

PROTEINS OF THE POSTSYNAPTIC DENSITY

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ABSTRACT

An analysis was made of the protein composition of a fraction of postsynaptic densities (PSDs) prepared from rat brain. Protein makes up 90% of the material in the PSD fraction. Two major polypeptide fractions are present, based on sodium dodecyl sulfate polyacrylamide gel electrophoresis. The major polypeptide fraction has a molecular weight of 53,000, makes up about 45% of the PSD protein, and comigrates on gels with a major polypeptide of the synaptic plasma membrane. The other polypeptide band has a molecular weight of 97,000, accounts for 17% of the PSD protein, and is not a prominent constituent of other fractions. Six other polypeptides of higher molecular weight (100,000–180,000) are consistently present in small amounts (3–9% each). The PSD fraction contains slightly greater amounts of polar amino acids and proline than the synaptic plasma membrane fraction, but no amino acid is usually prominent. The PSD apparently consists of a structural matrix formed primarily by a single polypeptide or class of polypeptides of 53,000 molecular weight. Small amounts of other specialized proteins are contained within this matrix.

The postsynaptic density (PSD)¹ is a characteristic feature of most synapses within the central nervous system. In the preceding paper (Cotman et al., 1974) we reported that PSDs may be isolated by detergent treatment of synaptic membrane fractions that contain intact synaptic junctional com-

plexes. In the isolation process the PSDs are detached from the plasma membrane, but retain their characteristic barlike or disklike shape and their special cytochemical properties. The isolation of a PSD fraction now enables a direct chemical characterization of PSD. This paper describes the protein composition of fractions of PSDs isolated from rat brain.

Previous studies have indicated that proteins are the principal macromolecular constituents of the PSD. Treatment with proteolytic enzymes degrades PSDs (Bloom and Aghajanian, 1966, 1968; Cotman and Taylor, 1972) while treatment with enzymes that hydrolyze DNA, RNA, or complex carbohydrates has no obvious effect (Bloom and Aghajanian, 1966, 1968). Other studies indicate

¹ *Abbreviations used in this paper:* C, bisacrylamide concentration expressed as 100 × grams bisacrylamide/T; INT, *p*-iodonitrotetrazolium violet; K_R , retardation coefficient; MW, molecular weight; M_o , free electrophoretic mobility; NLS, *N*-lauroyl sarcosinate; PSD, postsynaptic density; R_r , relative mobility; SDS, sodium dodecyl sulfate; T, acrylamide concentration expressed as grams of acrylamide plus grams of bisacrylamide per 100 ml of solution.

that specialized proteins are present in PSDs. PSDs have a special affinity for certain heavy metal stains that has been attributed to the presence of distinctive proteins at this site (Bloom and Aghajanian, 1968; Pfenninger, 1971). Histochemical studies have also revealed a prominent phosphodiesterase activity that is associated with PSDs in the cerebral cortex (Florendo et al., 1971). These studies, although based on indirect methods, all point to the importance of proteins as constituents of the PSD. Direct chemical studies are now needed to verify and extend these results.

MATERIALS AND METHODS

Subcellular Fractionation

Subcellular fractions were prepared from the forebrains of Sprague-Dawley rats, 40–60 days of age. Preparation methods are thoroughly described in the preceding paper (Cotman et al., 1974). A synaptic membrane fraction containing intact synaptic junctional complexes was obtained by sucrose density gradient centrifugation and treated with *N*-lauroyl sarcosinate (NLS). The residue was separated by density gradient centrifugation to yield a fraction of highly purified PSDs (banding at the interface between 1.4 and 2.2 M sucrose). A fraction of NLS-insoluble membrane vesicles was also obtained (banding at the interface between 0 and 1.0 M sucrose). For comparison, fractions enriched in myelin, mitochondria, and synaptic complexes were prepared as previously described (Cotman et al., 1974) and were treated with NLS under the same conditions used to prepare PSDs.

Sodium Dodecyl Sulfate (SDS)

Gel Electrophoresis

The methods for SDS gel electrophoresis were based on those of Fairbanks et al. (1971) and have been essentially described previously (Banker et al., 1972; Banker and Cotman, 1972). Samples were solubilized in 2% (wt/vol) SDS, 40 mM dithiothreitol, 1 mM EDTA, 10 mM Tris-HCl, pH 8.0 and heated at 90°–100°C for 2–5 min (Weber et al., 1972). Residual insoluble material, which consisted chiefly of aggregated formazan, was then removed by centrifugation at 144,000 *g* for 60 min. Electrophoresis was routinely carried out in gels containing 5.3% (wt/wt) acrylamide, 0.21% *N,N'*-methylenebisacrylamide. For measurements of molecular weight, gels contained 2.5 *C* and 3, 4, 5, or 6 *T*. Electrophoresis was performed until the tracking dye, Pyronine Y, had migrated 8 cm. Then the gels were stained with Coomassie blue as described by Fairbanks et al. (1971) and the mobility (R_f) of each polypeptide relative to Pyronine Y was determined.

