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Purification of the saxitoxin receptor of the sodium channel from rat brain

(electrical excitability/tetrodotoxin/ion transport)

ROBERT P. HARTSHORNE AND WILLIAM A. CATTERALL

Department of Pharmacology, University of Washington, Seattle, Washington 98195

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ABSTRACT **The saxitoxin (STX) receptor has been purified 740-fold from rat brain by a combination of ion exchange chromatography, wheat germ agglutinin chromatography, and sedimentation on sucrose gradients to a specific activity of 1488 pmol/mg of protein. The best fractions were estimated to be 47% pure from their specific activity or 66% pure on the basis of $NaDodSO_4$ gel electrophoresis. Two polypeptides, α ($M_r \approx 270{,}000 \pm 10{,}000$) and β ($M_r \approx 38{,}300 \pm 2000$) (mean ± SD) copurify with STX binding activity. Two polypeptides of the same apparent M_r are specifically covalently labeled by photoreactive derivatives of ^{125}I-labeled scorpion toxin in rat brain synaptosomes and are likely to be identical to α and β. The solubilized STX receptor has a M_r of 316,000 ± 63,000, limiting its composition to one α polypeptide and one or more β polypeptides per soluble receptor. Our results suggest that the α and β polypeptides contain both the STX binding site and the scorpion toxin binding site of the mammalian sodium channel.**

Voltage-sensitive Na channels mediate the changes in Na conductance associated with the action potential in a wide variety of excitable tissues. Although a great deal is known about the function of the Na channel from electrophysiological studies, little is known about the molecular mechanisms underlying these functions or the molecular structure of the Na channel itself. A useful approach for obtaining this information is to use neurotoxins specific for the Na channel to identify its molecular components and to serve as markers for them during purification. Three separate neurotoxin receptor sites have been identified on the Na channel from neuroblastoma cells and rat brain synaptosomes (1–3). The first receptor site binds saxitoxin (STX) and tetrodotoxin (TTX), causing inhibition of ion flux (4). The second receptor site binds the lipid-soluble toxins batrachotoxin, veratridine, aconitine, and grayanotoxin which alter the voltage dependence of Na channel activation and inactivation, leading to persistent activation (1). The third receptor site binds the polypeptides scorpion toxin and sea anemone toxin II in a voltage-dependent manner, blocking inactivation of the Na channel (1).

The scorpion toxin receptor in rat brain synaptosomes and the neuroblastoma cell line N 18 has been covalently labeled by a photoactivable ^{125}I-labeled derivative of scorpion toxin (5). Autoradiograms of $NaDodSO_4$/polyacrylamide gels of covalently labeled rat brain synaptosomes showed two specifically labeled polypeptides of $M_r \approx 250{,}000$ and 32,000.

The receptor site for STX and TTX was successfully solubilized from garfish olfactory nerve membranes by nonionic detergents as early as 1972, but the extreme lability of the solubilized receptor prevented purification (6, 7). More recently, stabilization of the solubilized receptor by phosphatidylcholine and TTX (8, 9) or by phosphatidylcholine and Ca^{2+} (10) has allowed substantial purification of the STX/TTX receptor from eel electroplax (8), rat skeletal muscle sarcolemma (11), and rat brain (12).

In this report, we describe purification of the STX receptor from rat brain and compare the polypeptide components of the purified brain receptor with those covalently labeled with photoreactive scorpion toxin derivatives in intact brain membranes.

EXPERIMENTAL PROCEDURE

Materials. STX was obtained from the National Institutes of Health. Stock solutions of protease inhibitors active against each of the four classes of protease (13) were made fresh daily and just prior to use were diluted 1:1000 into every solution coming in contact with the STX receptor. The solutions were 1 M 1,10-phenanthroline, 1 mM pepstatin A, and 0.1 M phenylmethylsulfonyl fluoride in anhydrous acetone; and 1 M iodoacetamide in distilled water. STX was tritiated by the specific exchange procedure of Ritchie *et al.* (14) and was purified and characterized as described (10). The [^{3}H]STX used had a specific activity of 18.6 Ci/mmol (1 Ci = 3.7×10^{10} becquerels) and a radiochemical purity of 85%.

