Correlation of Glycosylation Forms with Position in Amino Acid Sequence

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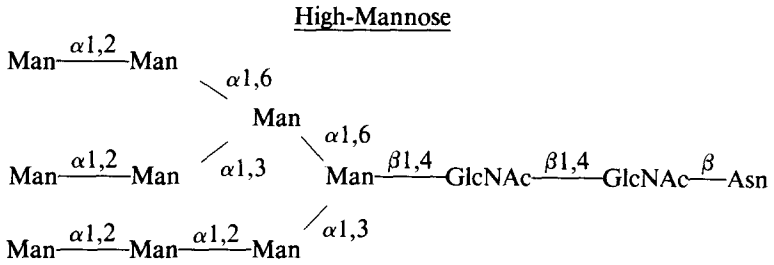
ABSTRACT We surveyed published reports on about 50 glycoproteins whose amino acid sequence, glycosylation sites, and type of glycosylation at a particular site have been established. We note that high-mannose substances were rarely found at the *N*-terminal side of a previously glycosylated complex site. There was a very definite distribution of complex sites about the *N*-terminal region. Furthermore, secreted glycoproteins usually contained only complex oligosaccharides whereas membrane proteins contained both types. We suggest that the position of the glycosylation site with respect to the *N*-terminus affects the extent of oligosaccharide processing and subsequent presentation of complex or high-mannose structures in the mature glycoprotein. This review relates glycosylation type to its position in the known sequence of given proteins and discusses these observations in light of known glycosylation processing reactions.

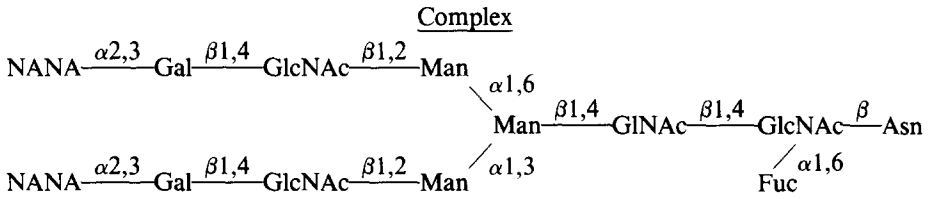
Although the pathways and reactions leading to the formation of asparagine-linked carbohydrates have been described in detail (71), regulation of the expression of a particular form of glycosylation at the cell surface or in other mature forms such as viruses is not understood. Regulatory effects are apparent in the glycoproteins of Sindbis, vesicular stomatitis, and influenza virus because exact structure depends upon the type of host cell (24, 39, 109). It was also found that in ovalbumin purified from different individual hens there were differences in the proportions of the five major glycopeptide classes characteristic of ovalbumin. These proportions were constant in a series of eggs from the same individual but were different between individuals (75). These differences may be partially explained by observations that the structure and conformation of the protein backbone are important in the final expressed form of oligosaccharide (109, 134, 169). It is also significant that Sindbis virus glycoproteins E1 and E2 are translated in the same intracellular compartment from a single 26S mRNA (31), yet they exhibit distinctly different forms of glycosylation (60), presumably because of differences in protein folding. In addition, physiological status may play an important role because the frequency of occurrence of particular oligosaccharide structures is seen to be growth dependent. This is illustrated by density-inhibited cells, which process oligosaccharides more completely (1), possibly a result of a novel α-mannosidase that appears in confluent cells (173).

The two major classes of asparagine-linked oligosaccharides are the high-mannose and complex types:[1]



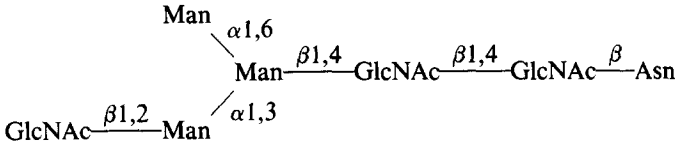
(e.g., in thyroglobulin $M_9GlcNAc_2Asn$).

[1] *Abbreviations used in this paper:* Man, mannose; GlcNAc, *N*-acetylglucosamine; NANA, *N*-acetylneuraminic acid; and Gal, galactose.



(e.g., in Sindbis virus).

