Synaptic Plasma Membrane Glycoproteins: Molecular Identification of Lectin Receptors†

James W. Gurd

ABSTRACT: Synaptic plasma membranes isolated from rat cerebral cortex reacted with concanavalin (con A), wheat germ agglutinin (WGA), *Lens culinaris* phytohaemagglutinin (LcH), and *Ricinus communus* agglutinin (RCA). Competition studies indicated that specific topographical relationships exist between receptors for con A and LcH and for WGA and LcH. Reaction of [^{125}I]con A with synaptic membrane proteins following polyacrylamide gel electrophoresis identified eight distinct molecular weight classes of glycoproteins possessing receptor activity for con A, ranging in apparent molecular weight from 27 000 to 165 000. Each of these also reacted to varying degrees with LcH, WGA, and RCA, indicating that a diverse population of carbohydrate moieties is associated with each molecular-weight class of glycoprotein. Following gel electrophoresis, competition between lectins did not occur, suggesting that each lectin reacts with a distinct group of carbohydrates and that specific relationships between these groups are destroyed by the solubilization and electrophoretic procedure. Synaptic junctional complexes isolated by Triton-X100 extraction of synaptic membranes exhibited a simplified glycoprotein composition with only three major molecular weight classes of glycoproteins possessing receptor activity for con A being present.

Glycoproteins have been implicated in a variety of cell-surface recognition phenomena (Hughes, 1973). Concentration of glycoproteins at central nervous system synapses has been demonstrated by histochemical (Rambourg and Leblond, 1967; Pfenninger, 1973) and chemical analysis (Gombos et al., 1971; Margolis et al., 1975). Isolated synaptic plasma membranes have been shown to possess seven to eight molecular-weight classes of glycoproteins (Banker et al., 1972; Gurd et al., 1974). By analogy with other systems, it has been suggested that these synaptic glycoproteins may be involved in specific aspects of synaptic function, such as recognition, adhesion, etc. (Brunngraber, 1969).

Lectins exhibit a high degree of discrimination between complex carbohydrates (Lis and Sharon, 1973) and have been widely used as probes for carbohydrates present on cell surfaces (Rabin and Burger, 1974). The presence of receptors for concanavalin A and *Ricinus communus* lectins in the synaptic cleft has been demonstrated (Matus et al., 1973; Bittiger and Schnebli, 1974; Cotman and Taylor, 1974) and lectin affinity chromatography has provided preliminary information as to the molecular identity of these receptors (Gurd and Mahler, 1974; Zanetta et al., 1975). In the present paper, several lectins, namely, con A[1] and LcH, both having specificity for α-D-mannose-like residues, WGA specific for *N'*-acetyl-D-glucosamine residues, and RCA I and II specific for D-galactose and *N'*-acetyl-D-galactosamine residues (Lis and Sharon, 1973) are used to further characterize synaptic membrane glycoproteins and lectin receptors following their separation by electrophoresis in the presence of sodium dodecyl sulfate (Gurd and Evans, 1976). In addition, the topographical relationships between lectin receptors in the intact membrane are investigated. A preliminary report of this work has appeared (Gurd, 1976a).

Experimental Procedures

Methods

Subcellular Fractionation. Synaptic plasma membranes (SPM) were prepared from the cortices of 28-day-old male Sprague Dawley rats as previously described (Gurd et al., 1974), except that centrifugation on the Ficoll gradient to purify synaptosomes was extended from 3 to 16 h. This modification had no detectable effect on the properties of the final surface membrane fraction. Fractions enriched in synaptic junctional complexes (SJC) were prepared from the cortices of 28–35-day-old rats exactly as described by Cotman et al. (1974) and were similar to their fraction when examined by electron microscopy using both osmium and ethanol–phosphotungstic acid staining procedures (Cotman and Taylor, 1972).

