Identification of Glycoproteins and Proteins at Synapses in the Central Nervous System*

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Synaptic plasma membranes, synaptic junctions, and postsynaptic densities have been isolated from rat brain, the proteins resolved by polyacrylamide gel electrophoresis, and the glycoproteins identified.

The synaptic junction fraction is composed of a spectrum of polypeptides which range in $M_r$ from 13,000 to 250,000. The overall pattern is similar to synaptic plasma membranes; however, the relative proportions of the polypeptides are distinctive. The postsynaptic density fraction consists primarily of one band with an $M_r$ of 52,000. Polypeptides with an $M_r$ of 55,000, and another five of higher $M_r$, make up the remaining protein. The polypeptides of the postsynaptic density fraction must be reduced with mercaptoethanol in order to permeate the polyacrylamide gel. Therefore, postsynaptic density proteins are cross-linked by disulfide bonds into supramacromolecular aggregates.

Glycoproteins which bind concanavalin A were identified in synaptic junctions by the binding of $^{125}$I-concanavalin A directly to the polypeptides resolved on the polyacrylamide gels. Only four bands, each with an $M_r$ greater than 95,000, bind concanavalin A. In contrast, the pattern of concanavalin A-binding polypeptides in synaptic plasma membranes is distinctive and more complex. In the postsynaptic density fraction, most of the concanavalin A binding occurs to a glyco-component which migrates at the dye front. These data, together with previous cytochemical data using concanavalin A-ferritin conjugates, indicate a limited and select group of high $M_r$ concanavalin A glycoproteins resides within the synaptic cleft of asymmetric type synapses.

Whereas a select group of polypeptides bind concanavalin A, all polypeptides resolved in the synaptic junction fraction are glycoproteins and contain galactosyl or galactosyl-like residues, since they label with tritiated borohydride following galactose oxidase treatment. This suggests that the carbohydrate composition of individual glycoproteins is different.

An increasing body of evidence in a variety of systems indicates that membrane glycoproteins are involved in intercellular recognition and adhesion (1–4). In the nervous system membrane glycoproteins probably play a major role in the recognition and formation of specific synaptic connections. Synaptic junctions in the central nervous system appear to contain glycoproteins. Cytotochemical studies using concanavalin A and Ricinus communis agglutinin-ferritin conjugates have shown that in several neuronal types in brain, the external surface of the postsynaptic membrane within the cleft is rich in Con A and R. communis agglutinin binding sites (5). However, at present specific glycoproteins located at the SJ have neither been identified nor characterized.

The analysis of macromolecules at the synapse has been greatly facilitated by the development of methods to isolate pure subsynaptic fractions. Methods are now available to isolate synaptic plasma membranes, SJ, and postsynaptic membranes which have lost their attached presynaptic membrane (40%) (8). The PSD fraction is about 80% pure in PSDs (9).

Previously the glycoproteins of SPM fractions have been partially characterized (10–12) and the carbohydrate composition of isolated SPM, SJ, and PSD fractions has been reported. All fractions contain protein-bound carbohydrates (8) at similar concentrations except for sialic acid, which is highest in SPM and lowest in PSD fractions, and mannose and glucose which are highest in PSD and lowest in SPM fractions. As determined by electron microscopic studies using Con A-ferritin conjugates, isolated SJs retain the Con A-binding sites present on the external surface of the postsynaptic membrane just within the synaptic cleft (33). Significantly, Con A-binding sites are absent from the extrajunctional membrane present and there is little or no labeling of the PSD. Therefore, the majority of Con A-binding sites are located on the external surface of the postsynaptic membrane in SJ fractions.

The aim of this study was to characterize the proteins and glycoproteins at the synapse and identify those glycoproteins which reside on the external face of the postsynaptic membrane. We have employed gradient polyacrylamide slab gels to electrophoretically separate the protein and glycoprotein com

* The abbreviations used are: Con A, concanavalin A; SJ, synaptic junctions; PSD, postsynaptic density; SPM, synaptic plasma membranes; SDS, sodium dodecyl sulfate.