There are a large number of different high-mannose oligosaccharides that in mature form contain four or more mannose groups, in various polymers, linked to dichitobiose. Their synthetic precursors, in animal cells and yeast, contain three glucose and nine mannose residues. In animal cells, the final products of these polymers have no more than nine mannoses, but in yeast they may be much larger (168). Complex oligosaccharides contain various numbers of other sugars, i.e., fucose, *N*-acetylglucosamine, galactose, and sialic acid linked to a core of three mannoses, including a β1,4 linked mannose, 3-0- and 6-0-substituted with α-mannose, and dichitobiose. Complex types also contain varying numbers of branched arms termed bi-, tri-, or tetra-antennary, where substitutions with β-GlcNAc occur on the α(1,3)- or α(1,6)-linked mannoses of the core. Hybrid structures (25) also exist, e.g., that of rhodopsin glycopeptide (50; for review see reference 105):



Thus some oligosaccharides can be of the high-mannose type with respect to composition but are actually completely processed by mannosidases as are complex-type molecules. Further processing to complex substances does not occur. It follows that because these glycosyltransferases act posttranslationally, there must be some additional structural aspect of a protein that specifies its oligosaccharide. In this regard, it has been suggested (7, 108, 134, 163) that the local environment surrounding the asparagine determines the accessibility of the processing enzymes and therefore the final form of the oligosaccharides.

Delineation of glycoprotein amino acid sequence and oligosaccharide structure has facilitated increasingly detailed correlations between protein primary and secondary structure and glycosylation. Sites of *N*-linked carbohydrate attachment can be predicted because the sequence Asn-X-Ser(Thr) has been shown to be a necessary but not sufficient condition for glycosylation (40, 73, 97, 112, 113, 147). Such a sequence, however, does not always result in glycosylation (e.g., ovalbumin [70, 100]); thus other factors must apply. More recently, reverse B-turns have been correlated with glycosylation of this tripeptide (2–4, 14, 107). There is currently no definitive explanation as to the regulation of expression of the numerous asparagine-linked oligosaccharide structures that have been reported.

In this paper, we review the available information on oligosaccharide type, i.e., complex or high-mannose, with regard to its position in the amino acid chain. We note that, in general, complex oligosaccharides are located towards the amino terminus of the polypeptide and that high-mannose types are located farther towards the carboxyl terminus. Processing (see reference 71 for review) is more complete towards the *N*-terminus. We also note that serum glycoproteins usually contain complex structures only and that membrane glycoproteins contain both types.

GENERAL OBSERVATIONS

Fig. 1 depicts the results of a literature search in which the type of glycosylation was compared with the location of the glycosylation site. Generally, complex oligosaccharides appear near amino termini and high-mannose structures are farther downstream towards carboxyl termini. The 105 complex sites are located at a mean amino acid site 109.1 ± 115.2 (standard deviation [SD]) residues from the amino terminus and the 24 high-mannose sites are located at a mean residue 224 ± 174.4 (SD) amino acids from the *N*-terminus. These means are significantly different in the Student's *t* test ($P < 0.01$). The different distributions of complex and high-mannose structures on glycoproteins in general are clearly seen in Fig. 2, in which the percentages of the two oligosaccharide types falling within 100 amino acids of the amino terminus, between 100 and 199 amino acids, and greater than 199 amino acids from the *N*-terminus are graphed. The ratio of the percentage of high-mannose to complex oligosaccharides at 100–199 amino acids from the *N*-terminus (0.66 or 12.5% ÷ 19%) is higher than at sites within 100 amino acids from it (0.51 or 33.3% ÷ 65.7%). This ratio is much higher at sites farther than 199 amino acids from the *N*-terminus (3.56 or 54.2% ÷ 15.2%), i.e., the farther an attachment site is from the amino terminus, the greater the likelihood that it will contain a high-mannose structure.

Of the 54 glycoproteins represented in Figs. 1 and 2, 15 contain both complex and high-mannose oligosaccharide types. This subset of glycoproteins shows an asymmetric distribution of oligosaccharides (Fig. 3) which is similar to that of glycoproteins in general (Fig. 2). In 12 of the 15 glycoproteins that contain both oligosaccharide types, the complex oligosaccharide is on the amino terminal side of the oligomannosyl structure. This 80% fit is significant in the Chi-squared test ($P < 0.05$).