Measurement of [^{3}H]STX Binding. [^{3}H]STX binding was measured by the rapid gel filtration method, as described (8, 10), after incubation for 10 min with 5 nM [^{3}H]STX. Under the conditions of this assay, the K_d for saxitoxin is 0.16 nM. Nonspecific binding was determined in the presence of 1 μM TTX and subtracted from total binding in the data shown.

Membrane Preparation and Solubilization. A crude synaptosomal preparation was made by a procedure modified from Gray and Whittaker (15), and a lysed P3 preparation was obtained as described (10). The lysed brain membranes were solubilized as described (10). $CaCl_2$ (1.0 M) was added to a final concentration of 10 mM, the pH of the membrane extract was adjusted to 6.5 with 0.25 M histidine·Tris (pH 5.5), and the conductivity was adjusted to 7.2 mmho (1 mho = 1 S) with 1 M choline chloride.

Ion Exchange Chromatography. A 5 × 12.5 cm column of DEAE-Sephadex A-25 was preequilibrated with solution A (175 mM choline chloride/20 mM histidine·HCl adjusted to pH 6.5 with Tris base/10 mM $CaCl_2$/0.1% Triton X-100/0.02% phosphatidylcholine) containing protease inhibitors. The column was loaded with membrane extract, washed with 300 ml of solution A, and eluted with 600 ml of 175–1000 mM choline chloride gradient in solution A. Fractions eluting between choline chloride concentrations of 270 and 480 mM were loaded directly on a wheat germ agglutinin column.

Wheat Germ Agglutinin (WGA)-Sepharose 4B Chromatography. WGA-Sepharose 4B was prepared at a final coupling

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Abbreviations: STX, saxitoxin; TTX, tetrodotoxin; WGA, wheat germ agglutinin.

density of 10 mg of WGA per ml of swollen resin exactly as described in the Pharmacia literature supplied with CNBr-activated Sepharose 4B. Resin (10 ml) was packed in a 1 × 15 cm column and equilibrated with solution B (0.4 M KCl/20 mM Hepes adjusted to pH 7.4 with Tris base/10 mM $CaCl_2$/0.1% Triton X-100/0.02% phosphatidylcholine) containing protease inhibitors. After the peak from the ion exchange column had run through, the WGA column was washed with 50 ml of solution B. The column was then eluted with 50 ml of 0–40 mM *N*-acetylglucosamine gradient in solution B at 0.8 ml/min.

Sucrose Gradient. The six fractions from the WGA column containing the most STX binding activity were pooled and 0.4-ml aliquots were layered on 3–14% or 5–20% linear sucrose gradients in solution C (100 mM KCl/5 mM Hepes adjusted to pH 7.4 with Tris base/10 mM $CaCl_2$/0.1% Triton X-100/0.02% phosphatidylcholine) containing protease inhibitors. The samples were sedimented at 200,000 × *g* for 18 hr at 4°C in an SW 40 rotor. Alternatively, 1-ml aliquots were layered on 3–20% linear sucrose gradients in solution C and sedimented for 5.5 hr at 210,000 × *g* in a Ti 50 vertical rotor. The gradients were fractionated from the bottom, and equivalent fractions were pooled.

Protein Assay. Protein concentrations were determined by using Peterson's (16) modification of the Lowry assay with bovine serum albumin as a standard.

$NaDodSO_4$ Gel Electrophoresis. Samples for Coomassie blue-stained gels were prepared by precipitation from 10% trichloroacetic acid and sedimentation at 1500 × *g* for 30 min. The pellets were washed with 95% acetone and then 95% ethanol and finally were dissolved by boiling for 3 min in 80 μl of 3% $NaDodSO_4$/50 mM Tris, pH 9.5/1 mM EDTA/10% sucrose/10 mM dithiothreitol/0.01% phenol red under N_2. The samples were incubated for 30 min at 22°C, iodoacetamide was added to a final concentration of 40 mM, and the samples were incubated 1 hr under N_2 in the dark. 2-Mercaptoethanol was added to a final concentration of 5%, the pH was adjusted to 7.4 with 0.8 M NaOH, and the samples were boiled 5 min and loaded on the gel. Samples for silver-stained gels were similarly prepared except that the precipitation and washing steps were omitted.