‡ Previously referred to in the literature as synaptic junctional complex. We have adopted the term synaptic junction here to conform to current usage for the equivalent structure in tissue sections (6).
Membrane specialization called the PSD. SPMs, when treated with detergent in SDS. Since SPM, SJ, and PSD fractions are rich in both functional membrane and SJs can be isolated by density gradient centrifugation (7). Treatment of SPMs with sodium lauroyl sarcosinate solubilizes synaptic membranes. SPMs prepared by lysis of synaptosomes consist of a matrix and 10~15% of the total protein is present in SPM fractions. In this procedure, the free amino groups of proteins react with pyridoxal phosphate and form a Schiff's base which is subsequently reduced with tritiated NaBH₄. In a typical reaction, 100 μg of SJ (100 μl of 0.05 m sodium phosphate, pH 7.5) containing 5% (w/v) nonradioactive NaBH₄, washed extensively to remove NaBH₄, and resuspended in 100 μl of 0.05 m sodium phosphate (pH 7.5). Pyridoxal phosphate was then added to a final concentration of 5 mM. After incubation at 37° for 20 min, the mixture was cooled to 4°, NaBH₄ was added to a final concentration of 20 mM, and the incubation was continued for an additional 15 min. The residual NaBH₄ was consumed by the addition of excess pyridoxal phosphate. Samples were then washed extensively to remove unbound radioactivity and SJs were solubilized in SDS. In order to obtain highly specific labeling of SJ polypeptides, it was necessary to precede with nonradioactive NaBH₄.

**Polyacrylamide Gel Electrophoresis** - The discontinuous SDS-buffer system of Laemmli (16) was used, together with the slab gel and electrophoresis apparatus described by Studier (17). Exponential radiolabeled slab gels were excised from the gels which were otherwise gels were fixed and stained with Coomassie blue as previously described (18). Following the separation of proteins by electrophoresis the patterns of radioactivity were determined by cutting the gels into 1- to 2-mm segments (perpendicular to the direction of protein migration). Each gel slice was placed into a scintillation vial and solubilized in 100 μl of 30% (w/v) hydrogen peroxide (50° for 2 h). After cooling to room temperature, each vial received 0.5 ml of NCS tissue solubilizer (Amersham/Searle) followed by 10 ml of Omnifluor (New England Nuclear)-toluene scintillation fluid. Radioactivity was measured in a Nuclear Chicago UniLux II liquid scintillation counter. Counting efficiency correction were calculated on the basis of internal standards. Coomassie blue-staining profiles were quantitated with a Joyce-Loeb scanning microdensitometer.

**Con A-Horseradish Peroxidase and "125I-Con A Binding to Polyacrylamide Gels** - Following electrophoresis, proteins and glycoproteins were fixed in the gel matrix by overnight incubation in 7% (w/v) acetic acid and 25% (w/v) isopropyl alcohol. Samples to be compared were electrophoresed in the same slab gel so that a side-by-side comparison could be made of the different samples. Horseradish peroxidase visualization of Con A binding glycoproteins was performed by a modification of the method of Wood et al. (19). Following overnight fixation in acetic acid isopropyl alcohol, gels were washed with three changes of Con A buffer (0.4 M NaCl, 50 mM sodium phosphate, pH 6.5). The gel was then incubated in 50 to 70 ml of buffer containing Con A (0.75 mg/ml) for 20 min at room temperature. Following removal of the Con A solution, the gel was washed at 10-min intervals with fresh buffer (100 ml) for 60 min. The gel was then incubated 15 min in a solution containing 65 μg/ml of horseradish peroxidase (Sigma type VI) in 0.1 m NaCl, 50 mM sodium phosphate, pH 7.0. The horseradish peroxidase solution was removed and the gel was washed extensively as above with buffer for 60 to 90 min. Because Con A is multivalent, it binds specifically to glycoproteins in the gel as well as to the added glycoprotein, horseradish peroxidase. Con A-binding glycoproteins in the gel can then be visualized by the enzymatic reaction product of horseradish peroxidase.