Lectin Binding. Binding of [^{125}I]lectins to synaptic plasma membranes was assayed in microfuge tubes (Beckman Instruments) by incubating 4 μg of membrane protein at room temperature for 45 min in 0.4 ml of 4 mM Tris-HCl buffer, pH 7.6, containing 0.1% BSA and 0.5–3 μg of [^{125}I]lectin. The incubation was terminated by centrifuging at 20 000*g* for 25 min, and washing the pellet once with 5 mM Tris-HCl buffer, pH 7.6, containing 0.1% BSA. Washed pellets were dissolved in 1 ml of 1 N NaOH (60 °C, 45 min), 0.1 ml of concentrated HCl was added, and the samples were counted in 10 ml of scintillation fluid containing 5 g of 2,5-diphenyloxazole and 0.3 g of 1,4-bis[2-(5-phenyloxazoly)]benzene/l. of toluene–Triton X-100 (2:1, v:v). In the experiment in which the effect of neuraminidase on RCA binding to membranes was determined, assay tubes were prepared as above, except that [^{125}I]lectin was omitted. Neuraminidase (10^{-3} unit/ml) was added and membranes were incubated for 2 h at 37 °C prior to the addition of [^{125}I]lectin. Competition between lectins was determined by adding a 15–20-fold excess of unlabeled lectin

† From the Department of Biochemistry, Scarborough College, University of Toronto, West Hill, Ontario M1C 1A4, Canada. *Received May 21, 1976.* This work was supported by a grant from the National Research Council of Canada.

[1] Abbreviations used are: con A, concanavalin A; WGA, wheat germ agglutinin; LcH, *Lens culinaris* phytohaemagglutinin; RCA, *Ricinus communus* agglutinin; SPM, synaptic plasma membranes; SJC, synaptic junctional complexes; BSA, bovine serum albumin.

at the same time as [^{125}I]lectin and treating as above. Specific binding was determined as the difference in lectin bound in the presence and absence of 2.5% (0.13 M) α-methyl-D-mannopyranoside (con A and LcH), 2.5% (0.11 M) *N'*-acetylglucosamine (WGA), or 2.5% (0.14 M) D-galactose (RCA). Nonspecific binding varied with the different lectins and was generally between 5 and 10% of the total counts bound with WGA and RCA, between 15 and 25% with LcH, and between 25 and 35% with con A. In all cases, results are presented as specific binding and are expressed as microequivalents of [^{125}I]lectin bound/mg of membrane protein, equivalents being calculated on the basis of the following equivalent weights per binding site: con A, 32 000 (Lis and Sharon, 1973); LcH, 23 500 (Lis and Sharon, 1973); WGA, 17 000 (Nagata and Burger, 1974); and RCA I and II, 60 000 (Nicolson et al., 1974).

Gel Electrophoresis and Lectin Binding to Glycoproteins following Electrophoresis. For sodium dodecyl sulfate gel electrophoresis, membrane samples were dissolved in 4 M urea, containing 1% sodium dodecyl sulfate, and 1% β-mercaptoethanol by heating at 100 °C for 4–5 min. Electrophoresis in 7% polyacrylamide gels was as previously described (Gurd et al., 1974). Following electrophoresis, gels were stained for protein with Coomassie blue or for carbohydrate by the periodic acid–Shiff procedure (Zacharius et al., 1969).

Lectin binding to individual molecular-weight classes of proteins was determined by reacting [^{125}I]lectins with membrane proteins following electrophoresis on 7% acrylamide gels essentially as described previously (Gurd and Evans, 1976). Gels were sliced longitudinally in half or into four pieces following electrophoresis. The two center flat pieces or the two halves were washed against 2-propanol–acetic acid to remove sodium dodecyl sulfate (Fairbanks et al., 1971) and then against 5 mM Tris-HCl, pH 7.6, as described (Gurd and Evans, 1976). One piece of each gel was incubated with gentle agitation in 7.5 ml of 5 mM Tris-HCl, pH 7.6, containing the indicated amounts of [^{125}I]lectin for 2 h at room temperature, followed by 16 h at 4 °C and then washed at 4 °C for 5 h against several changes of 5 mM Tris-HCl, pH 7.6, to remove unreacted lectin. The other piece of each gel served as a control for nonspecific binding and was treated similarly, except that incubation and washing solutions contained 2.5% of the appropriate sugar hapten. Gels were stained with Coomassie blue and the major proteins were marked with wires to serve as internal references and sliced. Gel slices were counted in 10 ml of scintillant containing 0.16 g of *p*-bis(*O*-methylstyryl)benzene, 7.84 g of 2,5-diphenyloxazole, and 30 ml of Protosol (New England Nuclear) in 1 l. of toluene after being allowed to swell overnight at 37 °C in the scintillation fluid. For competition studies, unlabeled lectin was added to the gel 1 h prior to the addition of [^{125}I]lectin and the procedure described above was followed. In some experiments, gels were treated with neuraminidase following the removal of sodium dodecyl sulfate. Washed gels were placed in 7.5 ml of 0.1 M citrate–phosphate buffer, pH 6.0, and 0.5 unit of neuraminidase was added. After 2 h at room temperature, the gels were washed overnight at 4 °C against 5 mM Tris-HCl buffer, pH 7.6, to remove neuraminidase and lectin binding was then determined as above.