The discontinuous gel system used was that described by Maizel (17). A 4.5–15% acrylamide gradient was used in the running gel, and the cathodic buffer reservoir contained 2 mM sodium thioglycolate. Gels were stained with Coomassie brilliant blue R-250 from Bio-Rad or the silver stain described by Oakley *et al.* (18). Protein M_r standards were thyroglobulin (330,000), ferritin (220,000 and 18,000), bovine serum albumin (68,000), catalase (60,000), and lactate dehydrogenase (36,000) from Pharmacia and myosin (200,000) and β-galactosidase (117,000) from Sigma.

RESULTS

The STX receptor solubilized from rat brain membranes was purified by a combination of conventional techniques: ion exchange chromatography, affinity chromatography on WGA-Sepharose, and velocity sedimentation through sucrose gradients. These techniques resulted in 740-fold purification of the STX receptor from rat brain or 470-fold purification from the original detergent extract to a final specific activity of 1488 pmol/mg of protein for the best fraction. The results of each purification step averaged from several preparations are summarized in Table 1.

Solubilization and Stabilization of the STX Receptor. A lysed crude synaptosomal fraction prepared from rat brain by differential centrifugation was used as the starting material for purification. This fraction included both axonal membrane fragments and synaptosomes and contained 80% of the STX receptor sites in the starting brain homogenate (10). We have shown (10) that the STX receptor can be solubilized from this membrane fraction by the nonionic detergent Triton X-100; with an 8-mg/ml suspension of lysed brain membranes, the optimal Triton X-100 concentration, 1% (wt/vol), solubilized 35–40% of the STX receptor and a similar percentage of the total membrane protein (10).

Table 1. Purification of STX receptor

Step	STX receptor pmol	%	Protein mg	%	Specific activity, pmol/mg* Mean	Best
Triton extract	2789	100	878	100	3.2	—
DEAE-Sephadex	811	29	29	3.3	28.1	53.1
WGA-Sepharose	426	15	1.4	0.16	310.2	408
Sucrose gradient	212	8	0.20	0.02	1046	1488

Mean specific activity represents the values for the pooled fractions used for further purification. Best specific activity represents the values for the individual fractions of highest specific activity.

The solubilized STX receptor from rat brain is very labile [$\tau_{1/2} < 1$ min at 22°C (10)]. Agnew *et al.* (8, 9) have shown that the stability of the solubilized TTX receptor from eel electroplax is critically dependent upon the phospholipid-to-detergent ratio and the temperature. The solubilized rat brain STX receptor similarly requires 10 mM Ca^{2+}, low temperature, and a phosphatidylcholine-to-Triton X-100 ratio of 1:5 for stability (10). Therefore, all purification steps were carried out at 0–4°C in buffers containing 0.1% Triton X-100, 0.02% egg yolk phosphatidylcholine, and 10 mM $CaCl_2$ to stabilize the STX receptor.

Ion Exchange Chromatography. Preliminary studies showed that the solubilized STX receptor from rat brain binds to the anion exchange resin DEAE-Sephadex A-25 in the pH range 5.0–8.5. These results indicated that the STX receptor is an acidic protein and suggested that anion exchange chromatography could be a useful purification tool. The solubilized STX receptor was loaded onto a preequilibrated DEAE-Sephadex column at the highest ionic strength at which the STX receptor is bound by the column. The column then was washed with 2 vol of buffer of the same pH and ionic strength. Under these conditions, >90% of the STX receptor was bound and >95% of the protein was washed through the column (Fig. 1). The bound STX receptor and much of the bound protein were eluted

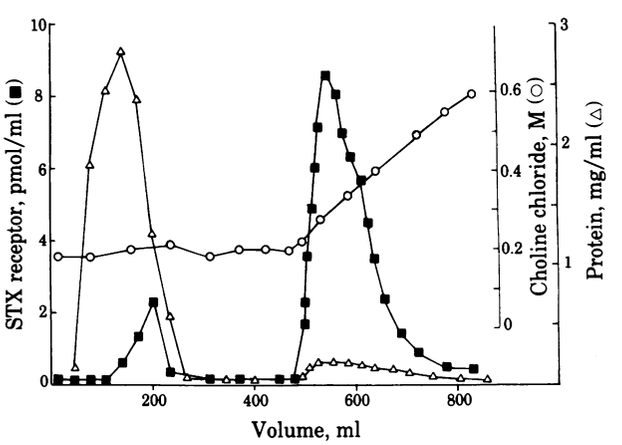

FIG. 1. Profile of elution of solubilized STX receptor from DEAE-Sephadex A-25 column. STX receptor concentrations were determined from specific [^{3}H]STX binding.

together with a linear 175–1000 mM choline chloride gradient. The result was an 8.9-fold purification (Table 1).

