by JEANLOZ and KOJIMA (115) and THOMAS and WINZLER (116). Recent progress in this area was summarized by MONTGOMERY (9). The monosaccharide sequence of certain heteropolysaccharides derived from erythrocytes (117), M-immunoglobulin (118), pancreatic ribonuclease (119), thyroglobulin (119a), and transferin (120) have now been elucidated to a large extent. The latest achievement in this area of study represents the complete elucidation of the structure of the heteropolysyccharide of Taka-amylase A reported by YAMAGUCHI, IKENAKA and MATSUSHIMA (75).

Secondary and Tertiary Structure

The conformations of glycoproteins have been investigated to a small extent and their tertiary structures very little. Recent studies by GREENFIELD and FASMAN (121) demonstrated that the three classes of conformations (helices, pleated-sheets and random structure) of at least certain proteins can now be determined relatively

[2] The hydrolases required to cleave all bonds of the carbohydrate moiety have now been described, and efforts are being made to make them available to the investigators in this area of study. These enzymes are isolated from Jack-bean meal (113a) or *Aspergillus niger* (113b).

accurately using circular dichroism. Yet, the exact secondary structure of no one glycoprotein is known yet. As judged by corresponding investigations of glycopeptides derived from globular glycoproteins, it seems that the carbohydrate units do not contribute significantly to or affect the conformations or the tertiary structures of glycoproteins (121a).

The secondary structures of many glycoproteins reversibly change their conformations when dissolved in polar organic solvents (formation of α-helices) of concentrated urea solutions (random coils) (122). Hence, the conformations of many proteins, for instance, those derived from certain membranes and from brain, when determined in aqueous media, do not represent their state of nature, since these proteins are originally present in a lipophilic milieu.

The precise secondary (123) and tertiary structures (124) of glycoproteins can be determined by X-ray diffraction pattern analysis. While the complete structures of over thirty "simple" proteins have been reported, to date the three-dimensional structure of no glycoprotein has as yet been established, although they are presently under intense investigation (125–127). Nevertheless, certain investigations regarding the tertiary structure have been carried out, such as "mapping" of the surface of the glycoprotein molecule (128). Specific methods are now available for this purpose (42, 129) permitting determination of the "free" (reactive) and "buried" (unreactive) residues of certain amino acids of proteins (for reviews see 42, 86, 87, 129, 130).

Metabolism

The biosynthesis of the carbohydrate moiety of globular glycoproteins, under investigation in many laboratories, has been elucidated essentially by ROSEMAN and his coworkers (108, see also refs. 2, 3, 9, 95, 131, 136–138, 140–143). These investigators were the first who described a large number of the *in-vitro* enzymatic synthesis of the carbohydrate moiety of these and related macromolecules including that of α_1-acid glycoprotein. They isolated several glycosyltransferases that are involved in the biosynthesis of these compounds and demonstrated that the synthesis proceeds in a stepwise manner by attaching one sugar at a time to its acceptor. The specificity of these enzymes for the activated monosaccharides and for their acceptors and the type of the terminal sugars primarily determine the sequence of the polysaccharide units (108). The mechanism of attachment of the first sugars to the nascent polypeptide chain as indicated above (3, 38, 88, 95) has been studied very little, excepting that of galactose to carbohydrate-free basement membranes and collagens (139). The formation of the core of the carbohydrate units has also not been elucidated. On the subcellular level, it has been shown that the biosynthesis of the carbohydrate units takes place after the nascent polypeptide chain has been released from the ribosomes (except for a small amount of N-acetylglucosamine (95, 140) and as it moves along the membranes of the rough and smooth endoplasmic reticulum (2, 95, 131, 141). Whereas the synthesis of the polypeptide chain is under strict genetic control, the postribosomal synthesis of the carbohydrate moiety is not. However, indirect genetic control, i.e. genetic control of the synthesis of the glycosyltransferases, has become apparent, especially from the study of the blood group substances (131, 138, 144). Another example of the indirect control of the biosynthesis of carbohydrates was revealed by the study of the polymorphism of α_1-acid glycoprotein. The polymorphic patterns of individual α_1-acid glycoprotein preparations may differ from each other suggesting that certain individuals may lack one of the sialyltransferases (143). It should be noted that the biosynthesis of certain glycosaminoglycans has already been elucidated to a considerable extent (132, 134, 144).