Molecular weight estimates were made by relating the

relative mobility of unknown proteins to those of standard proteins of known molecular weight, assuming a linear relationship between R_f and the logarithm of molecular weight (Shapiro et al., 1967; Weber and Osborn, 1969). In addition, the retardation coefficient (K_R), a measure of effective molecular size, and the free electrophoretic mobility (M_o) were measured for each polypeptide. Protein mobility was measured at four different gel concentrations ($T = 3, 4, 5, 6$) and K_R and M_o were obtained according to the following equation (Ferguson, 1964; Rodbard and Chrambach, 1970, 1971):

$$\log R_f = \log M_o - K_R T.$$

The calculated retardation coefficients were then used to provide a second estimate of molecular weight (MW), assuming a linear relationship between $K_R^{1/2}$ and $MW^{1/2}$ (Rodbard and Chrambach, 1971; Banker and Cotman, 1972).

Proteins used for molecular weight calibration, together with their assumed molecular weights, were: cytochrome *c* (11,700), ribonuclease A (13,700), myoglobin (17,200), gamma-globulin, light chain (23,500), chymotrypsinogen A (25,700), lactate dehydrogenase (36,000), aldolase (40,000), ovalbumin (43,000), gamma-globulin, heavy chain (50,000), glutamate dehydrogenase (56,000), catalase (60,000), bovine serum albumin (68,000), phosphorylase A (94,000), beta-galactosidase (130,000), and myosin (194,000). All were obtained from commercial sources except myosin, which was prepared from rat skeletal muscle by the method of Perry (1955), as modified by Trayer and Perry (1966).

Alternate Methods of Solubilization

Solubilizing agents other than SDS were also used in attempts to reduce large polypeptides to smaller subunits. Samples were incubated briefly in lithium diiodosalicylate (1.0 M in 4 mM EDTA, 2% β -mercaptoethanol, 40 mM Tris, pH 7.0) at 80°C (Marchesi and Andrews, 1971), or in 2-chloroethanol (75% in 0.01 N HCl) at room temperature (Zahler and Wallach, 1967). Other samples were solubilized by boiling in 8 M guanidine-HCl and reduced and alkylated according to the method of Weber et al. (1972). After solubilization the samples were centrifuged at 144,000 *g* for 60 min to remove any remaining particulate material. They were then prepared for electrophoresis by dialysis against 1% SDS, 1% β -mercaptoethanol, 1 mM EDTA in 10 mM Tris, pH 8.0, and any insoluble residue was removed by centrifugation as before.

Analytical Methods

Protein concentrations were estimated by the method of Lowry et al. (1951). Amino acid analysis was performed by the method of Spackman et al. (1958) after hydrolysis in 6 N HCl at 105°C for 24 h. Lipid was extracted with chloroform:methanol following the proce-

dure of Bligh and Dyer (1959) as modified by Churchill et al.²

Reagents

N-Lauroyl sarcosinate, sodium salt was obtained from K and K Laboratories, Inc. (Plainview, N. Y.). Sodium dodecyl sulfate and Coomassie blue were obtained from Schwarz/Mann Div., Becton, Dickinson & Co. (Orangeburg, N. Y.). Acrylamide and *N,N'*-methylene-bisacrylamide were obtained from Bio-Rad Laboratories (Richmond, Calif.). Lithium diiodosalicylate and 2-chloroethanol were obtained from Eastman Kodak Co. (Rochester, N. Y.). Pyronine Y was obtained from Mann Research Labs. Inc. (New York). Other reagents were obtained from standard suppliers and were of analytical grade.

RESULTS

Composition of the PSD

The high buoyant density of the PSD, about 1.24 g/cm³, immediately suggests that this structure is high in protein and low in lipid content. To investigate its composition directly, material from the PSD fraction was dried and extracted with chloroform and methanol (1:1 by volume). The chloroform-methanol-insoluble residue contained 79 ± 6% of the dry weight. The chloroform-methanol extract includes bound formazan, the product resulting from reduction of *p*-iodonitrotetrazolium violet (INT) during the isolation procedure, as well as lipid. Attempts to estimate the amount of bound formazan by absorbance measurements at 495 nm after extraction into chloroform² indicated that it represented up to 50% by weight of the material in the chloroform-methanol extract. On this basis, only about 10% of the material in the PSD fraction is lipid. Carbohydrates have been detected in this fraction in small amounts (about 3%²), but proteins must account for nearly 90% of the PSDs.