In a typical experiment, 100 μl of SJ or SPM fraction (300 to 350 μg of protein) was preincubated with nonradioactive NaBH₄, (0.5 to 1.0 mM) for 5 min at room temperature. Samples were then washed extensively to remove nonradioactive NaBH₄ and resuspended in 100 μl of 5 mM sodium phosphate (pH 8.0). Fifty microliters of 50 mM sodium phosphate (pH 8.5) containing 0.5 to 0.5 μl of galactose oxidase and 10 μl of NaBH₄, (freshly prepared in 0.01 n NaOH) was added to a final NaBH₄, concentration of 0.5 to 1.0 mM (100 to 200 μg). After a 10- to 15-min period at room temperature, the reaction was terminated by the addition of a 5- to 10-fold excess of nonradioactive NaNBH₄ in 2.0 ml of 50 mM sodium phosphate buffer (pH 7.5). Fractional proteins were washed four times to remove residual NaBH₄. The fractions were then solubilized in SDS (2.3%, w/v) and protein determinations were carried out by the method of Lowry et al. (14), using crystalline bovine serum albumin as a standard.

**Pyridoxal Phosphate-Sodium Borohydride Labeling of SJ Proteins** - The procedure of Rikfin et al. (15) was used to label proteins present in SJ fractions. In this procedure, the free amino groups of proteins react with pyridoxal phosphate and form a Schiff's base which is subsequently reduced with tritiated NaBH₄. In a typical reaction, 100 μg of SJ (100 μl of 0.05 m sodium phosphate, pH 7.5) containing 5% (w/v) nonradioactive NaBH₄, washed extensively to remove NaBH₄, and resuspended in 100 μl of 0.05 m sodium phosphate (pH 7.5). Pyridoxal phosphate was then added to a final concentration of 5 mM. After incubation at 37° for 20 min, the mixture was cooled to 4°, NaBH₄ was added to a final concentration of 20 mM, and the incubation was continued for an additional 15 min. The residual NaBH₄ was consumed by the addition of excess pyridoxal phosphate. Samples were then washed extensively to remove unbound radioactivity and SJs were solubilized in SDS. In order to obtain highly specific labeling of SJ proteins, it was necessary to precede with nonradioactive NaBH₄.

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Synaptic Proteins and Glycoproteins

raphy or liquid scintillation counting of gel slices. Con A was iodinated by the method of Greenwood et al. (20) and purified by affinity chromatography on Sephax G-100 (Pharmacia Fine Chemicals). Briefly, gels were incubated in buffer containing 125I-Con A (100 µCi/ml, 100 µCi/mg) for 20 min. Following incubation in the presence of 125I-Con A, gels were washed exhaustively to remove unbound lectin. Gels were then dried under vacuum on filter paper and placed next to Kodak No-screen medical X-ray film and exposed for 18 to 36 h. In all cases, specific lectin binding was determined following subtraction of values obtained from experiments in which lectin incubations were carried out in the presence of 0.2 M α-methylglucoside.

Materials—Triton X-100, pyridoxal phosphate, pronase, trypsin, bovine serum albumin, α-methylglucoside, and 3,3'-diaminobenzidine were obtained from Sigma Chemical Co. NaB3H4 was obtained from Amersham/Searle. Reagents used in electrophoresis were purchased from Eastman Kodak Co. Galactose oxidase was obtained from Worthington Biochemical Corp. Reagents used for electron microscopy were obtained from Polysciences, Inc. SDS was obtained from BDH Chemicals Ltd. Sodium lauroyl sarcosinate was purchased from K and K Laboratories, Inc. All other chemicals were of reagent grade. Con A was purchased from Calbiochem. Tubulin, purified by repeated cycles of polymerization-depolymerization, was a kind gift from Dr. Richard Olsen (University of California, Riverside). Actin, prepared from muscle, was a gift from David Shelton (this laboratory).

