Structural Features of Microsomal, Synaptosomal, Mitochondrial, and Soluble Glycoproteins of Brain†

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ABSTRACT: This study is concerned with the glycoproteins of brain microsomal subfractions whose morphology, enzyme composition, and distribution and metabolism of glycosaminoglycans and glycoproteins have been described in the previous paper (Kiang, W.-L., et al. (1978) *Biochemistry 17* (preceding paper in this issue)). Glycoproteins have been analyzed in terms of their sugar composition, the relative proportions of two classes of acidic type glycopeptides, and of neutral type glycopeptides (containing only mannose and *N*-acetylglucosamine). The five different O-glycosidically linked oligosaccharides of brain have also been quantitated in the various subfractions. For comparison with the microsomal membrane subfractions we have examined the same glycoprotein structural features in brain mitochondria, synaptic membranes, the synaptosomal soluble glycoproteins, and in soluble and particulate fractions obtained from low density microsomal membranes. The microsomal subfractions of intermediate density were generally very similar to one another with respect to their high concentration of glycoproteins and their glycoprotein composition. According to these criteria the intermediate microsomal subfractions also closely resembled synaptic membranes, with which they shared a high concentration of gangliosides. On the other hand, membrane fractions with a low concentration of glycoproteins (such as mitochondria or microsomal subfraction 5, which is enriched in rough endoplasmic reticulum) have a relatively high proportion of neutral oligosaccharides and a low concentration of O-glycosidically linked oligosaccharide units. The "soluble" glycoproteins associated with microsomal subfraction 1 share certain features with the soluble glycoproteins of whole brain. However, the synaptosomal soluble glycoproteins differ considerably from other soluble fractions in their carbohydrate composition, but resemble synaptic membranes insofar as both have a low proportion of O-glycosidically linked oligosaccharides. The data are discussed with respect to the interrelationships between the various types of membranes and their biosynthetic implications.

An understanding of the structure and distribution of nervous tissue complex carbohydrates is necessary before it will be possible to evaluate their functional roles in relation to such processes as intercellular adhesion, neural histogenesis, brain development, and other problems of neurobiological interest involving various types of cell–cell interactions. Although data have been reported on the concentrations and sugar composition of glycoproteins in various subcellular fractions of brain (Margolis et al., 1975a; Morgan & Gombos, 1976; Churchill et al., 1976; Quarles & Everly, 1977), there is no information available concerning structural features of glycoproteins in purified and characterized preparations of neural membranes or subcellular organelles. Structural information published previously has been exclusively derived from analyses of whole brain.

A general method for the fractionation of glycopeptides has recently been described (Krusius & Finne, 1977), permitting the isolation of four structurally distinct types of glycopeptides or oligosaccharides. These consist of O-glycosidically linked oligosaccharides, two fractions (A and B) of acidic type N-glycosidic glycopeptides, and N-glycosidic glycopeptides of neutral type (fraction C). (For reviews of glycoprotein structure and terminology, see Montreuil, 1975; Kornfeld & Kornfeld, 1976.) The O-glycosidically linked oligosaccharides have been shown (Margolis & Margolis, 1973b; Finne, 1975) to contain Gal(β1→3)GalNAc and its monosialosyl and disialosyl derivatives, while a fifth oligosaccharide, which appears to be present only in brain tissue (Finne & Krusius, 1976), has the structure Gal(α1→3)GalNAc. Although the complete structures of the N-glycosidically linked oligosaccharides are not known, there is considerable information available concerning their sugar sequences and substitution patterns. Thus, the acidic type of carbohydrate unit contains a core composed of mannose and *N*-acetylglucosamine to which varying numbers of sialosylgalactosyl-*N*-acetylglucosamine branches are attached. Fraction A glycopeptides probably contain an average of 3 to 4, and fraction B glycopeptides 2, peripheral branches, while the neutral type glycopeptides present in fraction C contain only mannose and *N*-acetylglucosamine (Krusius & Finne, 1977).