The polypeptides present in the PSD fraction were examined after solubilization in SDS. Treatment of the PSD fraction with SDS (2% at 90°–100°C) solubilizes most of the PSD protein, but leaves a small residue of protein (9 ± 3%) that

is not solubilized (pelleted after 6.3 × 10⁶ g-min). The solubilized polypeptides were separated by electrophoresis on polyacrylamide gels containing SDS. A photograph of a typical gel stained with Coomassie blue, and a densitometric tracing of a similar gel, are presented in Fig. 1. The pattern of polypeptides present is remarkably simple. A single polypeptide band (*B* in Fig. 1) is clearly the major constituent in the PSD. Estimates obtained from densitometry of stained gels indicate that this polypeptide fraction accounts for about 45% of the protein present on gels of the PSD fraction (Table I). A second polypeptide band of lower mobility (*A* in Fig. 1) is also a prominent constituent of the PSD fraction, accounting for about 17% of the total protein. Six other polypeptides of still lesser mobility can be consistently detected on gels of the PSD fraction, which account for all but a few percent of the remaining protein on the gel. No one of these accounts for more than 3–9% of the protein present.

Since a small amount of material remained insoluble after SDS treatment, it seemed possible that additional different polypeptides might be present in the SDS-insoluble residue. To examine this possibility, the PSD fraction was treated with several other strong dissociating agents before incubation with SDS and electrophoresis on SDS gels. Furthermore, because some of the material in the PSD fraction was relatively resistant to SDS solubilization, it seemed possible that some of the polypeptides separated by electrophoresis might represent protein aggregates. If such aggregates were present, they might be further dissociated by such agents. Several dissociating agents were tried. Samples of the PSD fraction were solubilized in 1.0 M lithium diiodosalicylate (Marchesi and Andrews, 1971) or 75% 2-chloroethanol (Zahler and Wallach, 1967), then dialyzed against SDS, and subjected to gel electrophoresis. Alternatively, the PSD fraction was solubilized and denatured by boiling in guanidine-HCl and reduced and alkylated by the method of Weber et al. (1972). No new components were revealed by any of these procedures although the relative amounts of the six polypeptides of low mobility varied slightly, depending on the solubilization procedure used. Furthermore, none of these treatments resulted in conversion of the two major PSD polypeptides, A and B, to lower molecular weight species of higher mobility.

²Churchill, L., G. Banker, and C. W. Cotman. 1974. Protein and carbohydrate composition of central nervous system synapses: Analysis of isolated synaptic junctional complexes and postsynaptic densities. Manuscript submitted for publication.

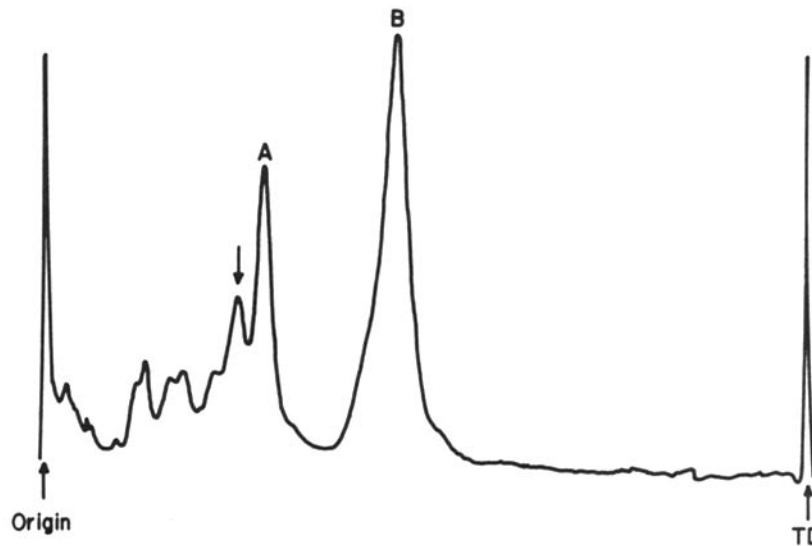
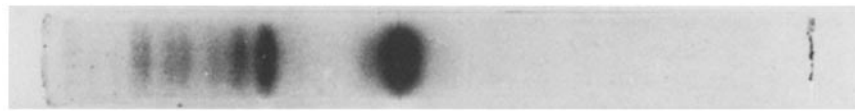


FIGURE 1 A photograph and densitometric tracing of a typical SDS gel of the PSD fraction. The two major polypeptides present are labeled *A* and *B*. A third, less prominent polypeptide (arrow) is also consistently present in this fraction. Gel origin is to the left, the location of the tracking dye (*TD*) to the right. Gels contained 5.3 (wt/wt) acrylamide.

TABLE I
Polypeptides of the PSD Fraction

Polypeptide	Relative amount (% of total)	Molecular weight based on		Mole %
		K_R	R_f	
A	17 ± 2	95,000	98,300	13
B	45 ± 8	54,400	52,600	65
Others (6)	35 (3-9% each)	105,000-175,000	100,000-180,000	2-7 each

Polypeptides were separated by electrophoresis on SDS gels. Relative amounts of each polypeptide were obtained from densitometry of gels stained with Coomassie blue using a Gilford spectrophotometer. Mole % of each polypeptide in the PSD fraction was estimated from relative amount and the average of molecular weight measurements. Measurements were made from five acrylamide gels each utilizing PDSs from a separate isolation.