Chromatography on WGA-Sepharose. Many cell surface proteins are glycoproteins. Therefore, Sepharose columns with covalently attached lectins of different sugar specificity were tested for their ability to bind the solubilized STX receptor. Concanavalin A, which binds D-glucose and D-mannose (19), and WGA, which binds *N*-acetylglucosamine and sialic acid (19), could bind the STX receptor. However, the recovery of bound STX receptor from the concanavalin A column was only 7–12%, whereas recovery from the WGA column was 65–85%. Similar results with WGA columns were obtained by Barchi *et al.* (11) using STX receptor solubilized from rat skeletal muscle sarcolemma. After loading of a 10 ml WGA-Sepharose column with the eluate from a DEAE-Sephadex column, the column was washed with 50 ml of a high ionic strength buffer to minimize nonspecific ionic interactions with the column. Essentially all the STX receptor bound to the column and 94% of the protein flowed through (Fig. 2). The bound receptor was eluted with a 0–40 mM *N*-acetylglucosamine gradient to yield an additional 11-fold purification of the STX receptor with a recovery of 54%. The peak fractions were pooled (average specific activity, 310 pmol/mg) and used for further purification.

Sucrose Gradient Sedimentation. Previous studies of the size of the solubilized STX receptor from rat brain by gel filtration and sucrose gradient sedimentation have shown that the receptor protein has a M_r of 316,000 ± 63,000 and binds 0.9 g of phosphatidylcholine and Triton X-100 per g of protein to give a complex with a total M_r of 601,000 ± 48,000 (20). Because most of the proteins solubilized from brain membranes are smaller than this, separation by molecular size gives additional purification of the receptor.

Upon velocity sedimentation of the purified STX receptor on a linear 3–14% sucrose gradient, the STX receptor migrated as a single peak centered at fraction 10 with an apparent sedimentation coefficient of 10.5 S (Fig. 3). The specific activity of the pooled peak fractions was 1046 pmol/mg of protein. This represents a 3.4-fold purification from the WGA column eluate. The best fractions had a specific activity of 1488 pmol of STX bound per mg of solubilized receptor protein. Scatchard analysis of the binding data indicated a single class of sites with K_d = 0.4 nM, essentially the same as that of the membrane-bound STX receptor (10).

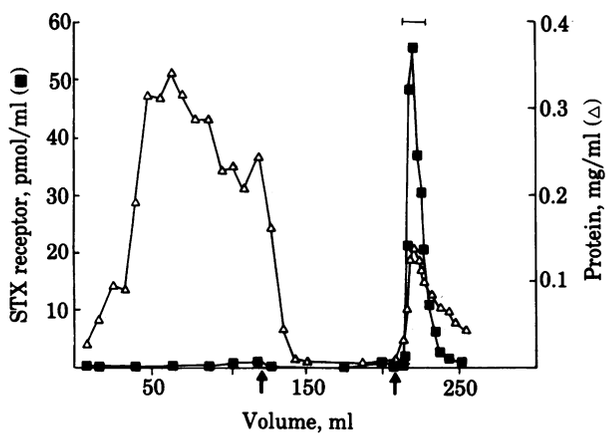

FIG. 2. Elution profile for WGA-Sepharose column. The STX receptor peak eluted from the DEAE-Sephadex column between 270 and 480 mM choline chloride was adsorbed to a WGA-Sepharose column and eluted. The first arrow on the abscissa indicates the beginning of the wash; the second arrow indicates the application of a 0–40 mM *N*-acetylglucosamine gradient to elute bound STX receptor. The six fractions of the eluted STX receptor peak pooled and saved for further purification are indicated.