Membrane Glycoproteins

Membrane glycoproteins often contained both complex and high-mannose glycosylation sites (Fig. 1, Table I) and, in general, the latter were located with a greater frequency toward the C-terminus. Semliki Forest virus precursor P62 is posttranslationally cleaved to E2 and E3 (30, 146). E3, the amino terminal portion of the polypeptide, contains one complex

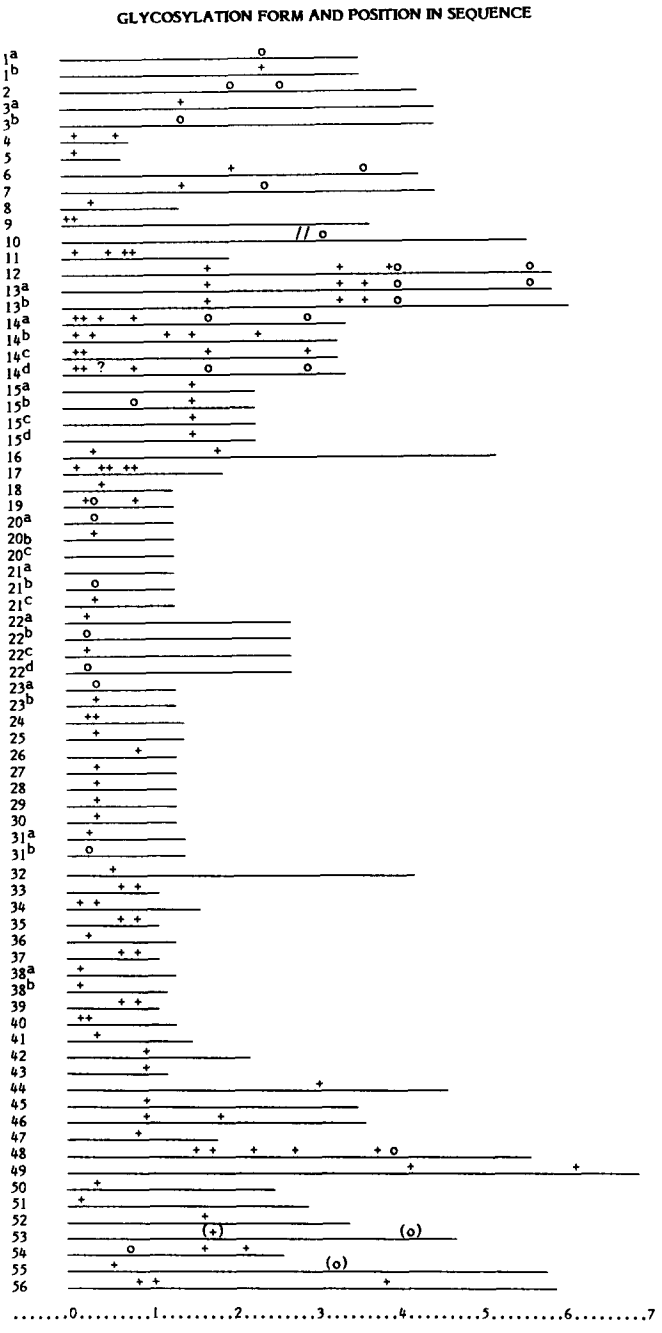


FIGURE 1 Glycosylation form and position in sequence. Each number represents an individual glycoprotein and corresponds to the same number in Table I (wherein the sequence and glycosylation parameters of the glycoprotein are described). The length of the line next to each number represents the number of amino acids in the glycoprotein. Each plus (+) represents the location of a complex oligosaccharide on the glycoprotein and each circle (O) represents an high-mannose structure. Parentheses surrounding on oligosaccharide indicate that the location of the glycosylation site(s) is within the parentheses but has not been precisely determined.

oligosaccharide (48, 98, 119). Its carboxyl portion, which forms E2, has two glycosylation sites and contains predominantly high-mannose oligosaccharides (48, 98, 119). Sindbis virus E3 also contains the complex forms (60). Sindbis virus E2 has a complex oligosaccharide at amino acid number 196 and a high-mannose oligosaccharide at 358 (24). Sindbis E1 has a complex structure at 138 and a high-mannose structure

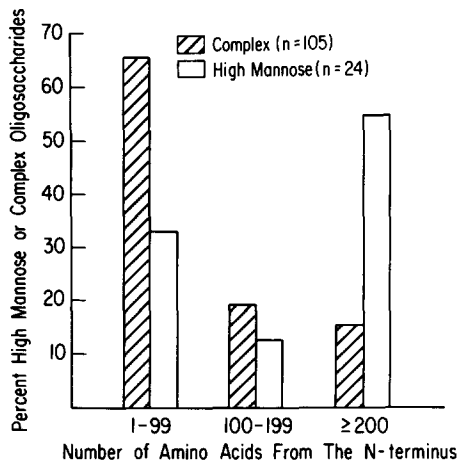


FIGURE 2 Location of complex and high-mannose oligosaccharides on glycoproteins in general. Complex and high-mannose oligosaccharides are separately graphed according to the percentages of their oligosaccharide attachment sites falling within 100 amino acids of their amino termini, between 100 and 199 amino acids, and at greater than 199 amino acids from their amino acid termini. Except for glycosylation sites containing hybrid oligosaccharides, all glycoproteins described in Fig. 1 and Table I are included.