Measurement of Polypeptide Molecular Weight

The mobilities of the PSD polypeptides during SDS gel electrophoresis were used to determine their molecular weights, taking care to verify that

their electrophoretic properties were comparable to those of the standard proteins used. It is now known that the molecular weights of some proteins cannot be correctly estimated by SDS gel electrophoresis because of unusual relationships between their molecular weight and their effective size or

charge when dissolved in SDS (Trayer et al., 1971; Segrest et al., 1971; Tung and Knight, 1971; Furthmayer and Timpl, 1971). It is possible to detect such anomalies, even without knowledge of a protein's molecular weight, by determining its retardation coefficient (K_R), a measure of effective size, and its free electrophoretic mobility (M_o), a function of charge and size (see Materials and Methods for details). When values of K_R and M_o are compared for different proteins in SDS gel electrophoresis, a linear relationship is normally seen. This relationship is shown for several soluble proteins in Fig. 2 (small circles and line). If a protein deviates from this relationship, it migrates anomalously on SDS gels and its electrophoretic mobility cannot be used to determine its molecular weight (Banker and Cotman, 1972). As shown in Fig. 2 (triangles), however, the values of K_R and M_o for the major PSD polypeptides follow the same linear relationship as the soluble proteins examined. This indicates that valid measurements of molecular weight can be made for the PSD polypeptides using SDS gel electrophoresis.

Accordingly, molecular weight measurements were made for the PSD polypeptides. Determinations were made by two methods. The relative mobilities (R_f) of the PSD proteins were measured and their molecular weights computed by comparison with a plot of $\log MW$ and R_f for standard proteins (Shapiro et al., 1967; Weber and Osborn, 1969). In addition, measurements of molecular

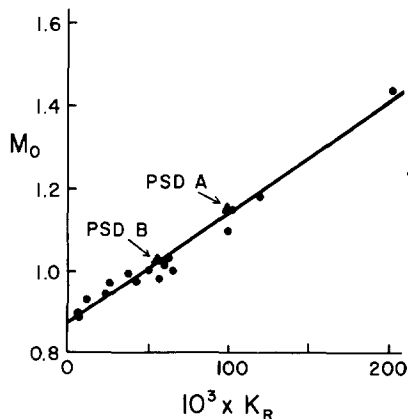


FIGURE 2 The relationship between retardation coefficient (K_R) and free electrophoretic mobility (M_o) for 13 soluble proteins (●) and for the two major polypeptides of the PSD fraction (▲). The line shown is the line that best fits the data for the soluble proteins. The two PSD polypeptides follow the same linear relationship.

weight were made based on the relationship between retardation coefficient and the two-thirds power of molecular weight (Rodbard and Chrambach, 1970, 1971; Banker and Cotman, 1972). As predicted, both relationships were linear for polypeptides of molecular weight from 20,000 to nearly 200,000. Molecular weights of the PSD polypeptides based on these two methods are shown in Table I (columns 2 and 3). In general, the molecular weights calculated by the two methods agreed quite well. The major PSD polypeptide, B, has a molecular weight of about 53,000, and the second most prominent polypeptide is about 97,000. The six minor polypeptides present are all of higher molecular weight.

Based on these values of molecular weight and on the relative amount of each polypeptide present, it is possible to estimate the molar composition of the PSD. This is presented in Table I, column 4. On this basis, polypeptide B accounts for about seven of every 10 polypeptide molecules in the PSD, polypeptide A for about one in 10.

Comparison with Other Fractions

It is of interest to know whether the particular polypeptides present in the PSD are unique to this structure or are common to other structures in the synaptic area such as the plasma membrane. To examine this question, the polypeptides present in a plasma membrane fraction were also examined by SDS gel electrophoresis (Fig. 3). A prominent membrane polypeptide (corresponding to polypeptide C-7 of Banker et al., 1972) had a mobility indistinguishable from that of PSD polypeptide B. (Differences in relative mobilities between the two polypeptides were insignificant: -0.002 ± 0.001 , $+0.002 \pm 0.008$, $+0.006 \pm 0.006$, -0.006 ± 0.004 at $T = 3, 4, 5,$ and 6 , respectively.) Measurements made from electron microscope observations (Cotman et al., 1974) indicate that plasma membrane accounts for only about 10% of the material within the PSD fraction and that PSDs account for only about 4% of the material in the plasma membrane fraction. Therefore, cross-contamination of the fractions could not explain these results. This polypeptide was also present in a membrane vesicle fraction that had been treated with NLS (Fig. 3 c). Thus, polypeptides of 53,000 molecular weight and with similar solubility characteristics are prominent constituents in both the PSD and the neuronal membrane. This does not, of course, establish that these polypeptides are identical.