Iodination of Lectins. Lectins were iodinated using a modification of the lactoperoxidase procedure (Marchalonis, 1969). Lectins (1 mg) were dissolved in 0.1 ml of 0.1 N sodium acetate buffer, pH 5.0, 0.5–1 mCi of ^{125}I in 0.1 N NaOH was added followed by sufficient 1 N HCl to neutralize the NaOH present in the iodine solution. Ten microliters of lactoperoxidase (200 μg/ml in acetate buffer) was added and the reaction was started by the addition of 5 μl of H_2O_2 (0.06 g/l.). Additional 5-μl portions of H_2O_2 (0.03 g/l.) were added at 10 and 20 min and a further 10 μl of lactoperoxidase (200 μg/ml) was added at 15 min. After 30 min, the reaction mixture was transferred to a Sephadex G-25 column equilibrated with 5 mM Tris-HCl buffer, pH 7.6, to separate free and bound iodine. The column buffer also contained 2.5% α-methyl-D-mannopyranoside when the [^{125}I]lectins were con A and LcH. Radioactive lectins were dialyzed against 5 mM Tris-HCl buffer, pH 7.6, to remove any residual free iodine and sugars. [^{125}I]Lectins were stored at −20 °C until use. Specific activities of the iodinated lectins were generally in the range of 0.2–0.7 mCi/mg.

Additional Methods. Protein was determined by the procedure of Lowry et al. (1951) using BSA as standard.

Materials

Lactoperoxidase, *p*-iodonitrotetrazolium violet (INT), concanavalin A, and neuraminidase (type V) were purchased from Sigma Chemical Co. Wheat germ agglutinin was obtained from Miles. LcH was prepared from lentils obtained locally as described previously (Gurd and Mahler, 1974) and *Ricinus communus* lectins were prepared from castor beans exactly as described by Nicolson and Blaustein (1972). RCA I and II were not separated. ^{125}I was purchased from New England Nuclear.

Results

Reaction of [^{125}I]Lectins with Synaptic Membranes. Con A, LcH, WGA, and RCA lectins reacted to varying degrees with the synaptic membrane fraction. The effect of increasing concentrations of lectin on the amount bound is presented in Figure 1. At concentrations in excess of 5 μg/ml, the amount of lectin bound to the membrane in each case approached a plateau value, indicating saturation of available receptor sites. Because the lectins used here are each multivalent, the present data cannot be used to directly calculate the number of receptors present on the membrane. However, the different plateau values approached by the various lectins suggest that they are each reacting with a distinct population of membrane receptors, although receptors common to more than one lectin may occur.

Competition studies were performed in order to assess topographical relationships between receptors for the various lectins (Table I). Reciprocal inhibition occurred between con A and LcH and between LcH and WGA, suggesting either that these lectins react with identical carbohydrate groupings or with oligosaccharides located in the membrane in such a way that steric interference between lectins occurs. Results presented below favor the second alternative.