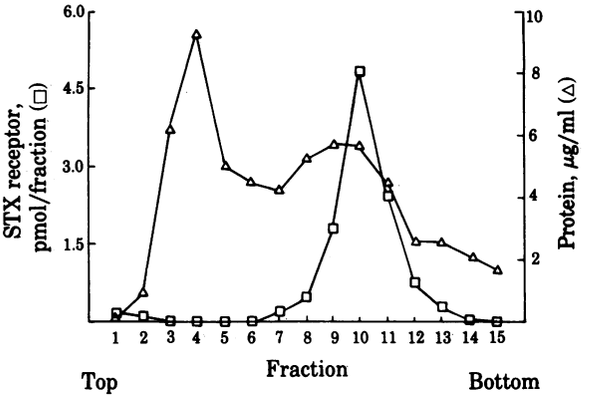

FIG. 3. Purification by sucrose gradient sedimentation. Purified STX receptor from the WGA-Sepharose column peak was sedimented through a 3–14% sucrose gradient for 18 hr at 200,000 × *g*.

The purity of these fractions can be estimated from their specific activity. If one saxitoxin molecule is bound per 316,000 daltons of receptor protein, the specific activity of the pure receptor would be 3160 pmol/mg of protein. By this criterion the pooled peak fractions are 33% pure and the best fraction is 47% pure. Because the purified fractions are likely to contain some denatured STX receptor, this method will underestimate the polypeptide purity of the preparation.

Polypeptide Components of the STX Receptor. The specific activities of the peak fractions from the sucrose gradients indicate that they were up to 47% pure. This suggests that the polypeptide components of the STX receptor should appear as prominent bands when these fractions are subjected to NaDodSO$_4$/polyacrylamide gel electrophoresis. Trace A in Fig. 4 represents a NaDodSO$_4$ gel of fraction 10 of the sucrose gradient in Fig. 3. The five protein bands that comprise more than 3% of the stain intensity are numbered. Band 5 often appears

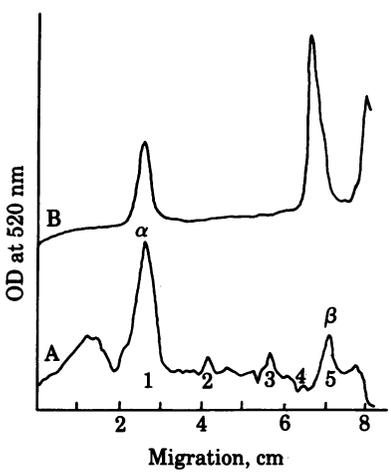

FIG. 4. Comparison of the NaDodSO$_4$ gel profiles of purified STX receptor and synaptosomes covalently labeled with ^{125}I-labeled scorpion toxin. Trace A. Densitometric scan of a Coomassie blue-stained gel of fraction 10 from Fig. 3. Each polypeptide containing more than 3% of the total stain is numbered. The apparent M_rs of these polypeptides are as follows: 1, 270,000; 2, 145,000; 3, 76,000; 4, 60,000; and 5, 38,300. Trace B. Synaptosomes were covalently labeled with a photoreactive ^{125}I-labeled scorpion toxin derivative as described (5), dissolved in NaDodSO$_4$/mercaptoethanol, and analyzed by electrophoresis in the same 4.5–15% gradient polyacrylamide gel as trace A. A densitometric scan of an autoradiogram of the gel is presented.

as a closely spaced doublet. The broad peak to the left of band 1 is an artifact caused by the trichloroacetic acid precipitation used to concentrate the sample prior to application to the gel.

To determine which of these polypeptides are components of the STX receptor, we analyzed each fraction from a sucrose gradient on a $NaDodSO_4$ gel. Fig. 5 shows the concentration of STX receptor and the relative amounts of the five polypeptides determined by measuring the area under their peaks in a densitometric scan of a $NaDodSO_4$ gel that had been stained with silver by the method of Oakley *et al.* (18). This staining method was used because it is much more sensitive than Coomassie blue and eliminated the need to concentrate the samples by trichloroacetic acid precipitation.

The relative amounts of polypeptides 1 and 5 corresponded well with the amount of STX receptor in each fraction and therefore are likely to be subunits of the STX receptor. Polypeptide 2 reached its peak in fraction 4, where there was no STX binding activity, and only a small tail of this polypeptide ran through the peak of STX binding. Therefore, polypeptide 2 is likely to be a contaminant. Polypeptide 3 also peaked in fraction 4 and slowly declined through fraction 8, where STX binding was increasing. However, it did increase in fraction 9 before continuing its decline toward the bottom of the gradient. Polypeptide 3 is unlikely to be a component of the STX receptor because most of it was present in fractions containing no STX binding activity. In other preparations, an increase in polypeptide 3 was not observed in the fractions containing the STX receptor. Polypeptide 4 peaked broadly in fractions 5–7 and gradually declined through the peak of STX binding activity. Therefore, it is not likely to be a component of the STX receptor.