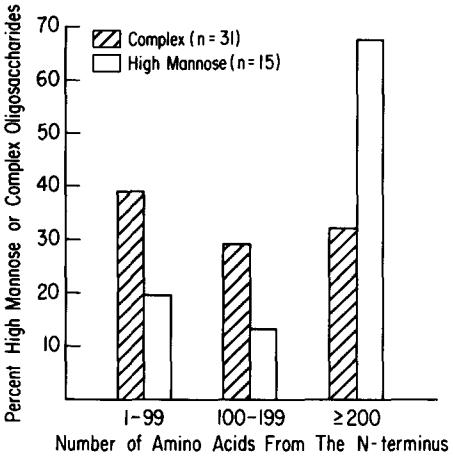


FIGURE 3 Location of glycosylation sites on the subset of glycoproteins containing both complex and high-mannose oligosaccharides. Same as Fig. 2, except that only glycoproteins containing both oligosaccharide types are shown. Includes SbV-E3 and SFV-P62.

at 244 (24). The membrane-bound form of mouse MOPC IgM contains three complex oligosaccharides at amino acids 171, 332, and 394, and a high-mannose oligosaccharide at number 402 (79). Influenza virus HA1 has six oligosaccharides which in both A/Memphis 72 (163) and A/Aichi/1/68(X-31) (162) are located at identical sites. Although there is still some doubt as to the exact structure of the oligosaccharide at amino acid 38 in X-31, in the HA1 of both strains its two high-mannose structures are located farther toward the carboxyl terminus than its four complex oligosaccharides. The amino terminal two-thirds of Rauscher Murine Leukemia Virus gp70 contains only complex oligosaccharide(s) (64, 81, 82). Since gp70 also contains high-mannose oligosaccharides, these structures must be located in its carboxyl terminus (81, 82). Examination of the gp70 amino acid sequence (143) confirms the presence of many potential glycosylation sites in the C-terminal part of gp70. The Fc fragment of the heavy

TABLE I

Sequence and Glycosylation Parameters

No.	Glycoprotein	Amino acid number		Amino acids in polypeptide	References
		Complex site	Hi-man site		
1a.	Ovalbumin	239°		346	70, 100
1b.	Ovalbumin		239°	346	70, 100
2.	SFV-E2		200, 262	422	48, 98, 119*
3a.	SFV-E1	141		438	48, 98, 119*
3b.	SFV-E1		141	438	48, 98, 119*
4.	SFV-E3	13, 60		66	48, 98, 119*
5.	SbV-E3	14		64	59, 129
6.	SbV-E2	196	358	423	24
7.	SbV-E1	138	244	439	23
8.	Glycophorin A	26		131	155, 166
9.	Bov. Rhodopsin	2, 14		360	45, 61
10.	procollagen (C-terminal)		11	250	118, 144
11.	ovomucoid	10, 53, 69, 75		186	14
12.	IgM(Waldenstroms)	170, 332, 395	402, 563	576	125, 141
13.	IgM(MOPC) 104E				
	Soluble form	170, 332, 364	402, 563	576	79, 80
	Membrane form	170, 332, 364	402	597	79, 80
14.	Influenza HA1				
	Memphis	8, 22, 38, 81	165, 285	328	163
	FPV	12, 28, 123, 149, 231		319	123, 84
	Japan/305/57	11, 23, 168, 285		324	51, 109, 164
	A/Aichi/2/68/(x-31)	8, 22, 81, 38?	165, 285, 38?	328	160, 162
15.	Influenza HA2				
	Memphis	154		221	163
	Fowl Plague	154	82	221	123, 84
	A/Japan/305/57	154		222	51, 109, 164
	A/Aichi/2/68/O31	154		221	160, 162
16.	VSV G protein	33, 178		511	39, 133
17.	a1-acid glycoprotein	15, 38, 54, 75, 85		181	44, 137, 138, 167
18.	a-lactalbumin	45		123	22, 68
19.	Porcine RNAase	21, 76	34	124	76, 77
20.	Bovine RNAase				
	RNAase B		34	124	67, 122
	RNAase C and D	34		124	67, 121
	RNAase A	—	—	124	67, 121
21.	Ovine RNAase				
	RNAase A			124	13, 85
	RNAase B		34	124	13, 85
	RNAase C	34		124	13, 85
22.	Deoxy-RNAase				
	DNAase A	18		257	91, 135
	DNAase B		18	257	91, 135
	DNAase C	18		257	91, 135
	DNAase D		18	257	90, 91, 135
23.	Chinchilla RNAase				
	Type 1		34	124	15
	Type 2	34		124	15
24.	Guinea pig RNAase	21, 34		128	15
25.	Coypu RNAase	34		128	15
26.	Lesser Rorqual B RNAase	76		?	15, 38
27.	Giraffe RNAase	34		124	47
28.	Okapi RNAase	34		?	15
29.	Moose RNAase	34		?	15
30.	Horse RNAase	34, 62		124	136‡
31a.	Avidin	17°		128	36, 69
31b.	Avidin		17°	128	36, 69
32.	Fibrinogen Y	52		410	65, 156
33.	HCG (alpha)	56, 82		96	120
34.	HCG (beta)	13, 30		147	120
35.	TSH (alpha)	56, 82		96	120
36.	TSH (beta)	23		119	120
37.	LH (alpha)	56, 82		96	120