In the present investigation we have studied the carbohydrate composition as well as the amounts of the different types of oligosaccharide units in several subcellular fractions of brain. In addition to the five microsomal subfractions described in the preceding paper (Kiang et al., 1978), we have also carried out comparable studies on mitochondria, synaptosomal membrane and soluble glycoproteins, the soluble glycoproteins from whole brain, and those associated with a low density microsomal subfraction enriched in glycosaminoglycans. The distribution and metabolism of gangliosides in these microsomal and synaptosomal subfractions will be described elsewhere.

Materials and Methods

Subcellular Fractionation. Cerebra of 30- to 40-day-old rats were used for the preparation of all subcellular fractions.

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TABLE I: Glycoprotein Carbohydrate Composition of Microsomal Subfractions.

	fraction number									
	1		2		3		4		5	
mannose	2.45[a]	1.00[b]	3.59	1.00	4.42	1.00	3.90	1.00	3.24	1.00
galactose	2.04	0.83	1.96	0.55	2.43	0.55	2.06	0.53	1.20	0.37
glucosamine	1.81	0.74	2.43	0.68	3.38	0.76	2.49	0.64	1.37	0.42
galactosamine	0.33	0.13	0.27	0.08	0.31	0.07	0.23	0.06	0.16	0.05
fucose	0.74	0.30	1.07	0.30	1.47	0.33	1.38	0.36	0.63	0.19
sialic acid	1.36	0.56	1.86	0.52	2.57	0.58	1.67	0.43	0.70	0.22
total	8.73		11.18		14.58		11.73		7.30	

[a] μmol/100 mg of lipid-free dry weight. Values are the averages from two experiments showing good agreement. [b] Molar ratios.

Previous studies have demonstrated that at this age the concentrations of glycosaminoglycans and glycoproteins are essentially the same as those present in adult brain (Krusius et al., 1974; Margolis et al., 1975b, 1976). Microsomal subfractions were isolated as described in the preceding paper (Kiang et al., 1978), and mitochondria were prepared using a sodium diatrizoate density gradient (Manthorpe et al., 1976).

For the isolation of synaptic membranes, a crude mitochondrial fraction was prepared and washed three times as described by Gurd et al. (1974). This was then lysed by suspending in 5 mM Tris buffer (pH 8.1 at 5 °C) containing 50 μM $CaCl_2$ and allowed to stand in an ice bath for 30 min, after which the suspension was mixed by six up-down strokes in a Teflon–glass homogenizer with a loose-fitting pestle. The lysate was then rapidly made to a concentration of 10% in sucrose and the particulate fraction was obtained by centrifugation for 90 min at 146 000*g*.

The supernatant was dialyzed and saved as the synaptosomal soluble proteins. Although the crude mitochondrial fraction contains myelin and mitochondria in addition to synaptosomes, it has previously been demonstrated that only small amounts of soluble protein are released by lysis of these first two fractions in hypotonic media (Barondes, 1968). Moreover, myelin and mitochondria contain very low concentrations of glycoproteins (Margolis et al., 1975a; Quarles & Everly, 1977) which are apparently firmly bound to the membranes. Since the glycoprotein and ganglioside concentration and composition of the synaptosomal soluble fraction prepared by the method described above are almost identical with that previously reported for soluble glycoproteins and gangliosides obtained by lysis of purified synaptosomes isolated by Ficoll density gradient centrifugation (Margolis et al., 1975a; Ledeen et al., 1976), it would appear that hypotonic lysis of a well-washed crude mitochondrial fraction provides a relatively simple method for obtaining the soluble glycoproteins and gangliosides present in synaptosomes.

The particulate fraction obtained as described above was resuspended in 34% sucrose, and synaptic membranes were isolated by the method of Jones & Matus (1974) with the modification that all solutions contained 50 μM $CaCl_2$ and were buffered with 5 mM Hepes,[1] pH 7.4 (Walters & Matus, 1975).