Polyacrylamide Gel Electrophoresis of Synaptic Membrane Components. The results of polyacrylamide gel electrophoresis of the synaptic membranes are presented in Figure 2 and are similar to those described previously (Gurd et al., 1974). The membranes contain two major proteins with molecular weights of 54 000 and 107 000. Seven glycoproteins with apparent molecular weights (Segrest et al., 1971) ranging from 50 000 to 150 000 were revealed by the Schiff stain.

Identification of Con A Receptors on Polyacrylamide Gels. Molecular identification of individual lectin receptors was achieved by reacting [^{125}I]lectins with membrane proteins following sodium dodecyl sulfate gel electrophoresis, as described under Methods (see also Gurd and Evans, 1976). Using this procedure, a minimum of eight distinct molecular-weight

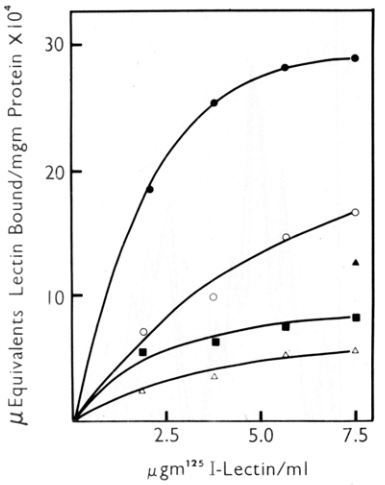

FIGURE 1: Binding of [^{125}I]lectins to synaptic plasma membranes. Synaptic plasma membranes (4 μg) were incubated in the presence of the indicated amounts of [^{125}I]lectin. Specific binding was determined as the difference between the amount of lectin bound in the presence and absence of competing hapten, as described under Methods. Lectins used were: WGA (● - ●), con A (○ - ○), LcH (■ - ■), and RC (△ - △). (▲) RC binding following pretreatment of the membrane with neuraminidase.

TABLE I: Competition between Synaptic Plasma Membrane Lectin Receptors.[a]

[^{125}I]Lectin	Competing Unlabeled Lectin		
	Con A	LcH	WGA
Con A	0	84	100
LcH	47	0	55
WGA	100	60	6

[a] Synaptic plasma membranes (4 μg) were incubated with 3 μg of the indicated [^{125}I]lectin plus or minus 40 μg of competing unlabeled lectin. Specific binding was determined as the difference between binding in the presence and absence of 2.5% of the appropriate sugar hapten (see Methods). Results are expressed as percent of the value obtained in the absence of competing lectin.

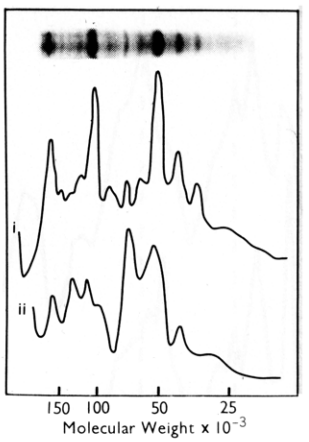

FIGURE 2: Electrophoresis of synaptic plasma membranes on 7% polyacrylamide gels containing 0.1% sodium dodecyl sulfate. Duplicate gels were stained for protein with Coomassie blue (i) or for carbohydrate by the periodic acid–Schiff procedure (ii). The photograph represents the protein stain. Each gel contained 160 μg of membrane protein.