TABLE I

Sequence and Glycosylation Parameters

No.	Glycoprotein	Amino acid number		Amino acids in polypeptide	References
		Complex site	Hi-man site		
38.	LH (beta)				
	porcine, ovine, bovine	13		121	120
	human	13		116	120
39.	FSH (alpha)	56, 81		96	120
40.	FSH (beta)	7, 24		120	120
41.	Bence Jones SM-light chain)	25		135	26, 49
42.	Bence Jones NEI-light-chain	93		214	50
43.	Bence Jones Wh-V region	94		107	83
44.	IgG	297		446	37, 87
45.	HLA-B7	86		336	116, 117
46.	H2-K^b	86, 176		346	32, 111
47.	Interferon (IFN-B)	80		166	20, 151
48.	IgE(Heavy chain)	145, 173, 219, 265, 371	394	547	18
49.	Human transferrin	414, 608		676	58, 103
50.	MOPC-46K-Kappa	28		~240	101, 102
51.	Myelin P O protein	14		~280	74
52.	MOPC 47A	155		330	131
53.	IgD (mouse, membrane-bound)	In *N*-terminal two thirds	C-terminal third	~460	56
54.	IgD Fc fragment (human soluble, C-ter.)	159, 210	68	226	142, 92‡
55.	MuLV gp70	45, possibly others in C-ter.	In C-ter. only		64, 81, 82, 143
56.	bovine prothrombin	77, 101, 376		582	96, 104

Results of a literature search in which the type of glycosylation was compared with the location of the glycosylation site. The number of amino acids from the *N*-terminus of each complex or high-mannose asparagine-linked oligosaccharide is listed for each polypeptide. Proteins for which only partial information was available, i.e., amino acid sequence data or carbohydrate analysis alone, are not shown.

* Denotes Atkinson et al., unpublished observations.

‡ Denotes Scott Mellis and Jaques Baenziger, personal communication; and *o* denotes a hybrid structure.

chain of murine cell surface IgD binds only partially to lentil lectin but binds completely to concanavalin A. In contrast, the Fab and Fab' fragments bind completely to both columns (56). The binding specificity of these lectins (86, 110) indicates that complex biantennary oligosaccharides are located in the Fab and Fab' fragments (amino terminus) and that high-mannose oligosaccharides are in the Fc portion (C-terminus).