In the peak fraction of STX binding activity in the sucrose gradient of Fig. 3, polypeptides 1 and 5 accounted for 55% and 11% of the total stain intensity, respectively (Fig. 4, trace A). No other polypeptide accounted for more than 8% of the total

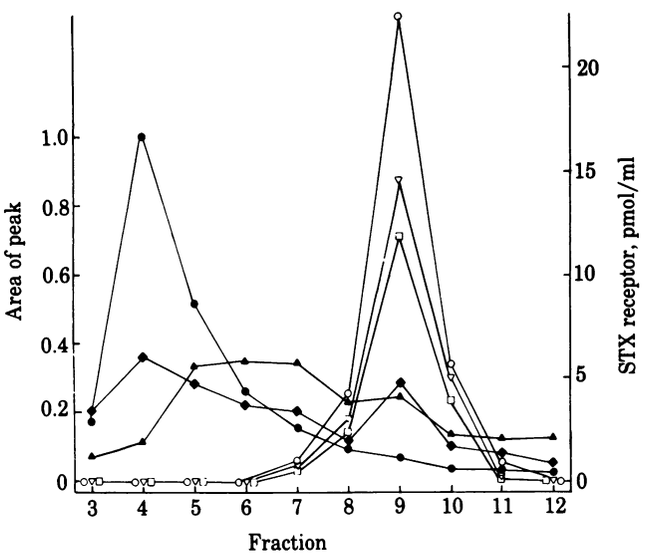

FIG. 5. Comparison of polypeptide composition with STX binding activity in sucrose gradient fractions. An aliquot of the pooled peak fractions from a WGA column was run on a 5–20% sucrose gradient. Sixteen fractions were collected (no. 1 was the top of the gradient). Aliquots of each fraction (50 μl) were analyzed by electrophoresis on a 4.5–15% acrylamide gradient $NaDodSO_4$ gel and silver stained. Each gel lane was scanned with a densitometer and the areas under the peaks of the five polypeptides numbered in Fig. 4 were electronically integrated. These areas are plotted for each fraction containing significant amounts of protein as follows: ▽, polypeptide 1; ●, polypeptide 2; ◆, polypeptide 3; ▲, polypeptide 4; and □, polypeptide 5. Also plotted is the amount of STX receptor in each fraction (○).

intensity. Thus, polypeptides 1 and 5 which correlate with STX binding activity were the two most prominent bands in this fraction, accounting for 66% of the stain intensity. Polypeptide 5 stained more intensely in silver-stained gels than in Coomassie blue-stained gels and therefore appeared to comprise a larger fraction of the protein in the peak area measurements in Fig. 5.

Polypeptide Components of the Purified STX Receptor Have Same M_r as Those Covalently Labeled by Scorpion Toxin in Intact Synaptosomes. Further evidence that polypeptides 1 and 5 are components of the Na channel comes from comparison with polypeptides covalently labeled by photoreactive scorpion toxin derivatives (5). Photoactivable derivatives of ^{125}I-labeled scorpion toxin specifically label two polypeptides in intact synaptosomes (5). Under the electrophoresis conditions used in previous studies (5), these polypeptides had apparent M_r of 250,000 and 32,000. In order to determine whether bands 1 and 5 of the purified STX receptor preparation are related to these polypeptide components of the scorpion toxin receptor site identified previously (5), covalently labeled synaptosomes were solubilized in $NaDodSO_4$ and mercaptoethanol and analyzed by $NaDodSO_4$ gel electrophoresis on the same slab gel as purified STX receptor fractions. The apparent M_rs of the specifically labeled bands were 280,000 and 44,000. Subtracting the contribution of scorpion toxin (M_r, 6700) gives mean (± SD) M_rs for the covalently labeled polypeptides of 273,000 ± 15,000 and 37,000 ± 2700 from six separate experiments. These apparent M_rs are similar to those of bands 1 (270,000 ± 10,000) and 5 (38,000 ± 2000) of the purified STX receptor preparation.