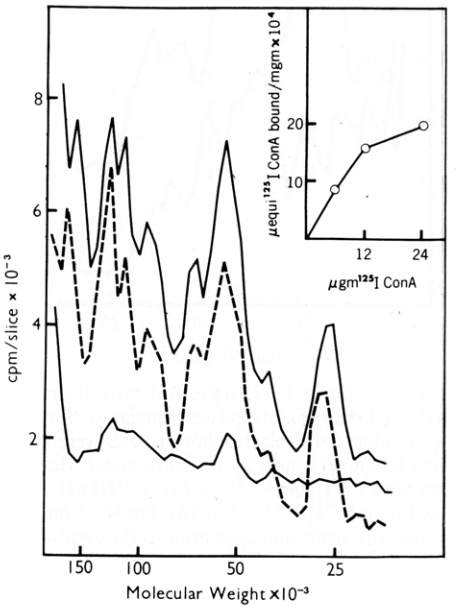

FIGURE 3: Reaction of [^{125}I]con A with synaptic plasma membrane glycoproteins separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. eighteen micrograms of membrane protein were subjected to electrophoresis. The gel was sliced longitudinally into four pieces and the two center pieces were washed and reacted with 26 μg of [^{125}I]con A, as described under Methods. The dashed line represents specific binding obtained as the difference between counts bound in the absence (upper solid line) and presence (lower solid line) of 2.5% α-methyl D-glucopyranoside. Insert: the effect of [^{125}I]con A concentration on binding to membrane proteins separated by gel electrophoresis. Gel slices containing 4.5 μg of membrane protein were reacted with the indicated amounts of [^{125}I]con A in the presence or absence of 2.5% α-methyl D-glucopyranoside and total specific counts bound to the gel were determined.

classes of glycoproteins carrying receptors for [^{125}I]con A were identified (Figure 3). In general, these corresponded closely to glycoproteins stained with the Schiff reagent, although quantitative differences between the two procedures were apparent (compare Figures 2 and 3). The Schiff procedure stains predominately terminal sialic acid residues, whereas con A binding depends on the presence of oligosaccharides containing mannose residues. The different profiles obtained with these two procedures therefore indicate a differential distribution of these carbohydrate moieties among the various molecular-weight classes. [^{125}I]Con A binding identified an additional glycoprotein with an apparent molecular weight of 27 000, which stains poorly, if at all, with the Schiff procedure. Binding patterns similar to those presented in Figure 3 were obtained over a wide range of con A to membrane protein ratios (0.23–3.7 mg of con A/mg of membrane protein), indicating that the profile reflects absolute amounts of receptors present in the gel, rather than relative affinities of different receptors for con A. When the con A/membrane protein ratio exceeded 2.7, the amount of con A bound to the gel approached a plateau value, indicating saturation of available receptors (Figure 3, insert). This value is similar to that obtained with intact membranes (Figure 2), suggesting that no new con A receptor sites were exposed following solubilization and electrophoresis.

Identification of Receptors for Other Lectins. [^{125}I]LcH, [^{125}I]WGA, and [^{125}I]RCA also reacted with membrane glycoproteins following sodium dodecyl sulfate gel electrophoresis (Figure 4), although it was necessary to treat the separated proteins with neuraminidase in order to obtain reproducible RCA binding profiles. Pretreatment with neuraminidase resulted in a two- to threefold increase in RCA binding to intact membranes (Figure 1) and a similar increase