Analytical Methods. Subcellular fractions were dialyzed, lyophilized, extracted with chloroform–methanol, and the lipid-free protein residues were digested with Pronase (Margolis et al., 1975a). The chloroform–methanol extracts were pooled, evaporated to dryness, and stored at −40 °C until analyzed for gangliosides. The Pronase digest was desalted by gel filtration on Sephadex G-15 and glycosaminoglycans were precipitated with cetylpyridinium chloride (Margolis et al., 1975a). Excess cetylpyridinium chloride was extracted with *n*-amyl alcohol and the glycopeptides were analyzed for total sugar composition by gas–liquid chromatography (Bhatti et al., 1970). The glycopeptides were also fractionated by affinity chromatography on concanavalin A–Sepharose using a two-step gradient of α-methyl D-glucoside (Krusius, 1976). Glycopeptides not bound to the column were subjected to $NaOH$–$NaBH_4$ degradation and the alkali-stable glycopeptides (fraction A) were separated from the released O-glycosidically linked oligosaccharides by gel filtration on Sephadex G-50 (Krusius & Finne, 1977). Glycopeptides interacting weakly with concanavalin A–Sepharose were obtained by elution with 20 mM α-methyl D-glucoside (fraction B) and glycopeptides interacting strongly were eluted with 200 mM α-methyl D-glucoside (fraction C). The different O-glycosidically linked oligosaccharides were fractionated by DEAE-Sephadex A-25 chromatography and quantitated by gas–liquid chromatography (Finne & Krusius, 1976). Since the structures of the oligosaccharides obtained from rat brain have previously been determined (Finne, 1975; Finne & Rauvala, 1977), the amount of β-galactosyl-(1→3)-*N*-acetylgalactosaminitol observed in the monosialosyl fraction was taken as a measure of oligosaccharides III + IV, and that observed in the disialosyl fraction was taken as a measure of oligosaccharide V.

Results and Discussion

Glycoproteins of Microsomal Subfractions. The electron microscopic appearance and the concentrations of various enzyme markers in the microsomal subfractions are described in the preceding paper (Kiang et al., 1978). Although the relative proportions of smooth membranes and golgi cisternae (as well as contaminating particles derived from sources such as mitochondria and lysosomes) varied somewhat in subfractions 2, 3, and 4, these fractions were generally quite similar to one another and showed identical protein patterns on polyacrylamide gel electrophoresis in sodium dodecyl sulfate.

The membranes of subfraction 3 had the highest concentration of glycoproteins (14.6 μmol of sugar/100 mg of lipid-free dry weight, compared with 11.2 and 11.7 μmol/100 mg in fractions 2 and 4, respectively), but the three subfractions have almost identical glycoprotein compositions according to the criteria used in this study. These include the molar ratios of the individual sugars (Table I), and, as summarized in Table II, the proportions of the two classes of acidic type glycopeptides (groups A and B), neutral type glycopeptides (group C), and O-glycosidically linked oligosaccharides. Moreover, the percentages of the five different O-glycosidically linked oli-

[1] Abbreviation used: Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

TABLE II: Distribution of Neutral, Acidic, and *O*-Glycosidically Linked Oligosaccharide Units in Microsomal Subfractions and Whole Rat Brain.

	fraction number					whole
	1	2	3	4	5	brain
N-glycosidic						
group A glycopeptides						
concn[a]	3.10	4.30	5.18	4.66	3.14	2.79
% of recovered sugar	48	53	51	54	52	56
group B glycopeptides						
concn	1.45	1.74	2.41	1.77	1.18	1.02
% of recovered sugar	22	21	24	20	20	21
group C glycopeptides						
concn	1.04	1.48	1.72	1.66	1.43	0.74
% of recovered sugar	16	18	17	19	24	15
O-glycosidic						
concn	0.90	0.64	0.80	0.60	0.25	0.42
% of recovered sugar	14	8	8	7	4	8
total concn	6.48	8.16	10.11	8.69	5.99	4.97
% recovery	74	73	69	74	82	73

[a] Concentration expressed as μmol of sugar/100 mg of lipid-free protein residue.