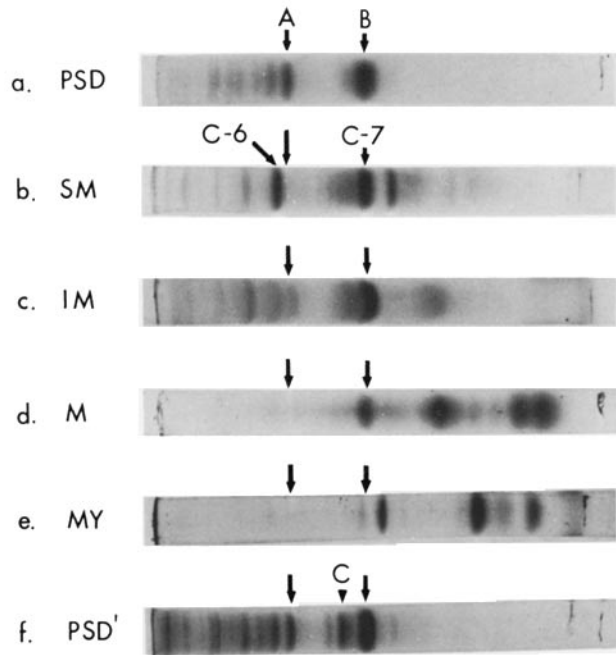


FIGURE 3 Comparison of the polypeptides present in the PSD fraction with those in other fractions. Gels shown from top to bottom are: PSD; SM; the NLS-insoluble residue of synaptic membranes obtained as the membrane fraction banding on 1.0 M sucrose after NLS treatment (IM); the insoluble residue of mitochondria pelleted at 105,000 g for 30 min after NLS treatment (M); the insoluble residue of myelin pelleted at 105,000 g for 30 min after NLS treatment (MY); PSDs prepared with 0.4% NLS (PSD'). Polypeptides were separated on SDS gels containing 5.3% (wt/wt) acrylamide. Arrows in columns indicate the distance of migration of PSD polypeptides A and B. Origin is to the left.

The other major PSD polypeptide, A, is specifically localized to the PSD fraction. It was not discernible in gels of synaptic plasma membranes (Fig. 3 b). Measurements of its mobility show that it is clearly different from the major membrane polypeptide, C-6, migrating ahead of it on gels of four different acetylamine concentrations. (Differences between the two were: $+0.02 \pm 0.001$, $+0.02 \pm 0.007$, $+0.02 \pm 0.004$, $+0.02 \pm 0.004$ at $T = 3, 4, 5,$ and 6 , respectively.) Polypeptide A may be one of the minor components of the fraction containing NLS-extracted membrane vesicles (Fig. 3 c), but it is a prominent component only of the PSD fraction. Some of the polypeptides of higher molecular weight present in the PSD fraction seem to correspond to polypeptides present in the neuronal membranes (Fig. 3 c), while others do not. Because these polypeptides are present in such small amounts, some of them may not be constituents of PSDs, but may be present in contaminants within the PSD fraction.

Estimates based on chemical, enzymatic, and

electron microscope analyses indicate that contamination of the PSD fraction by components other than plasma membrane, such as mitochondria or myelin, cannot be more than a few percent. Nonetheless, if one or two polypeptides were highly concentrated in the NLS-insoluble residue from these contaminant particles, they might be discernible as minor bands on gels of the PSD fraction. To investigate this possibility, fractions enriched in myelin and mitochondria were treated with NLS and the total insoluble residue was subjected to SDS gel electrophoresis. As shown in Fig. 3 (d,e), low molecular weight polypeptides are characteristic of the NLS-insoluble residues of both the myelin and the mitochondrial fraction. None of these low molecular weight polypeptides are present in the PSD fraction, nor do any myelin or mitochondrial polypeptides correspond to minor polypeptides of the PSD fraction. The mobility of one prominent mitochondrial polypeptide appears similar to that of PSD polypeptide B and membrane polypeptide C-7, but measure-

ments of its relative mobility indicate that it migrates slightly slower (see Banker et al., 1972).

It also seemed possible that some of the proteins present in the PSD fraction were soluble proteins or NLS-solubilized proteins that became adsorbed to the PSD during the isolation procedure. Attempts were made to remove any such adsorbed proteins by incubation in solutions of high ionic strength or treatment with chelating agents. Samples were treated with 1 M NaCl or with 5 mM EDTA for 12 h at 2°C, then centrifuged to pellet the remaining insoluble proteins, and subjected to gel electrophoresis. None of the polypeptides in the PSD fraction was removed by this treatment. In addition, incubation in 1 M urea did not result in the loss of substantial quantities of the total PSD protein.

PSDs Prepared with Lower Concentrations of NLS

The detergent treatment used to isolate PSDs may selectively solubilize some PSD constituents. In this connection we examined the polypeptides present in a fraction of PSDs prepared with much lower concentrations of NLS. Such fractions prepared with 0.4% NLS are greatly enriched in PSDs, but also contain substantial membrane contamination (Cotman et al., 1974). The pattern of polypeptides present in such fractions was examined by SDS gel electrophoresis (Fig. 3 *f*). The major PSD polypeptides, A and B, together with the higher molecular weight bands, were present in about the same proportions as before. A few polypeptides typical of plasma membrane fractions were also present. However, one major polypeptide was present, indicated as C in Fig. 3 *f*, that was not present in PSDs prepared with 3% NLS or in plasma membrane (compare with Fig. 3 *a* and 3 *b*). This polypeptide may be a component of the PSD that is selectively solubilized with the high concentration of NLS ordinarily used to prepare PSDs.