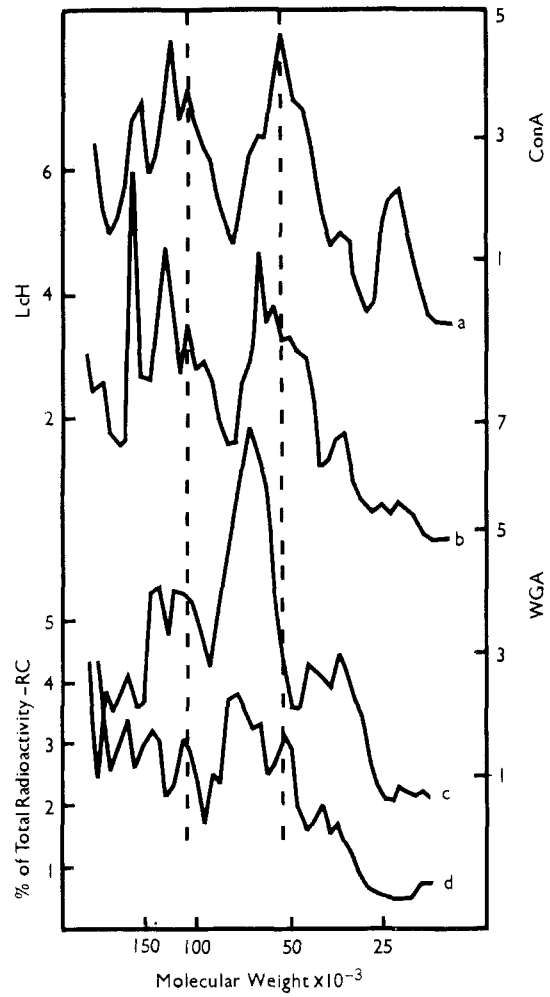

FIGURE 4: The reaction of [^{125}I]lectins with synaptic membrane proteins following sodium dodecyl sulfate gel electrophoresis. Synaptic membranes (160 μg) were subjected to electrophoresis, gels were sliced in half and lectin binding was determined, as described under Methods. Gel halves were reacted with [^{125}I]con A, 22 μg (a); [^{125}I]LcH, 16 μg (b); [^{125}I] WGA, 20 μg (c); or [^{125}I]RCA, 15 μg (d). For RCA binding, the washed gel was treated with neuraminidase prior to the addition of [^{125}I]RCA. Each profile represents specific binding obtained as the difference between counts bound in the presence and absence of the appropriate sugar hapten (see Methods). The ordinate represents the percent of total radioactivity bound to the gel which was present in each slice and is repeated for each lectin as indicated.

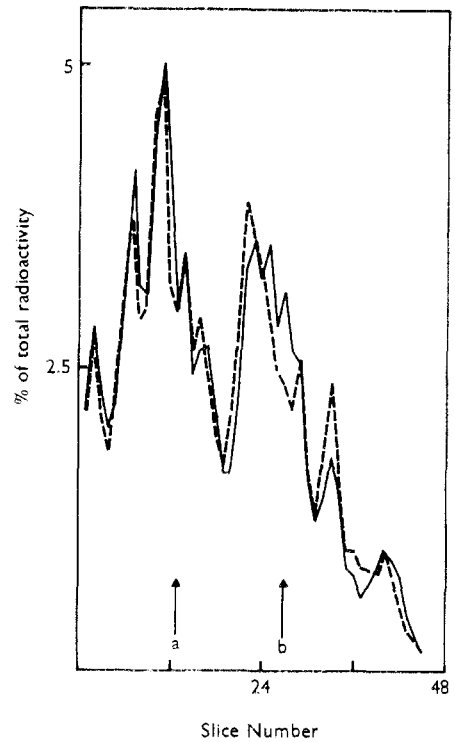

FIGURE 5: The effect of unlabeled con A on the reaction of [^{125}I]LcH with synaptic membrane glycoproteins. 175 μg of membrane protein was electrophoresed, the gels were sliced into four pieces and the two center pieces were used to determine lectin binding. Gel slices were reacted with 14 μg of [^{125}I]LcH in the presence (dashed line) or absence (solid line) of 700 μg of unlabeled con A. When present, con A was added 1 h prior to the addition of [^{125}I]LcH. Profiles represent specific binding of [^{125}I]LcH. Arrows represent the positions of synaptic membrane proteins of molecular weights 107 000 (a) and 54 000 (b).

in binding to the gels (not shown) in agreement with the previously demonstrated stimulatory effect of neuraminidase on RCA binding to membrane receptors (Nicolson, 1973). Of the lectins tested, only LcH gave any evidence of binding to the con A binding protein of molecular weight 27 000, and this was variable (compare Figures 4 and 5). With the exception of the 54 000-dalton protein which did not react with WGA, each of the other con A positive proteins also reacted to some extent with the other lectins tested. Two additional glycoproteins of apparent molecular weights of 71 000 (RCA positive) and 44 000 (WGA positive) were also clearly identified.