The only exception observed for a membrane protein is Fowl Plague virus HA2. It has a high-mannose oligosaccharide at amino acid 82 and a complex oligosaccharide at 154 (84). In some cases, HA is apparently not cleaved intracellularly. Cleavage is dependent on the host cell and is also strain-dependent (19, 150). When uncleaved HA is cleaved in vitro with trypsin, the HA1s in some strains contain both oligosaccharide types and the corresponding HA2s contain complex oligosaccharides (109, 139). Since HA1 contains the amino terminus of HA and since HA2 contains HAs carboxyl terminus, this is seemingly an exception to the general order of appearance of high-mannose and complex oligosaccharides. However, it is difficult to electrophoretically separate in vivo cleaved HA1 and HA2, even in the presence of SDS and a reducing agent (63). Although cleavage in vivo was not detected (109, 139), it is not certain that the electrophoretic conditions used in these studies would separate in vivo cleaved HA1 from HA2. It is therefore possible that at least some HA had been cleaved prior to the in vitro trypsinization. Nonetheless, the structure of HA2 has been demonstrated to affect the extent of processing on HA1 in A/Udorn/72 (H3N2): deletions were introduced near the carboxyl terminus of HA2 and the mutant HA was expressed using an simian virus 40–HA construct. Whereas the wild-type HA contained high-mannose oligosaccharides, the mutant (169), which would normally be located in HA1 of the H3N2 serotype (139), did not. The env protein (G2) of Rous Sarcoma virus has both complex and high-mannose oligosaccharides in a ratio of 3:1 and probably has ten complex and three high-mannose structures (88), though the sequence distribution of its glycosylation sites is not known. Likewise, HA of Influenza virus A/USSR has three complex and two high-mannose tryptic peptides of known composition (11) though position in the sequence (33) is not known. Fitting the peptides to the appropriate sites, as was done with Sindbis virus (129), would seem an elegant way in which to obtain this information for these cases and in general.

Soluble Glycoproteins

Most glycoproteins found in serum contain only complex oligosaccharides (Table I). In ovomucoid, α-1 acid glycoprotein, α-lactalbumin, avidin (which probably has a rhodopsin-like structure based on its sugar composition [69]), fibrinogen, human chorionic gonadotropin, thyroid-stimulating hormone, porcine luteinizing hormone, interferon, ceruloplasmin, the PO protein from myelin, α-1 microglobulin, *inter alia*, one or more complex oligosaccharides are located within 100 amino acids of the amino terminus (Table I). Human transferrin is an exception, having complex structures at amino acids 414 and 608, as is prothrombin (96, 104) which contains complex structures at amino acids 77, 101, and 376. Bovine lactotransferrin, however, contains high-mannose oligosaccharides, although their locations in the glycoprotein are not known (159).

Two other soluble glycoproteins also contain high mannose oligosaccharides. The oligosaccharide of procollagen is located at residue 180 of its 250 amino acid carboxyl propeptide. Ovalbumin's oligosaccharide is at amino acid 239. These high-mannose sites are located markedly farther from the *N*-terminus than the glycosylation sites of most soluble glycoproteins.

The soluble forms of the epsilon, mu, and delta immunoglobulin chains contain both complex and simple carbohydrates. The epsilon chain contains five complex and one high-mannose oligosaccharide per chain (6, 7). Of these, the high-mannose structure is closest to its carboxyl terminus (18). The soluble chains of both human (Waldenstrom) and mouse (MOPC 104E) IgM have a similar arrangement; i.e., three complex oligosaccharides towards the amino terminus and two high-mannose structures farther towards the carboxyl terminus. It is remarkable that the site at 563 on the 572 amino acid IgM heavy chain is glycosylated at all since a minimum of 30 amino acids must be added beyond an acceptor site for glycosylation to occur during translation (54). Soluble forms of gamma and alpha chains contain only complex oligosaccharides (6–8, 93, 95). However, Dawson and Clamp (34) concluded that a glycopeptide from an IgA myeloma contained only mannose and glucosamine and was contaminated with galactose and fucose. Low et al. (95) found that the oligosaccharide of IgA closest to its carboxyl terminus has a sugar composition similar to that of the glycopeptide studied by Dawson and Clamp (34). Its mannose to glucosamine ratio (1.58:1) is considerably higher than those of two other *N*-linked oligosaccharides in IgA (0.69:1 and 0.79:1). It is therefore possible that this substance is actually high-mannose or hybrid type.

The Fc fragment of soluble human IgD delta chain has three glycosylation sites (92, 142). It is an exception to the usual locations of high-mannose and complex oligosaccharides since the site at residue 68 is high-mannose and those at 159 and 210 are complex. Furthermore, the glycosylation of the high-mannose site is unusual because ~20% of its oligosaccharides are glucosylated (Scott Mellis and Jaques Baenziger, personal communications). IgD is also exceptional in that the two primary translation products of the human delta chain (which correspond to membrane and secreted forms of IgD) are differentially *N*-glycosylated to four discrete forms (99). There is evidence that glycosylation protects against proteolysis (27–29, 42, 161). The exquisite protease sensitivity of IgD (148) shows an inability of glycosylation to exert this effect. It is also notable that although secretion of murine IgM and IgE is inhibited by tunicamycin, that of IgD and IgG, which is glycosylated at only one site, is not inhibited (145).