RESULTS

Polypeptides of SPM, SJ, and PSD Fractions—SPM, SJ, and PSD fractions all contained polypeptides which ranged in apparent M, from 13,000 to >250,000 (Fig. 2). The major polypeptides of the SPM fraction had M, of 85,000, 55,000, 52,000, and 45,000. The SJ fraction had a broad spectrum of polypeptides which resembled those of the SPM fraction. Upon closer examination, however, the SJ profile contained many polypeptides which were present in different relative proportions when compared to SPM. Most noticeable and enriched in the SJ over the SPM fraction were those polypeptides in Table I (Footnote b), listed by M, and per cent of total. The only polypeptides markedly enriched in the SPM fraction were a major 85,000 component and several minor bands with M, of 54,000, 17,000, and 10,000 to 14,000 (Fig. 2).

Considerably fewer polypeptides were resolved in the PSD fraction. Most of the components in SPM and SJ fractions were absent from the PSD. However, all PSD polypeptides possessed comparable electrophoretic counterparts in either SPM or SJ fractions. Most notable of the PSD polypeptides was the

![Fig. 2](left). Polyacrylamide slab gel electrophoresis of subsynaptosomal fractions. Densitometric tracings of: (a) SPM, 60 µg; (b) SJ, 60 µg; (c) PSD-60 µg. Electrophoretic patterns of: (a') SPM, 60 µg; (b') SJ, 60 µg; (c') PSD, 60 µg. Gels were 8 to 20% exponential-linear gradients (18).

![Fig. 3](right). Relative specific activity (a) and total incorporation (b) of galactose oxidase-NaB3H4 incorporation in prereduced galactosyl-containing glyco-components in SJ. (a) Relative specific activity plotted as a ratio of per cent galactose oxidase (Gal. Ox.-NaB3H4) incorporation per band (calculated as a per cent of total incorporation) to the corresponding amount of Coomassie blue (C.B.) staining per band (calculated as a per cent of the total area of all peaks). Values are the average of three determinations, which all yielded indistinguishable patterns. The asterisk denotes the dye front where the per cent Coomassie blue is approximately zero. (b) Radioactivity profile obtained by liquid scintillation counting of gel slices. Individual values varied ±5% of each value (average of four experiments). Background profile (b.g.) represents incorporation in the absence of galactose oxidase. Inset shows Coomassie blue staining pattern of SJ fraction (75 µg) from which radioactive determinations were obtained (8.5 to 20% exponential-linear gradient gel).
major 52,000 component. This polypeptide(s) constituted ~43% of the total Coomassie blue-staining material in this fraction (Table I). Other polypeptides which comprised significant amounts of this fraction are listed by $M_r$ and per cent of total in Table I.

In separate experiments using purified tubulin and actin as $M_r$ standards, the band at 55,000 co-migrated with tubulin, and that at 45,000 co-migrated with actin. The major PSD polypeptide always migrated ahead of tubulin ($M_r$ 55,000) and behind actin ($M_r$ 45,000). We have also extracted the major PSD polypeptide from gels and upon re-electrophoresis it again migrated as a single peak between those of purified tubulin and actin. Therefore, it appeared to represent a dis- major 52,000 component. This polypeptide(s) constituted ~43% of the total Coomassie blue-staining material in this fraction (Table I). Other polypeptides which comprised significant amounts of this fraction are listed by $M_r$ and per cent of total in Table I.

Pyridoxal Phosphate Labeling of SJ Proteins—Pyridoxal phosphate-dependent labeling was carried out to provide a check for the quantification of proteins based on Coomassie blue staining and in order to determine the type of domain in which SJ proteins reside. Pyridoxal phosphate reacts primarily with free amino groups and is known not to penetrate the hydrophobic domains of intact membranes (15).