Information on the catabolism of proteins and glycoproteins has only very recently been forthcoming. The degradation of both the polypeptide chain and carbohydrate units of proteins and glycoproteins seems to reside with the lysosomes. The various lysosomal proteases and glycosidases effecting this important role were first reported by MAHADEON *et al.* (145) and summarized by ROTHSCHILD and WALDMAN (146) and PATEL and TAPPEL (147). Of particular interest is the observation that desialized glycoproteins are catabolyzed at a very much higher rate. For example, the half-life of desialized α_1-acid glycoprotein was reported to be decreased from 5.5 days of the native protein to 2 minutes of the desialized protein (148, 149).

Considerations Related to Genetics

The genetic aspects of the variants of plasma proteins have been reviewed earlier by ROBSON and HARRIS (150), and the author (1) and more recently by EPSTEIN (151), HEREMANS (62) and others (54, 84). It may be concluded that probably all plasma proteins are present as variant proteins. Usually, apparently only one amino acid residue is replaced by another due to a single point mutation. Such amino acid replacements may result in a change in the electrostatic net charge and, hence, in the electrophoretic heterogeneity (62). A typical example of this class of proteins are the hemoglobins (84). In contrast to this observation are the well known multiamino acid substitutions of the immunoglobulins resulting in polymorphism and, hence, in a large number of bands on electrophoresis (54, 152, 153).

With the knowledge of the primary structures of proteins (84), it is now possible to correlate some of these biopolymers with each other based on their structural homologies. The evolution from the ancestral immunoglobulin to the L and H chains of the present-day immunoglobulins (15, 154) and the formation of the α-

chain of haptoglobin illustrate this point (155). Partial duplications of the amino acid sequences of certain proteins, reflecting partial gene duplication, represent a probable mechanism of elongating the polypeptide chain during evolution (156, 157).

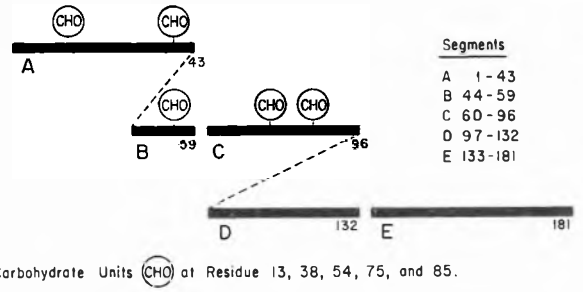

Fig. 1. Schematic presentation of the linear amino acid sequence of α_1-acid glycoprotein. For the sake of clarity, some of the amino acid residues are denoted by arabic numbers and the polypeptide sections by capital letters. The carbohydrate units are designated with S, and their positions on the polypeptide chains are also indicated. For the description of the relationship of α_1-acid glycoprotein with the immunoglobulins, see text

Recent studies on α_1-acid glycoprotein are of particular interest with regard to the evolution of proteins and homologies with other proteins. The elucidation of the amino acid sequence of this human plasma α_1-globulin revealed a relatively large series of amino acid substitutions reminiscent of the immunoglobulins (158). Analysis of the linear amino acid sequence of α_1-acid glycoprotein for internal homologies demonstrated two partial sequence duplications (segment *B* was found to be homologous with the carboxyl-terminal section of segment *A*, and segment *D* homologous with segment *C*) (Fig. 1). Comparison of the amino acid sequence of this glycoprotein with that of other plasma proteins demonstrated the following striking findings. The amino-terminal segment (*A*) consisting of 43 residues is homologous with the amino-terminus of the *K*-type L chain of human IgG, the direct identity being 28% and the total homology 68%. No gaps were required to obtain this alignment. The carboxyl-terminal segment (*E*) consisting of 49 residues was found to be homologous with a constant region of the H chain of rabbit IgG (158), the direct identity was 27% and the total homology 75%. A third homologous segment was found in segment *D*. The carboxyl-terminal 22-residue section of this segment showed a very high direct homology of 48% with the α-chain of haptoglobin (158). α_1-Acid glycoprotein thus appears to be the first protein that is homologous with two other plasma proteins. Moreover, if the two partial sequence duplications and the "haptoglobin" segment were deleted from the linear amino acid sequence of α_1-acid glycoprotein, a "reduced" sequence of approximately 110 residues would result (Fig. 2). As shown above, the amino-terminal half of the "reduced" α_1-acid glycoprotein is homologous with the variable region and the carboxyl-terminal half homologous with the constant region of the γ-G-immunoglobulin. From these findings it may be speculated that α_1-acid glycoprotein must have evolved directly from the primordial immunoglobulin or, in other words, this study (159) appears