Pancreatic ribonucleases and deoxyribonucleases are exceptions to the glycosylation patterns usually observed for soluble glycoproteins. They can have high-mannose structures near their amino termini and they can also have a complex oligosaccharide following a high-mannose. Porcine ribonuclease has a high-mannose oligosaccharide at amino acid 34 and this is followed at number 76 by a complex oligosaccharide. Ribonucleases from other species are generally glycosylated at amino acid 34 only, although horse ribonuclease has complex oligosaccharides at 21, 34, and 62, guinea pig at 21 and 34, and lesser rorqual at 76 only (15). The oligosaccharide at 34 may be complex (giraffe, okapi, moose, and coypu), either complex or high-mannose (chinchilla, sheep, and cow), or can occur with or without the oligosaccharide (topi, cow, sheep, goat, and roe deer). Deoxyribonuclease is similar; it also exhibits either high-mannose or complex oligosaccharides at its single glycosylation site, amino acid 18 (90). It may be difficult for such short molecules to achieve sufficient folding for processing to be interfered with in the manner in which it appears to occur in many other glycoproteins.

Although microheterogeneity within an oligosaccharide type is common (for review see reference 149) partial glycosylation and switching between oligosaccharide types at a specific glycosylation site is rarer, though in Semliki Forest virus E1 this does occur (P. Atkinson, unpublished observations). Bovine ribonuclease C and D (both glycosylated with complex oligosaccharides) and A (unglycosylated form) have half-lives in nephrectomized rats of at least 8 h, whereas ribonuclease B in the high-mannose form is cleared in 15 min from serum (12) and it is possible that this form is not physiologically relevant.

DISCUSSION

Oligosaccharides are processed by a well defined pathway (for details, exceptions, and references, see reference 71). Briefly, precursor oligosaccharides (Glc_3 Man_9 $GlcNAc_2$) are co-translationally transferred to each glycosylation site from a lipid intermediate. Glucosidases, located in the rough and smooth endoplasmic reticulum, rapidly alter the newly synthesized protein to form a $Man_9GlcNAc_2$ structure. Some or all of the four outermost mannose residues are then cleaved by membrane-bound mannosidases to produce the high-mannose series of oligosaccharides ($Man_{5-9}GlcNAc_2$). Several mannosidases are in or near the Golgi apparatus (126, 127, 153); others may be in the rough endoplasmic reticulum (55). $Man_5GlcNAc_2$ can be converted to complex oligosaccharides by the addition of a single GlcNAc to the exposed "core" $\alpha(1,3)$-linked mannose residue and the subsequent rapid release of the two terminal noncore mannose residues by the enzyme "late mannosidase" (62). The trimmed GlcNAc-$Man_5GlcNAc_2$ structure can then be further processed by the addition of fucose to the innermost GlcNAc residue and by additions of various amounts of GlcNAc, galactose, sialic acid, and fucose to the outer residues. These latter reactions all take place in or near the Golgi apparatus (16, 21, 115). Inasmuch as there four $\alpha(1,2)$-linked mannoses and two other α-mannoses are removed during processing to complex carbohydrates, there are quite conceivably six different mannosidases. As an example of site specificity of $\alpha(1,2)$-mannosidase, yeast contains an α-mannosidase which will cleave one specific α-mannose from precursor $Man_9GlcNAc_2$, leaving three others intact (171). Tabas and Kornfeld (153) and Tulsiani et al. (157) have purified several α-mannosidases from rat liver Golgi fractions although it is possible that there are other α-mannosidases in the rough endoplasmic reticulum. Forsee and Schutzbach (43) have purified an α-mannosidase from rabbit liver microsomes which has a substrate specificity similar to but a pH optimum lower than that of the Golgi enzymes. Furthermore, pulse-chase studies using carbonyl cyanide m-chlorophenylhydrazone, an inhibitor of intracellular protein transport, suggest that an α-mannosidase in the rough endoplasmic reticulum removes one of the terminal α-mannose residues of thyroglobulin $Man_9GlcNAc_2Asn$ (see above for its structure [55]). Our current studies show that nascent chain vesicular stomatis virus-G protein contains oligosaccharides processed to $Man_8GlcNAc$ (Atkinson, manuscript in preparation). The number and exact subcellular site of α-mannosidases remain to be precisely elucidated. Such studies may be complicated by the fact that

density inhibition of cell growth favors the formation of more processed structures (60), and that a possible induction of new enzymes (173), which may be an important regulatory event, may occur.