Within the limits of resolution, every SJ polypeptide visualized by Coomassie blue staining appeared to exhibit pyridoxal phosphate-dependent labeling. The resulting radioactive profile (not shown) resembled very closely the densitometric scan of the Coomassie blue profile of the same fractions (Fig. 1), except that a polypeptide of 32,000 incorporated a disproportionately large amount of radioactivity relative to Coomassie blue staining. The ratio of the amount of pyridoxal phosphate-dependent incorporation into each peak (per cent of the total incorporation) to the corresponding amount of Coomassie blue staining (per cent of the total area of all peaks) was calculated. The mean value of all ratios was 1.005 ± 0.149 S.D. ($n = 29$), excluding the 32,000 band, which was 2.2 ± 0.1, and the dye front values.

Therefore, the protein content of the majority of individual bands determined by Coomassie blue staining agrees quite closely with that determined by the pyridoxal phosphate reaction. Moreover, it is quite unlikely that most amino groups in these fractions are inaccessible because they are buried in a hydrophobic environment.

Galactose Oxidase Labeling of SJ Proteins—It was necessary to prereduce the samples prior to the labeling reaction. Following prereduction, the omission of galactose oxidase from the labeling reaction resulted in approximately one-tenth of the incorporation that took place in the presence of galactose oxidase (see background values, Fig. 3b). With increased reaction times (0 to 30 min), and increased enzyme concentrations (0 to 0.5 IU), the amount of galactose oxidase-dependent labeling increased while the patterns did not change. SJ and SPM fractions were labeled to about the same specific activity.

Within the limits of resolution, all bands in the SJ fraction labeled to some extent, indicating that all contained galactose, or galactosamine residues, or both. However, the extent of incorporation varied between bands. In order to compare the extent of incorporation in each band to the protein content, the relative specific activity was calculated. The highest relative specific activities were associated with polypeptides with $M_r$ of 160,000 to 170,000, 90,000 to 140,000, 70,000 to 80,000, 32,000 to 40,000, 20,000, and 15,000 (Fig. 3a).

It was not known whether all galactosyl-containing glyco- components were accessible to galactose oxidase during the labeling reaction. Some carbohydrates may have been sterically protected in the SJ and not labeled. However, labeling reactions conducted with SJ fractions subjected to repeated cycles of freeze-thaw to disrupt membrane structure resulted in radioactive profiles which were indistinguishable from those of freshly prepared SJs. This suggests that few, if any, carbohydrates were protected.

Additional experiments were conducted to examine the nature of the substrates being labeled in the galactose oxidase reaction. Between 10 to 15% of the total radioactivity was extracted from labeled SJs with a mixture of chloroform/methanol (2:1, v/v). Therefore, little radioactivity appeared to be associated with lipids. This result is compatible with the finding that little lipid is present in the SJ fraction (8). Subjecting radiolabeled SJs to acid hydrolysis in 0.3 N H$_2$SO$_4$ (80°C 60 min), a procedure which has been shown to disrupt saccha-

### Table I

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<th>Band*</th>
<th>per cent of total</th>
<th>Band*</th>
<th>per cent of total</th>
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<td>4.2 ± 0.1</td>
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</table>

* Individual electrophoretic bands of SJ and PSD fractions in Fig. 1.

### See "Results."
ride-saccharide covalent linkages (21), resulted in the release of 70 to 80% of the total radioactivity. The same hydrolytic conditions resulted in the release of 60 to 70% of the total protein-bound neutral sugar content (assayed by the phenol-
H$_2$SO$_4$ reaction (Ref. 22)) from SJs. These results suggest that little, if any, galactose oxidase-dependent labeling is associated with the amino acids of proteins.