TABLE III: *O*-Glycosidic Oligosaccharides in Microsomal Subfractions.

		percent[b]			
subfraction	concn[a]	I	II	III + IV	V
1	287	9	28	21	42
2	207	14	19	17	50
3	236	14	17	20	49
4	173	13	20	18	49
5	73	12	24	19	45

[a] Concentration expressed as total nmol of *oligosaccharide*/100 mg of lipid-free protein residue. Values given in the table are the averages from two experiments showing good agreement. [b] Molar percent contribution of oligosaccharides I–V to total *O*-glycosidically linked carbohydrate in each microsomal subfraction: (I) α-galactosyl-(1 → 3)-*N*-acetylgalactosaminitol; (II) β-galactosyl-(1 → 3)-*N*-acetylgalactosaminitol; (III) β-galactosyl-(1 → 3)-[*N*-acetylneuraminyl-(2 → 6)]-*N*-acetylgalactosaminitol; (IV) *N*-acetylneuraminyl-(2 → 3)-β-galactosyl-(1 → 3)-*N*-acetylgalactosaminitol; (V) *N*-acetylneuraminyl-(2 → 3)-β-galactosyl-(1 → 3)-[*N*-acetylneuraminyl-(2 → 6)]-*N*-acetylgalactosaminitol.

gosaccharides present in brain are also identical in membrane subfractions 2, 3, and 4 (Table III).

Subfraction 1 consists largely of smooth membranes with a high concentration of hyaluronic acid and chondroitin sulfate, whereas subfraction 5 is enriched in rough endoplasmic reticulum (Kiang et al., 1978). This latter fraction had the lowest concentration of glycoprotein carbohydrate (7.3 μmol/100 mg of lipid-free dry weight), and a much lower molar ratio of galactose, fucose, and sialic acid as compared with mannose (Table I). These analytical data are consistent with the relatively high proportion of neutral type glycopeptides in subfraction 5, where they account for 24% of the total sugar as compared with only 15% in whole brain (Table II). The synthesis of the N-glycosidically linked carbohydrate units begins in the rough endoplasmic reticulum by the transfer of the core structure from lipid intermediates to the protein. Since the core structure of N-glycosidically linked carbohydrate units is composed of only mannose and *N*-acetylglucosamine, a portion of the neutral type glycopeptides in subfraction 5 may represent neutral precursors of the acidic glycopeptides. On the other hand, subfraction 5 has the lowest concentration (4%) of O-glycosidically linked oligosaccharides, as compared with 7–8% in fractions 2–4 and in whole brain, and 14% in subfraction 1.

The relatively large proportion of O-glycosidically linked oligosaccharides is the major distinguishing feature of the glycoproteins of subfraction 1, which appears to be composed almost entirely of plasma membranes and smooth endoplasmic reticulum (Kiang et al., 1978). The composition of these oligosaccharides in subfraction 1 is also peculiar, insofar as there is relatively less of the brain-specific disaccharide α-galactosyl-(1→3)-*N*-acetylgalactosamine, but a correspondingly greater percentage of the β-linked anomer than is present in any of the other membrane subfractions (Table III). However, of the five O-glycosidically linked oligosaccharides it is clear that the brain specific disaccharide is the one which is most enriched in membrane as compared to soluble fractions of brain (see Table VI).

The presence of disialosyl (α-*N*-acetylneuraminyl-(2→8)-*N*-acetylneuraminyl) groups in glycoproteins from brain and other tissues has recently been reported (Finne et al., 1977). Since these are primarily localized in membrane glycoproteins, we studied their distribution in the five microsomal subfractions. The proportion of the total glycopeptide sialic acid which is 8-O-substituted was determined by mass fragmentography (Rauvala & Kärkkäinen, 1977), and found to be 6.0, 16.0, 16.2, 15.3, and 13.4% in microsomal subfractions 1, 2, 3, 4, and 5, respectively. The values for microsomal subfractions 2, 3, and 4 prepared from 1-month-old rat brains are therefore somewhat lower than those found for plasma membranes from 8-day-old-rat brain (19.8%), although considerably higher than the 9.5% of 8-O-substituted sialic acid present in the total brain microsomes of 14-month-old rats (Finne et al., 1977).