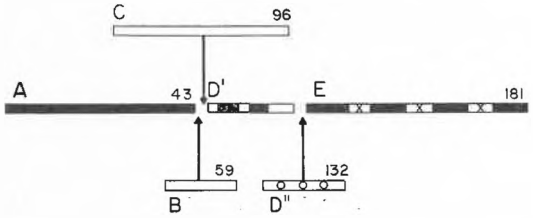

Fig. 2. Schematic presentation of the "reduced" amino acid sequence of α_1-acid glycoprotein. The two partial sequence duplications, segments *B* and *D*, and the "haptoglobin section" of segment *C* are boxed in. The "reduced" sequence consists of segment *A*, which is homologous with the variable region of the L chain of IgG, segment *E*, which is homologous with the constant region of the H chain of IgG, and part of segment *D* (*D'*), and thus is comprised of approximately 110 residues, equal to that of the hypothetical ancestral immunoglobulin

to have led to the discovery of the hypothetical ancestral L chain (154) now disguised with five carbohydrate units and the additional peptide segments in α_1-acid glycoprotein.

The Biological Significance of the Carbohydrate Moiety

The significance of the carbohydrate units of most glycoproteins, especially those of plasma, has remained a puzzle. It has been known for some time, however, that the activity of certain hormones (FSH and HCG) (160), the vitamine B_{12}-binding protein and certain blood-group types of the red cells and the viscosity of mucins depends on the presence of sialyl residues in these macromolecules. Further, the sialyl residues of certain high molecular weight glycoproteins are responsible for the inhibition of the influenza virus hemagglutination. Removal of sialic acid from some of the components of the clotting mechanism brings about a change in the kinetics of clotting. Partially desialized proteins are rapidly removed from the circulation (148, 149), their half-life being reduced to a fraction of that of the original glycoproteins. As yet, no studies have been reported on the effect of the penultimate monosaccharide, galactose, on the biological activity of glycoproteins. However, it should be noted that enzymatic removal of a large part of the carbohydrate moiety from a series of glycoproteins does not appear to directly affect their functions (161). Hence, since there is little more known about the significance of the carbohydrate moiety of the globular glycoproteins (for very recent findings on other glycoproteins see below), it has been proposed that the "non-essential" carbohydrate plays a "supportive" role, in that it helps the polypeptide chain to assume the cor-

rect three-dimensional structure, a preprequisite for performing the biological functions of these glycoproteins (2, 3). It is also conceivable, however, that some of the globular proteins, that possess the necessary structural requirements including the tripeptide:

$$-\text{Asn}-\text{X}-\overset{\text{Ser}}{\underset{\text{Thr}}{}}-,$$

exist as glycoproteins simply because of the presence of cellular transferases which attach carbohydrates to proteins, "rather than because of any functional usefulness in having the covalently bound carbohydrate moiety" (88). This concept is in contrast to the earlier hypothesis of EYLAR (91) according to which the carbohydrate moiety of plasma glycoproteins is required for the passage of these proteins through the cell membrane. Indeed, albumin which accounts for over 50% of the weight of the plasma proteins and is devoid of sugar, is readily secreted into the cirulation, thus supporting the recent theory. Furthermore, the glycoproteins as well as the nonconjugated proteins, when completely synthesized in the cell, are collected in the secretory granules that are derived from the GOLGI apparatus, moved in these "bags" through the cell membrane and are then released into the circulation. That the problems pertaining to the secretion of proteins by the cell are not yet completely resolved, is indicated by a more recent report suggesting that the carbohydrate moiety of the immunoglobulins may play a role in their secretion from the cell that produces these proteins (162), although according to the current concepts the carbohydrate units do not act as recognition signals for export.

Very important, however, are the recent studies on the carbohydrate moiety of glycoproteins derived from certain cell membranes (33, 34, 163–166) revealing that here it is the carbohydrate units which are the functional part of the macromolecules. Such carbohydrate units act, for example, as transplant antigens, others as recognition signals for (identical) cells. The carbohydrate composition of the constituents of the cell surface undergoes significant changes when cells are transformed into malignant ones (163–166). With these fundamental discoveries the study of glycoproteins has received a new impetus.

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