A significant amount of radioactivity was present at the dye front. This radioactivity represented 45 to 50% of the total incorporation in SJs. It was not removed by repeated washing of SJs with buffer following labeling, and it remained fixed in the polyacrylamide gel following staining and destaining. The fact that only 10 to 15% of the total galactose oxidase-dependent incorporation was extractable in chloroform/methanol suggests that, at most, only 20 to 30% of this material at the dye front could be lipid. There was very little, if any, Coomassie blue-staining material or pyridoxal phosphate-dependent incorporation at the dye front. The specific activity of galactose front could be lipid. There was very little, if any, Coomassie blue-staining material or pyridoxal phosphate-dependent incorporation in SJs with buffer following labeling, and it remained fixed in the polyacrylamide gel following staining and destaining. The specificity of 70 to 80% of the total radioactivity. The same hydrolytic conditions resulted in the release of 60 to 70% of the total protein-bound neutral sugar content (assayed by the phenol-
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The possibility was considered that proteolysis after or during isolation affected the pattern of polypeptides and glycopeptides observed and might account for the extensive amount of galactose oxidase-dependent labeling at the dye front. The electrophoretic patterns of SJs freshly prepared, and those stored at 4°C (pH 7.5) for 5 days, were indistinguishable so that endogenous degradative enzymes, if present, had a negligible effect after isolation. In addition, galactose oxidase did not introduce significant proteolytic activity, since the same patterns of incorporation were observed whether samples were incubated 5 or 20 min in the presence of the enzyme prior to reduction. The possibility that proteolysis occurred during isolation could not be evaluated directly, but circumstantial evidence argues against it. The isolation was carried out at 0°C except for a period of about 30 min at 30°C and at neutral pH, a condition unfavorable for brain acid proteases (23, 24). Brain contains neutral proteases, but these are present at low concentrations and are primarily soluble and microsomal (23) and would be removed early in the isolation procedure. It may be that the extensive galactose oxidase-dependent labeling at the dye front is due to intrinsic glycopeptides and glycolipids. A part of the labeling is perhaps analogous to the extensive labeling of globoside in erythrocytes (13).

$^{125}$I-Con A Binding to SPM, SJ, and PSD Glyco-components—SPM and SJ fractions prior to solubilization bound about the same quantities of $^{125}$I-Con A per mg of protein, while PSD fractions bound twice as much. The Con A-binding glyco-components in SPM, SJ, and PSD fractions were identified on gels by either autoradiography or liquid scintillation counting of gel slices. As shown elsewhere, both techniques show excellent quantitative agreement, but x-ray film autoradiography offers better resolution.

In the SJ fraction, four major components bound Con A. The four components, labeled I, II, III, and IV in Figs. 4 to 6, possessed apparent $M_r$ of 160,000 to 165,000, 123,000, 108,000 to 118,000, and 95,000 to 100,000, respectively. A densitometric scan (Fig. 6) showed that Component III bound 20% of the total $^{125}$I-Con A, IV and I bound 12%, and II bound 9%. Glyco-components at the dye front accounted for 6% of the total bound Con A. The major polypeptides of the SJ fraction (45,000 to 55,000) bound little, if any, $^{125}$I-Con A. As shown in Fig. 5, the relative specific activity of Con A binding in this 45,000 to 55,000 region was at least one-seventieth of either Components I or II (Fig. 5). Only background levels of bound $^{125}$I-Con A were observed when incubations were conducted in the presence of 0.2 m a-methylglucoside (Fig. 6), and the same relative labeling patterns were observed over a wide range of Con A concentrations. The indirect histochemical method using horseradish peroxidase to visualize glyco-components (19) gave qualitatively similar results (Fig. 4A).

Components I to IV were completely degraded by the proteolytic enzymes, trypsin (1 µg/ml), or pronase (2 µg/ml), so they
are glycoproteins. Protolysis resulted in the appearance of all binding to material at the dye front. Treatment of SJs with neuraminidase (5 μg/ml), which removed 85% of the trichloroacetic acid-insoluble sialic acid, did not alter the I-125-Con A binding pattern to SJs (not shown).