Since we found that approximately 65% of the proteins and glycoproteins and almost all of the glycosaminoglycans could be removed by simple washing of the membranes in microsomal subfraction 1 (Kiang et al., 1978), we compared the

TABLE IV: Glycoprotein Carbohydrate Composition of Subcellular Fractions from Brain.

	microsomal subfraction 1				whole brain		synaptosomes					
	membranes		"soluble"		soluble		soluble		membranes		mitochondria	
mannose	2.76[a]	1.00[b]	2.42	1.00	1.08	1.00	0.67	1.00	4.79	1.00	0.74	1.00
galactose	1.50	0.54	2.42	1.00	1.21	1.12	0.36	0.54	2.82	0.59	0.27	0.35
glucosamine	1.79	0.65	1.69	0.70	1.40	1.30	0.58	0.87	3.36	0.70	0.50	0.68
galactosamine	0.14	0.05	0.35	0.15	0.23	0.21	0.07	0.11	0.28	0.06	0.01	0.01
fucose	0.82	0.30	0.75	0.31	0.36	0.33	0.20	0.30	1.42	0.30	0.21	0.28
sialic acid	1.31	0.48	1.34	0.55	0.94	0.87	0.22	0.33	2.92	0.61	0.20	0.27
total	8.32		8.97		5.22		2.10		15.60		1.93	

[a] μmol/100 mg of lipid-free dry weight. [b] Molar ratio.

TABLE V: Distribution of Neutral, Acidic, and *O*-Glycosidically Linked Oligosaccharide Units in Subcellular Fractions of Brain.

	microsomal subfraction 1		whole brain	synaptic	
	membranes	"soluble"	soluble	membranes	mitochondria
N-glycosidic					
group A glycopeptides					
concn[a]	3.42	3.59	2.19	6.42	0.79
% of recovered sugar	61	52	54	59	50
group B glycopeptides					
concn	1.13	1.81	1.10	2.33	0.39
% of recovered sugar	20	26	27	21	25
group C glycopeptides					
concn	0.73	0.51	0.20	1.38	0.38
% of recovered sugar	13	8	5	13	35
O-glycosidic					
concn	0.30	0.93	0.56	0.71	0.02
% of recovered sugar	6	14	14	7	1
total concn	5.58	6.84	4.05	10.84	1.58
% recovery	67	76	78	70	82

[a] Concentration expressed as μmol of sugar/100 mg of lipid-free protein residue.

glycoprotein composition of this "soluble" fraction from subfraction 1 with washed membranes from the same subfraction and with the soluble glycoproteins of whole brain.

The concentration and composition of glycoproteins were very similar in both the "soluble" and membrane fractions of these low density microsomal membranes, the major differences being the relatively larger amounts of galactose and galactosamine in the proteins released by washing (Table IV). In this respect the soluble glycoproteins from microsomal subfraction 1 resemble the soluble glycoproteins from whole brain, but these latter have less than 60% as much carbohydrate, with proportionately much greater amounts of glucosamine and sialic acid (Table IV). However, the relative amounts of acidic, neutral, and O-glycosidically linked oligosaccharides are very similar in the two types of soluble glycoproteins (Tables V and VI). In view of these data and the metabolic differences described earlier (Kiang et al., 1978), it would appear that while one or several types of soluble glycoproteins from whole brain may be specifically associated with the subfraction 1 microsomes, these membranes do not simply occlude or adsorb a portion of the total population of soluble brain glycoproteins.

Glycoproteins of Mitochondria, Synaptic Membranes, and Soluble Fractions. For comparison with microsomal membranes and soluble glycoproteins of whole brain, we also analyzed the glycoprotein composition of mitochondria, synaptic membranes, and synaptosomal soluble glycoproteins.[2] As reported previously (Margolis et al., 1975a), mitochondria have a very low concentration of glycoproteins (Table IV). These contain a relatively large proportion (24%) of neutral type oligosaccharides and only traces of O-glycosidically linked oligosaccharides (Tables V and VI). The proportions of the five O-glycosidically linked oligosaccharides are similar to those present in other subcellular fractions (Table VI) and are probably a result of a low degree of contamination of the mitochondria.