Amino Acid Composition

The amino acid composition of the PSD fraction is shown in Table II and is compared with that of the synaptic membrane fraction. Table II shows the composition of the PSD fraction obtained by treatment of such membrane fractions with either 0.4 or 3% NLS and the range of data obtained for brain plasma membrane fractions. The differences

TABLE II
Amino Acid Composition of the PSD Fraction

Amino acid	PSD		SM
	0.4% NLS	3% NLS	
Lysine	58	57	59-66
Histidine	29	29	18-21
Arginine	60	57	40-48
Aspartic acid	90	100	96-105
Threonine	56	60	54-61
Serine	86	83	71-98
Glutamic acid	125	130	110-122
Proline	63	67	44-51
Glycine	78	86	70-78
Alanine	78	87	75-83
Valine	56	49	57-73
Methionine	21	13	19-30
Isoleucine	44	37	43-53
Leucine	88	79	83-93
Tyrosine	31	28	26-32
Phenylalanine	37	31	36-46

Data are expressed as residues/1,000 residues. Data for the PSD fractions are means from two separate experiments for material prepared with each concentration of NLS. Values for SM plasma membranes represent the range of values from two previous reports (Cotman et al., 1968; Churchill et al., footnote 2) and a single determination performed in the course of this investigation.

between the PSD and plasma membrane fractions are small. The PSD fraction shows slightly, but consistently, greater amounts (about 10 residues/1,000 residues) of histidine, arginine, glutamic acid, and proline and correspondingly lower amounts of hydrophobic amino acids.

DISCUSSION

Our results indicate that the macromolecular composition of the PSD is relatively simple. Proteins represent nearly 90% of the material in the PSD fraction. Since small amounts of membrane are present in the fraction, the lipid present could be associated with this contamination. If this is the case, the PSD is composed almost entirely of protein together with a small amount of carbohydrate. A single protein or class of proteins with a polypeptide chain molecular weight of 53,000 accounts for about 45% of the PSD protein. This component may also be a component of synaptic plasma membrane since it comigrates on SDS gels with a major membrane polypeptide band. The

molecular weight of the major PSD polypeptide fraction is also similar to that reported for tubulin, the protein subunit of microtubules (Feit et al., 1971; Olmsted et al., 1971; Weisenberg et al., 1968). Under our conditions of electrophoresis, tubulin prepared from a rat brain-soluble fraction by vinblastine precipitation migrates as a single zone with an apparent molecular weight of 55,000 (unpublished observations), a value very similar to that of the major PSD polypeptide. Further characterization of this polypeptide fraction is essential to determine if it consists of a single polypeptide chain and whether it is related to tubulin or a major membrane protein.

Several other higher molecular weight polypeptides are present in smaller amounts in the PSD fraction. The most prominent of these polypeptides, with molecular weight 97,000, is present in considerable amounts in the PSD fraction and is not a prominent constituent of any other fraction we have examined. Some of these high molecular weight polypeptides may be responsible for some of the special properties of the PSD.

We cannot be sure that only the polypeptides which we have described are present in intact PSDs. The detergent treatment used in preparing PSD fractions may selectively remove some PSD constituents. A possible example of this is the polypeptide labeled *C* in Fig. 3*f*. This polypeptide is present in fractions enriched in PSDs prepared with 0.4% NLS, but is absent from the purer preparations obtained with 3% NLS. From our data it is not possible to decide if this is a PSD polypeptide that is selectively solubilized at high detergent concentrations or is a constituent of some contaminant that is not present in the purer preparations obtained at high detergent concentrations. It is also possible that some of the constituents of the PSD fraction are soluble proteins that have become adsorbed during the isolation procedure. No adsorbed proteins were released from the PSD fraction by treatment with high salt or chelating urea agents, but the presence of adsorbed material resistant to these treatments cannot be ruled out particularly in the case of the minor polypeptides.

It has previously been suggested that PSD proteins are greatly enriched in basic amino acid residues because of the distinctive affinity of PSDs for ethanolic phosphotungstic acid and bismuth iodide uranyl lead stains (Bloom and Aghajanian, 1968; Pfenninger, 1971). The isolated PSDs dem-

onstrate these staining properties, though at a partially reduced intensity, but their amino acid composition is very similar to that of brain plasma membrane fractions which do not stain with these agents. Fractions enriched in synaptic junctional complexes also are very similar to brain plasma membrane in amino acid composition.² It seems very unlikely that the small increase in basic residues (about two residues/100 residues) could by itself account for the distinctive staining properties of the PSD. This raises doubts about the interpretation of the initial cytochemical evidence. It was originally assumed that lack of staining after acetic anhydride treatment implicated basic amino acid residues in the staining process. However, studies with soluble proteins indicate that, of the three basic amino acids commonly found in proteins, only lysine reacts with acetic anhydride (Cohen, 1968; Stark, 1970). In addition, several nonbasic amino acids also react (Cohen, 1968; Riordan and Vallee, 1967 *a, b*; Stark, 1970). The situation is further complicated since staining is performed after glutaraldehyde fixation, which would be expected to block many lysine amino groups (e.g., Habeeb and Hiramoto, 1968). It seems most likely that minor proteins or that special properties of the particular proteins present in the PSD—properties not apparent from their amino acid composition alone—are responsible for the special staining characteristics. This effect could be greatly enhanced by the denser packing of proteins, or by a greater accessibility to the stain, in the PSD than in the membrane lipid bilayer.