The I-125-Con A-binding patterns for all three synaptic fractions were compared (Fig. 6). The SPM fraction contained Con A-binding glycoproteins not present in the SJ fraction. Specifically, in the SPM fraction Con A bound to two broad Mr regions: (a) a rather complex region from 85,000 to 165,000, and (b), a large peak in the 45,000 to 52,000 region. SJs contained very little, if any, Con A binding to the polypeptides present in the 45,000 to 52,000 region.

The PSD fraction showed little labeling of the major glycoproteins present in the SJ fraction (Fig. 6). The major amount of Con A binding in the PSD fraction was localized to the dye front, with smaller amounts of I-125-Con A bound to components with the Mr of the SJ components, I and III. Since 8% acrylamide gels used in these experiments can only resolve glycoproteins whose Mr are ~25,000, additional binding studies were carried out using exponential gradient gels (8 to 20% polyacrylamide). Major Con A-binding components were not present in the 6,000 to 25,000 region for either PSD, SPM, or SJ fractions. Rather, the only Con A-binding components moved with the dye front, so they have an apparent Mr of 6,000 or less. Extraction of PSDs with chloroform/methanol (1/1, v/v) did not alter the Con A binding to the dye front so this material is unlikely to be lipid.

**DISCUSSION**

In this paper we have analyzed the protein and glycoprotein composition of isolated components of central nervous system synapses.

**Polypeptide Composition**—The polypeptides of the SJ fraction are distinct from SPM and PSD fractions, but closely resemble those of the SPM fraction. In the SJ fraction the 32,000 band appears enriched in free amino groups, probably lysine, since it is heavily labeled by pyridoxal phosphate-borohydride treatment. Previously it has been suggested that an enrichment of basic amino acids in SJ proteins is responsible for the distinctive staining properties of the synapse with ethanolic phosphotungstic acid (25) and bismuth iodide-uranyl lead ion (26, 27), and this band may account in part for these properties.

In contrast to the SJ fraction, in the PSD fraction about half of the total protein is a polypeptide(s) with an apparent Mr of 52,000. A band of 55,000 comprises about 14% of the total, while five other polypeptides of higher Mr make up the remaining protein.

The polypeptides with Mr of 45,000, 52,000, and 55,000 in the SJ and PSD fractions are particularly significant because they appear to represent the fibrous proteins, actin, tubulin, and possibly neurofilament protein. The SJ fraction contains a prominent band at 45,000 which co-migrates with actin. In electron microscopic studies using heavy meromyosin, actin had been shown to be associated with the PSD (28). Thus cytochemical and biochemical data indicate that this band probably contains actin. The band with a Mr of 55,000 migrates with tubulin and cross-reacts with an antibody produced against tubulin. Therefore, the 55,000 Mr component appears closely related or identical to tubulin. In tissue sections microtubules have been shown to contact the PSD (29), and an antiserum prepared against a tubulin fraction has been shown by histochemistry to bind to the PSD (30). The identity of the band at 52,000 in SJs in presently unknown, but it has a similar Mr, and cross-reacts, although rather weakly, with an antibody to neurofilament protein. It may be neurofilament protein or a closely related species. PSDs retain primarily the 52,000 component; the other bands appear less integral to the PSD, since they are lost in purification.

The major PSD proteins appear to be covalently linked by disulfide bonds into supramacromolecular aggregates. In the absence of reduction by mercaptoethanol, proteins are excluded from the gel so they must have an apparent Mr of more than 500,000. Thus it would appear that the fibrous proteins at

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the postsynaptic membrane are cross-linked into a distinctive matrix. However, a direct demonstration of intermolecular disulfide bonds is necessary before a final conclusion can be made. Tubulin has 8 to 10 sulphydryl groups (31), and Mellon and Rebhun (32) recently discovered that the in vitro polymerization of tubulin depends upon its free sulphydryl content. It may be that tubulin and other PSD proteins become cross-linked by disulfide bonds as they interdigitate with the membrane and differentiate into part of the SJ. The cross-linking of the PSD proteins by disulfide bonds may help explain why the PSD resists detergent treatment and can be isolated as an intact organelle (9).