Competition between Lectins following Gel Electrophoresis. Competition between lectins binding to the intact membrane (Table I) may have resulted from competition for the same receptor site or from steric interference between lectins bound to neighboring receptor sites. In an attempt to differentiate between these possibilities, competition studies were performed in which the effects of unlabeled lectins on the binding of [^{125}I]LcH and [^{125}I]con A to membrane proteins separated on sodium dodecyl sulfate gels were determined. Competition resulting from the reaction of different lectins with the same carbohydrate moiety should persist following separation of the proteins by electrophoresis. Competition reflecting primarily steric interference between adjacent receptor molecules, on the other hand, should be reduced or eliminated following solubilization and electrophoresis of membrane components. In contrast to the results obtained with intact membranes, in which con A inhibited the binding of [^{125}I]LcH by 50%, the reaction of [^{125}I]LcH with membrane glycoproteins was not inhibited by con A following gel electrophoresis (Figure 5). Identical results were obtained when the competing lectin was WGA. Similarly, unlabeled LcH, which reduced con A binding to intact membranes by 15–20%, did not inhibit con A binding following electrophoresis (not shown). These results indicate that different lectins do not compete for identical receptor sites but bind to carbohydrates which are topographically related to each other in the intact membrane, either on the same or adjacent glycoproteins, but which do not interact following solubilization of the membrane and separation of glycoproteins by gel electrophoresis.

Con A Receptors in Synaptic Junctional Complexes. The membrane fraction used for the experiments described above represents, primarily, membranes derived from the presynaptic region of the nerve terminal (Gurd et al., 1974). Con A receptors have, however, been localized in the synaptic cleft (Matus et al., 1973). In order to identify these receptors more specifically, synaptic junctional complexes were isolated by Triton-X100 extraction of a crude membrane fraction as described by Cotman et al. (1974). The Triton-X100 insoluble

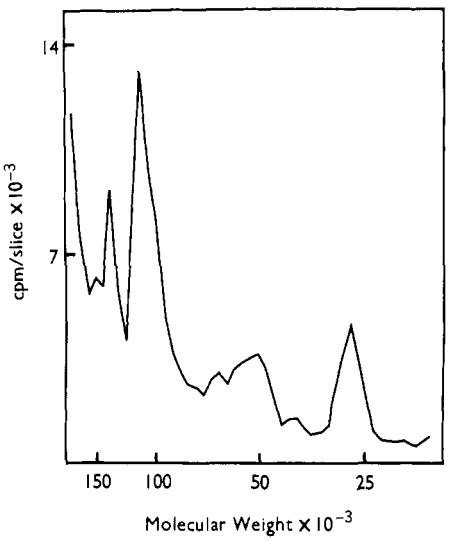

FIGURE 6: Reaction of [^{125}I]con A with synaptic junctional complex proteins following sodium dodecyl sulfate gel electrophoresis. Synaptic junctional complexes (48 μg) were subjected to electrophoresis and gels were sliced in half and reacted with [^{125}I]con A (15 μg), as described under Methods. Results represent specific binding of [^{125}I]con A.

residue obtained by pelleting through 1 M sucrose, which is enriched in intact junctional complexes (Cotman and Taylor, 1972; Gurd, unpublished observations) was analyzed for con A receptors (Figure 6). In contrast to the synaptic membrane fraction, the synaptic junctional complexes presented a relatively simple profile of con A binding activity, with only two major con A receptors, of high molecular weight, being apparent. When separated on 10% acrylamide slab gels, the smaller of those two components was further resolved into two bands with apparent molecular weights of 130 000 and 105 000 (Gurd, J. W., manuscript submitted for publication). Glycoproteins of intermediate molecular weight (65 000–35 000) were deficient in the junctional complex fraction and may represent real components of the synaptic complex or glycoproteins present in contaminating nonjunctional membrane elements.

Discussion

In the present paper, synaptic plasma membrane glycoproteins have been characterized on the basis of their reactivity with four lectins of differing specificities: con A, LcH, WGA, and RCA. Each of seven to eight molecular-weight classes of glycoproteins previously identified by staining procedures reacted to varying degrees with the lectins used in this study and each exhibited characteristic lectin binding properties. The quantitative differences in binding patterns exhibited by the different lectins presumably reflect variations in the oligosaccharide content of the various molecular-weight classes of glycoproteins, since each of the lectins possesses different carbohydrate specificity. It should be noted that the reaction of lectins with oligosaccharides is highly complex depending not only on the type of monosaccharides present but also on their sequence and on the nature of the glycosidic linkages involved (Kornfield and Ferris, 1975). Thus, although the present studies allow discrimination between glycoproteins on the basis of their lectin receptor properties, they provide, as yet, only superficial information pertaining to the chemical nature of the oligosaccharides. For example, the results suggest that the class of glycoproteins with molecular weights of approximately 54 000 contain α-mannoside residues (con A and LcH binding) but are deficient in N'-acetylglucosamine residues (WGA), confirming our earlier study in which [^{3}H]fucose-labeled proteins of a similar molecular weight constituted a major component of the LcH positive–WGA negative fraction (Gurd and Mahler, 1974).