Present evidence indicates that the protein primary structure can determine whether complex or high-mannose oligosaccharides will occur (7, 109, 134, 163, 169), and protein conformation affects processing of high-mannose oligosaccharide in yeast carboxypeptidase Y and invertase (170). Carbohydrate is necessary for vesicular stomatis virus–G protein to attain a proper conformation (52, 53). It is also necessary in the stability of yeast invertase (28, 29), carboxypeptidase Y (27), alkaline phosphatase (42), porcine ribonuclease (161), and interferon (46), for clearance of ribonuclease B (12), and in conformational changes which can affect the antigenicity of Semliki Forest virus glycoproteins (78). Since glycosylation does seem to affect conformation, it is possible that the addition of each oligosaccharide to a nascent chain increases the likelihood that the next glycosylation site will mature with a high-mannose structure. It seems likely that the final oligosaccharide form depends on steric hindrance since at least some glycosyltransferases act after folding of the protein has taken place (2). Indeed, x-ray crystallographic analysis of influenza HA (165) shows that, although both types of oligosaccharide are either at or near Ha's surface, the glycosylation sites of the complex oligosaccharides appear to be more exposed than those of the high-mannose structures. A physical explanation of why processing occurs more towards the *N*-terminus may be that the amino terminal sites are translated first or because the *C*-terminal sites are often closer to the membrane (94), where the *C*-terminal is buried.

The function of glycoprotein *N*-linked oligosaccharides and their asymmetric distributions remains to be demonstrated. The tissue specificity of the asialoglycoprotein receptor (12, 35, 106, 132) indicates that complex-type glycopeptides may be important in targeting these glycoproteins to a specific tissue. The exact fine structure in this type of recognition mediated by complex carbohydrates is also important because Baenziger and Fiete (5) have shown that triantennary complex oligosaccharides with three terminal galactose residues are endocytosed by rat hepatocytes, whereas biantennary complex oligosaccharides with one or two terminal galactose residues are not endocytosed. In other manifestations of the effect of glycosylation on function, inhibition of glycosylation of a polyprotein precursor inhibits its cleavage (172). Cleavage of such a polyprotein would also cause the glycosylation site(s) of its *C*-terminal polypeptide(s) to be closer to the amino terminus of the cleaved product. The time of cleavage of cotranslated proteins and their form of glycosylation prior to this cleavage (59) are important parameters to consider in these cases. High-mannose structures have been demonstrated as significant if not major components of *N*-linked oligosaccharides exposed on the surfaces of many cell types (Ceccarini and P. H. Atkinson, unpublished observations) as have complex structures (reviewed in 1). How the various forms of these types of oligosaccharides appear on the cell surface is not directly known, but they are involved in recognition functions in specific adhesion of cells (57, 128, 130) and in development (152). Aside from their involvement in cell surface recognition, high-mannose oligosaccharides are involved in directing lysosomal enzymes to their correct subcellular compartment (for review, see reference 114).

The functional significance, if any, of the different distributions of complex and high-mannose oligosaccharides on their protein backbones is not known. However, if, as current sequence and glycosylation data indicate, asymmetric distributions of oligosaccharide types occur, we believe that this could be a significant determinant as to how oligosaccharides participate in recognition events.

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Notes Added in Proof: The complete amino acid sequence of the heavy chain of human IgD has recently been determined by Takahashi, N., D. Tetatert, B. Debuiere, L.-C. Lin, and S. W. Putnam, (*Proc. Natl. Acad. Sci. USA.* 79:2850–2854, 1982).

Since the manuscript was submitted, we have completed a study using 500 MHz ^{1}H-nuclear magnetic resonance (NMR) spectroscopy on the oligosaccharides of MOPC 104E IgM (Anderson, D. R., P. H. Atkinson, and W. J. Grimes, Major carbohydrate structures at five glycosylation sites on murine IgM determined by high resolution ^{1}H-NMR spectroscopy, manuscript in preparation). We found a biantennary structure at position 171, triantennary structures, two arms of which terminated in galactose, at positions 332 and 364, a less processed triantennary structure terminating in GlcNAc at position 403, and a high-mannose structure at position 563.

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