The synaptosomal soluble fraction also has a very low concentration of glycoproteins, which differ considerably in sugar

[2] Although subcellular fractions were prepared by methods different from those used previously (Margolis et al., 1975a), the glycoprotein carbohydrate composition found in this study is generally comparable to that reported earlier. The only major exception concerns the concentration of mannose in certain subcellular fractions such as mitochondria and the synaptosomal soluble glycoproteins, which have a low concentration of mannose but very high amounts of bound glucose. In these two fractions, where the levels of glucose (presumably derived from material present in the sucrose used to prepare the density gradients) are two to three times the sum of the *total glycoprotein sugars*, an accurate correction for such high glucose concentrations is not possible using the enzymatic method previously employed for mannose determination. However, analyses of mannose in these fractions by automated ion-exchange chromatography of the sugar-borate complexes (Lee et al., 1969) and by gas chromatography, as reported here, agree very well.

composition from the nonsynaptosomal soluble glycoproteins of brain (Table IV). The low proportion of O-glycosidically linked oligosaccharides (13 nmol/μmol total sugar) also distinguishes the synaptosomal soluble glycoproteins from the nonsynaptosomal soluble fraction (35 nmol/μmol), but is very similar to the proportion of these oligosaccharides in synaptic membranes (15 nmol/μmol; cf. Tables IV and VI).

We and others have previously reported that synaptic membranes have a high concentration of glycoproteins (Margolis et al., 1975a; Morgan & Gombos, 1976; Churchill et al., 1976). The level of 15.6 μmol of glycoprotein sugar/100 mg of lipid-free dry weight is somewhat greater than the concentration in membranes prepared by a different procedure (Margolis et al., 1975a) and is comparable to that of microsomal subfraction 3 (14.6 μmol/100 mg), which also has a very similar composition (Tables I and IV). It is known that there are considerable similarities in the protein composition of microsomal and synaptic membranes (Wannamaker & Kornguth, 1973; Gurd et al., 1974; Jones et al., 1975), and the presence of many common protein and glycoprotein components in these two types of membranes is consistent with the membrane flow or endomembrane hypothesis, according to which membrane biogenesis involves the physical transfer of membranes from one subcellular compartment to another (Morré et al., 1974). Thus, if synaptic membranes are derived from endoplasmic reticulum which is in turn transferred to the golgi apparatus for transformation into plasma membrane, it would be expected that the fraction of endoplasmic reticulum destined for conversion to plasma membranes would resemble these latter in glycoprotein composition. Another probable reason for the similarities in composition between the microsomal and synaptic membrane fractions is that the microsomal fraction is itself heterogeneous, consisting both of endoplasmic reticulum and of plasma membranes derived mostly from nonsynaptic areas of the neuronal (and glial) cell surface.

TABLE VI: *O*-Glycosidic Oligosaccharides in Subcellular Fractions of Brain.

	concn[a]	percent[b] I	II	III + IV	V
microsomal subfraction 1 membranes	99	13	21	25	41
"soluble"	303	8	28	20	44
whole brain soluble	181	7	25	25	43
synaptosomal soluble	28				
synaptic membranes	236	16	24	21	39
mitochondria	7	15	24	17	44

[a] Concentration expressed as total nmol of oligosaccharide/100 mg of lipid-free protein residue. [b] Molar percent contribution of oligosaccharides I–V to total O-glycosidically linked carbohydrate. For identification of oligosaccharides, see footnote to Table III.

General Discussion

We have found that there are considerable differences in the oligosaccharide structure of glycoproteins present in various soluble and membrane fractions of brain. Although certain specific structural features and sugar sequences have been identified, other aspects can at present be described only in terms of different classes of oligosaccharides. Some of the most striking differences are between soluble and membrane glycoproteins. If one considers the soluble glycoproteins of whole brain and the glycoproteins of microsomal membranes as representative of these two classes, it can be seen that the former are characterized by a relatively high proportion of O-glycosidically linked oligosaccharides and smaller acidic type (fraction B) glycopeptides, whereas glycopeptides of the neutral type are enriched in microsomal and other membranes.

It is also interesting that the glycoprotein composition of myelin (Quarles & Everly, 1977) differs from that of any of the soluble or membrane fractions analyzed in the present study. Galactosamine was not detectable in glycopeptides prepared from either rat brain or peripheral nerve myelin, indicating the absence of O-glycosidically linked oligosaccharides in this specialized membrane. Moreover, the almost equimolar amounts of mannose and glucosamine, together with much smaller quantities of galactose, fucose, and sialic acid, suggest that myelin glycoproteins are enriched in neutral type oligosaccharides (represented by the group C glycopeptides isolated in the present investigation).