The lack of competition between lectins following solubilization and electrophoretic separation of membrane proteins indicates that each lectin reacts with a unique population of oligosaccharides and does not share common receptors with other lectins. The lack of cross-reactivity between con A and LcH following electrophoresis is of particular note in view of the similar simple sugar specificities of these two lectins and confirms earlier observations that con A and LcH discriminate between complex oligosaccharides (Young and Leon, 1974; Ahmann and Sage, 1974). Competition between lectins binding to receptors situated in the intact membrane, on the other hand, clearly indicates a defined topographical relationship between sets of membrane-associated lectin-binding proteins. Whether or not these associations exist independently of the presence of lectins or result from a rearrangement of membrane components induced by the presence of lectins remains to be determined and is of interest in view of the demonstrated con A induced clustering of synaptic membrane glycoproteins (Matus et al., 1973). In the case of LcH and WGA, at least some of the receptors appear to be present on the same molecule, since they could not be separated by serial lectin affinity chromatography (Gurd and Mahler, 1974).

The reduced number of con A binding proteins now found to be present in the synaptic junctional complex is in general agreement with the results of Cotman and Taylor (1974), in which fewer con A receptors were found in synaptic junctional complexes than in synaptic membranes, and of Margolis et al. (1975), in which the Triton-X100 residue of synaptic membranes was relatively deficient in carbohydrates. One or all of these proteins, which are likely candidates for specific involvement in synaptic function, may be identical with the con A receptors previously localized in the synaptic cleft by histochemical procedures. Assignment of these glycoproteins to specific topographical locations within the synapse is therefore an important goal. Preliminary results have indicated that extraction of the synaptic junctional complex fraction with sarcosyl or deoxycholate under conditions previously shown to solubilize the pre- and postsynaptic membrane (Cotman et al., 1974; Walters and Matus, 1975) solubilizes most of the high-molecular-weight glycoproteins, suggesting that these are predominately associated with the membrane components of the junctional complex. The insoluble residue obtained following these extraction procedures is enriched in postsynaptic densities and also contains receptors for con A, LcH, and WGA (Gurd, J. W., manuscript submitted for publication). Thus, glycoproteins appear to be distributed amongst the various components of the synaptic assembly. Specific characterization of glycoproteins present in each of these synaptic organelles remains a topic for future research.

Although specific biological functions cannot yet be associated with the individual glycoproteins described in this study, a variety of membrane-associated enzymes and receptors are glycoproteins and are presumably represented amongst the various species now described. For example, rat brain acetylcholinesterase, which is present in the postsynaptic membrane (McBride and Cohen, 1972) is a glycoprotein and binds to the four lectins used here (Gurd, 1976b) and preliminary studies have indicated that rat brain acetylcholine receptor is also a glycoprotein and reacts with con A (P. Salvaterra, personal communication). It is tempting to speculate that the con A

receptors, which are now found to be localized in the synaptic junctional complex, may play a specific role in synaptic function and specificity, involved in recognition, adhesion, receptor function, or ion translocation.

Characterization of the carbohydrate content of individual molecular-weight classes of synaptic membrane glycoproteins provides a basis of comparison of intact membrane glycoproteins present in different regions or subcellular fractions of the brain. It must be emphasized, however, that a single molecular-weight class may contain several glycoproteins of different lectin specificities (Gurd and Mahler, 1974). Differentiation between single glycoproteins of similar molecular weight will therefore necessitate combining the present procedures with fractionation techniques specific for glycoproteins (Gurd and Mahler, 1974; Gurd, 1976a). Such experiments are presently in progress and should provide additional insight into the role of glycoproteins in neuronal function.

Acknowledgments

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