Our results suggest some tentative conclusions about the organization of the PSD. If the 53,000 molecular weight fraction consists of a single polypeptide chain, then this polypeptide accounts for about seven of every 10 polypeptides present in the PSD. This suggests that it forms the essential structural matrix of the PSD, with smaller amounts of other specialized proteins associated with it.

The function of the PSD and its composite proteins is unknown at present. The PSD might provide a binding site for specialized proteins that need to be concentrated at the synaptic region (e.g., the localization of phosphodiesterase). In addition, as suggested previously (Cotman and Taylor, 1972, 1974), the PSD may serve to stabilize the overlying postsynaptic membrane and prevent specialized molecules of the postsynaptic membrane from undergoing lateral diffusion. A

number of specialized properties and molecules are associated with the section of the postsynaptic membrane delineated by the outreach of the PSD. Concanavalin A-binding sites (Cotman and Taylor, 1974), aggregates of intramembranous particles (Landis and Reese, 1974), and possibly transmitter receptors are closely confined to this specialized membrane region. The PSD may serve to stabilize these and other components in a manner analogous to the situation in erythrocytes where the lateral diffusion of proteins can be restricted by interactions with proteins on the cytoplasmic surface (Nicolson and Painter, 1973). Analysis and further characterization of PSD proteins may aid in understanding the function of this membrane specialization.

4 yr ago a review of macromolecules in synaptic junction (Bloom, 1970) emphasized the variety of important roles that proteins may play in synaptic function, but could provide little direct data about these constituents. The results described on the isolation of PSDs and the characterization of the proteins present in such fractions indicate one promising approach for studying the specialized macromolecules present at synaptic sites.

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REFERENCES

- BANKER, G. A., and C. W. COTMAN. 1972. Measurement of free electrophoretic mobility and retardation coefficient of protein-sodium dodecyl sulfate complexes by gel electrophoresis. *J. Biol. Chem.* **246**:5856.
- BANKER, G., B. CRAIN, and C. W. COTMAN. 1972. Molecular weights of the polypeptide chains of synaptic plasma membranes. *Brain Res.* **42**:508.
- BLIGH, E., and W. DYER. 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **37**:911.
- BLOOM, F. E. 1970. Correlating structure and function of synaptic ultrastructure. The Neurosciences: Second Study Program. F. O. Schmitt, editor. The Rockefeller University Press, New York. 729.
- BLOOM, F. E., and G. K. AGHAJANIAN. 1966. Cytochemistry of synapses. Selective staining for electron microscopy. *Science (Wash. D. C.)*. **154**:1575.
- BLOOM, F. E., and G. AGHAJANIAN. 1968. Fine structural and cytochemical analysis of the staining of synaptic junctions with phosphotungstic acid. *J. Ultrastruct. Res.* **22**:361.
- COHEN, L. A. 1968. Group-specific reagents in protein chemistry. *Annu. Rev. Biochem.* **37**:695.
- COTMAN, C. W., G. BANKER, L. CHURCHILL, and D. TAYLOR. 1974. Isolation of postsynaptic densities from rat brain. *J. Cell Biol.* **63**:441.
- COTMAN, C., H. MAHLER, and T. HUGLI. 1968. Isolation and characterization of insoluble proteins of the synaptic plasma membrane. *Arch. Biochem. Biophys.* **126**:821.
- COTMAN, C. W., and D. TAYLOR. 1972. Isolation and structural studies on synaptic complexes from rat brain. *J. Cell Biol.* **55**:696.
- COTMAN, C. W., and D. TAYLOR. 1974. Localization and characterization of concanavalin A receptors in the synaptic cleft. *J. Cell Biol.* **62**:236.
- FAIRBANKS, G., T. L. STECK, and D. F. H. WALLACH. 1971. Electrophoretic analysis of the major polypeptides of the human erythrocyte membrane. *Biochemistry*. **10**:2606.
- FEIT, H., L. SLUSAREK, and M. L. SHELANSKI. 1971. Heterogeneity of tubulin subunits. *Proc. Natl. Acad. Sci. U. S. A.* **68**:2028.
- FERGUSON, K. A. 1964. Starch gel electrophoresis—application to the classification of pituitary proteins and polypeptides. *Metab. (Clin. Exp.)*. **13**:985.
- FLORENDO, N., R. BARNETT, and P. GREENGARD. 1971. Cyclic 3',5'-nucleotide phosphodiesterase: Cytochemical localization in cerebral cortex. *Science (Wash. D. C.)*. **173**:745.
- FURTHMAYER, H., and R. TIMPL. 1971. Characterization of collagen peptides by sodium dodecyl sulfate-polyacrylamide electrophoresis. *Anal. Biochem.* **41**:510.
- HABEEB, A. F. S. A., and R. HIRAMOTO. 1968. Reaction of proteins with glutaraldehyde. *Arch. Biochem. Biophys.* **126**:16.
- LANDIS, D. M., and T. S. REESE. 1974. Differences in membrane structure between excitatory and inhibitory synapses in the cerebellar cortex. *J. Comp. Neurol.* **155**:93.
- LOWRY, O., N. ROSENBOUGH, A. FARR, and R. RANDALL. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**:265.
- MARCHESI, V. T., and E. P. ANDREWS. 1971. Glycoproteins: Isolation from cell membranes with lithium diiodosalicylate. *Science (Wash. D. C.)*. **174**:1247.
- NICOLSON, G. L., and R. G. PAINTER. 1973. Anionic sites of human erythrocyte membranes. II. Antispectrin-induced transmembrane aggregation of binding sites for positively charged colloidal particles. *J. Cell Biol.* **59**:395.
- OLMSTED, J. B., G. B. WITMAN, K. CARLSON, and J. L. ROSENBAUM. 1971. Comparison of the microtubule proteins of neuroblastoma cells, brain, and *Chlamydomonas flagella*. *Proc. Natl. Acad. Sci. U. S. A.* **68**:2273.