Differences in the composition of various types of nervous tissue membranes may reflect the different structures of the precursors involved in their biogenesis. For example, myelin membranes in brain are derived from oligodendrocytes whereas synaptic membranes probably represent specialized products of the neuronal endoplasmic reticulum, as discussed above. Our previous analyses of glycoproteins in neuronal perikarya, astrocytes, and oligodendroglia isolated in bulk from bovine brain (Margolis & Margolis, 1974) demonstrated that oligodendroglia contain only very small amounts of glycoprotein galactosamine (<1% of the total sugar), and that the other glycoprotein constituents are present in proportions close to those reported for brain myelin, while the neuronal cell bodies more closely resemble microsomal and synaptic membrane glycoproteins.

The anatomical localization of the soluble glycoproteins is not yet clear. They constitute a relatively small pool with an unusually rapid turnover of certain terminal sugars such as fucose and sialic acid (Margolis & Margolis, 1973a; Margolis & Gomez, 1973; Margolis et al., 1975a), but do not appear to be precursors of membrane glycoproteins in brain (unpublished results). Although a portion of these soluble glycoproteins may have been released from lysosomes or from the lumen of the endoplasmic reticulum, we have indirect evidence that at least certain classes of soluble glycoproteins and proteoglycans may be cytoplasmic constituents of neurons and glia, rather than being products destined solely for "export" as in other tissues.

It is apparent from the studies reported here that specific structural features of brain glycoproteins are associated with defined morphological components of nervous tissue. The extension of this approach to include a finer discrimination among related structures (e.g., synaptic junctional complexes, postsynaptic densities, synaptic plasma membranes) and the availability of more detailed information on the sugar sequences and linkages of the glycoprotein oligosaccharides can be expected to help elucidate the role of glycoproteins in such processes as neural histogenesis, regional brain differentiation, and the specificity of neuronal associations.

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Nitrogen-15 and Carbon-13 Nuclear Magnetic Resonance of Reduced Flavins. Comparative Study with Oxidized Flavins†

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ABSTRACT: Nitrogen-15 and carbon-13 nuclear magnetic resonance spectra of the fully reduced form of flavin were studied with riboflavin tetrabutyrate (RBUT), an organic solvent-soluble derivative of riboflavin. For the measurement of ^{15}N resonances, 99% enriched [1,3-^{15}N]RBUT and [1,3,5-^{15}N]RBUT were synthesized. In order to assign the ^{13}C resonances, 90% enriched [2-^{13}C]RBUT, [4a-^{13}C]RBUT, [4,10a-^{13}C]RBUT, and [8-$^{2}H_3$]RBUT were employed. The upfield shift of N(5) resonance upon reduction was remarkable (286 ppm), while the N(1) signal moved only by 79 ppm. The one-bond ^{15}N-H spin-spin coupling constant $^1J[^{15}N(5)-H]$ of the reduced RBUT was smaller than its $^1J[^{15}N(1)-H]$ and $^1J[^{15}N(3)-H]$. These observations indicate that N(5) changed into sp^3 hybridization upon reduction and lost the character of planar nitrogen. Most of the ^{13}C nuclei of the reduced form resonated at higher field than did those of the oxidized form, which is well explained by the increase in π-electron densities. Among the ^{13}C resonances, the upfield shift of C(4a) was remarkable (32 ppm), which explains the reactivity of C(4a) in oxygen flavoprotein complexation. ^{13}C-^{15}N spin-spin coupling constants were obtained from the measurements of ^{13}C magnetic resonance of ^{15}N-enriched RBUT. The values of the one-bond ^{13}C-^{15}N coupling constants increased markedly with protonation at N(1) and N(5) upon reduction.

The elucidation of the electronic and structural properties of the reduced flavin in comparison with those of the oxidized one is essential for understanding the redox reactions of flavoenzymes. Electronic states of the oxidized and reduced flavins have been examined by means of molecular orbital calculations and discussed in connection with their reactivities (Song et al., 1976) and with the electronic spectra (Grabe, 1974; Nishimoto et al., 1978). However, there is no experimental evidence which proves the calculated electronic distribution in a flavin molecule except for the X-ray analysis of flavin derivatives (Kierkegaard et al., 1971).

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