The major polypeptides comprising the PSD probably form its essential structural matrix with smaller quantities of other proteins associated with it. PSDs are prepared from heterogeneous cell populations and synaptic types. The relatively simple protein pattern of PSDs indicates that there are insufficient polypeptides to represent each synaptic type. Thus the PSD appears made primarily from polypeptides common to all asymmetric synapses in brain. The increase in the complexity in SJs over PSDs may reflect the addition of specialized proteins to a common macromolecular design shared by most synapses or it may represent subsets of proteins unique to specific synaptic types added to a common design. We have found that the pattern of SJ polypeptides is similar whether prepared from whole brain or the hippocampus, a brain region where there are primarily two cell types receiving on the order of five major synaptic inputs (Fig. 4B). This suggests that central nervous system synapses are composed of a common homogeneous synaptic population to test this directly.

Glycoprotein Composition—Most, if not all, polypeptides present in the SJ fraction are glycoproteins and contain galactosyl residues, as indicated by their labeling with tritiated borohydride following galactose oxidase treatment. The major polypeptides in the 45,000 to 70,000 region appear relatively poor in galactosyl residues. In the SJ fraction, four bands each with a Mr greater than 95,000 bind the majority of Con A. The SJ polypeptides in the 45,000 to 52,000 region bind little, if any, Con A but the SPM fraction shows extensive labeling in this Mr region. Thus SJ glycoproteins appear to be distinct from those present in synaptic membranes. The SPM glycoproteins with a Mr of 50,000 to 52,000 are probably primarily extrajunctional, since they are a major component of the SPM and a minor component in isolated junctions. The identification of SJ glycoproteins which bind £1-Con A is the first characterization of the macromolecules which bind this lectin at the synapse (33). These glycoproteins are likely to be located on the external face of the postsynaptic membrane just within the cleft, since this is the only site at which ferritin-Con A conjugates bind to isolated SJs. Thus the Con A-binding polypeptides in the SJ fraction appear to be the first identified glycoproteins which reside within the synaptic cleft in the central nervous system. All of the SJ glycoproteins binding Con A may not be present in the cleft, but certainly the majority must reside there.

The extent to which SJ polypeptides bind Con A differs from the extent to which they are labeled following galactose oxidase-borohydride treatment. This suggests that the carbohydrate composition of individual SJ glycoproteins is different.

In the PSD fraction the high Mr Con A glycoproteins present in the SJ (Components I to IV) are relatively sparse. The majority of Con A is bound to material at the dye front where there is little, if any, Coomassie blue staining. This component is highly unlikely to be lipid or ganglioside, since it was not extracted with chloroform/methanol. At present, it is unidentified, but it may be a specific type of lipopolysaccharide or a glycoprotein. Since isolated PSDs are very rich in mannose and glucose relative to SJs (8), this component must represent the major source of these sugars.

In summary, SJs are composed of proteins and glycoproteins. The polypeptides in the 45,000 to 55,000 region appear to represent the major components of the PSD and are probably actin, tubulin, neurofilament protein, or closely related proteins. Disulfide bonds appear to unite the PSD polypeptides into supramolecular aggregates. The majority of other SJ proteins either bind Con A or are labeled by galactose oxidase-borohydride and therefore are glycoproteins. The Con A-binding glycoproteins appear located primarily on the external surface of the postsynaptic membrane within the cleft. Thus the synaptic junction appears made primarily from membrane glycoproteins and a matrix of fibrous proteins interconnected by disulfide bonds.

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Identification of glycoproteins and proteins at synapses in the central nervous system.
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