- PERRY, S. V. 1955. Myosin adenotriphosphatase. *Methods Enzymol.* **2**:582.
- PFENNINGER, K. H. 1971. The cytochemistry of synaptic densities. I. An analyses of the bismuth iodide impregnation method. *J. Ultrastruct. Res.* **34**:103.
- RIORDAN, J. F., and B. L. VALLEE. 1967 *a*. Acetylation. *Methods Enzymol.* **11**:565.
- RIORDAN, J. F., and B. L. VALLEE. 1967 *b*. *O*-Acetyltyrosine. *Methods Enzymol.* **11**:570.
- RODBARD, D., and A. CHRAMBACH. 1970. Unified theory for gel electrophoresis and gel filtration. *Proc. Natl. Acad. Sci. U. S. A.* **65**:970.
- RODBARD, D., and A. CHRAMBACH. 1970. Estimation of molecular radius, free mobility and valence using polyacrylamide gel electrophoresis. *Anal. Biochem.* **40**:95.
- SEGRETT, J., R. JACKSON, R. ANDREWS, and V. MARCHESI. 1971. Human erythrocyte membrane glycoprotein: A re-evaluation of its molecular weight as determined by SDS polyacrylamide gel electrophoresis. *Biochem. Biophys. Res. Commun.* **44**:390.
- SHAPIRO, A. L., E. VIÑUELA, and J. V. MAIZEL, JR. 1967. Molecular weight estimation of polypeptide chains by electrophoresis in SDS-polyacrylamide gels. *Biochem. Biophys. Res. Commun.* **28**:815.
- SPACKMAN, D., S. MOORE, and W. STEIN. 1958. Automatic recording apparatus for use in the chromatography of amino acids. *Anal. Chem.* **30**:1190.
- STARK, G. R. 1970. Recent developments in chemical modification and sequential degradation of proteins. *Adv. Protein Chem.* **24**:261.
- TRAYER, H. R., Y. NOZAKI, J. A. REYNOLDS, and C. TANFORD. 1971. Polypeptide chains from human red blood cell membranes. *J. Biol. Chem.* **246**:4485.
- TRAYER, F. P., and S. V. PERRY. 1966. The myosin of developing skeletal muscle. *Biochem. Z.* **345**:87.
- TUNG, J., and C. KNIGHT. 1971. Effect of charge on the determination of molecular weight of proteins by gel electrophoresis in SDS. *Biochem. Biophys. Res. Commun.* **42**:1117.
- WEBER, K., and M. OSBORN. 1969. The reliability of molecular weight determination by dodecyl sulfate-polyacrylamide gel electrophoresis. *J. Biol. Chem.* **244**:4406.
- WEBER, K., J. R. PRINGLE, and M. OSBORN. 1972. Measurement of molecular weights by electrophoresis on SDS-acrylamide gel. *Methods Enzymol.* **26**:3.
- WEISENBERG, R. C., G. G. BORISY, and E. W. TAYLOR. 1968. The colchicine-binding protein of mammalian brain and its relation to microtubules. *Biochemistry.* **7**:4466.
- ZAHLER, P. H., and D. F. H. WALLACH. 1967. Isolation of lipid-free plasma membrane proteins by gel filtration on Sephadex LH-20 using 2-chloroethanol-water as solvent. *Biochim. Biophys. Acta.* **135**:371.