Fig. 4 compares a densitometric tracing of a Coomassie blue-stained gel of fraction 10 from the sucrose gradient of Fig. 3 (trace A) to a densitometric tracing of an autoradiogram of another lane of the same $NaDodSO_4$/polyacrylamide gel containing synaptosomes covalently labeled with the photoactivable ^{125}I-labeled scorpion toxin derivative (trace B). Fig. 4 shows that band 1 comigrated with the larger of the two polypeptides covalently labeled by scorpion toxin and band 5 migrated somewhat more rapidly than the smaller of the covalently labeled polypeptides. The difference between the apparent M_rs of band 5 and the smaller covalently labeled polypeptide is 5700 on this gel. This is approximately the difference expected from covalent attachment of scorpion toxin. These results indicate that the purified STX receptor has two principal polypeptide components and suggest that these two polypeptides also contain the receptor site for scorpion toxin. Because the apparent M_rs of these polypeptides are dependent on experimental conditions, we will designate the larger polypeptide α and the smaller polypeptide β (Fig. 4) rather than referring to them by apparent M_r as in previous work (5).

DISCUSSION

Although not purified to homogeneity, the STX receptor is highly enriched by the purification procedure described. The purity was assessed in two ways. The best fractions from our preparations were estimated to be 47% pure by taking the ratio between their specific activity, 1488 pmol/mg, and the theoretical specific activity, 3160 pmol/mg, for a pure receptor of 316,000 ± 63,000 daltons (20) which binds 1 STX per mol. The purity of our best fractions was also estimated by comparing the amount of Coomassie blue bound by the α and β polypeptides to the total amount of dye bound by the entire gel lane. In our four most recent preparations, the α polypeptide bound 43 ± 5% of the stain and the β polypeptide bound 12 ± 3% of the stain. On the assumption that the stain binds equally well to each protein and that the binding is linearly dependent on protein concentration, the polypeptide purity of our peak fractions

is 55%. No single polypeptide impurity comprises more than 8% of the protein staining in the preparation.

Polypeptides with the apparent M_rs of α and β have been identified as protein components of the Na channel from mammalian brain in two independent ways. In this work, we have found that these two polypeptides are the only ones whose presence correlated with STX binding activity in substantially purified STX receptor preparations from mammalian brain. Previous studies (5) showed that two polypeptides of similar electrophoretic mobility were specifically labeled by photoreactive derivatives of ^{125}I-labeled scorpion toxin in intact synaptosomes in which the Na channel is in its native membrane environment and is fully functional (2). Analysis of purified STX receptor and covalently labeled synaptosomes on the same $NaDodSO_4$ slab gel gives results consistent with the conclusion that the α and β polypeptides of the STX receptor preparations are the same as the large and small polypeptides labeled by scorpion toxin. Thus, our working hypothesis is that the α and β polypeptides are components of the Na channel which contain both the receptor site for TTX and STX and the receptor site for scorpion toxin and sea anemone toxin. Further work is required to demonstrate rigorously the identity of these polypeptides. In approximately half of our preparations, the β polypeptide appears as a poorly resolved doublet. More detailed analysis is necessary to determine whether the band we have designated β is composed of two polypeptides of similar M_r.

The work of Agnew *et al.* (21) provides independent evidence for an essential role of a polypeptide of the size of α in the STX/TTX receptor. These authors showed that purified preparations of the TTX receptor from eel electroplax having an average specific activity of 1500 pmol/mg contained a polypeptide of $M_r \approx 260,000$ as the major component. Preparation of these fractions by three different separation techniques showed that a polypeptide of $M_r \approx 260,000$ was closely associated with TTX binding activity. A polypeptide of the size of β was not shown to comigrate with the TTX binding activity in these studies, but a polypeptide of M_r 42,000 was present in the most highly purified fractions and could not be excluded as a component of the receptor (21). Barchi *et al.* (11) have obtained purified preparations of the STX receptor from mammalian skeletal muscle which have specific activities of approximately 1500 pmol/mg. The most recent preparations made by Barchi *et al.* (personal communication) contain two small polypeptides with M_rs of 55,000 and 38,000 and, in addition, contain diffusely staining polypeptides with M_rs of 250,000 and 130,000. Further work will be required to determine whether these differences in polypeptide chain composition reflect intrinsic differences among the Na channel structures in these three excitable membranes or differences that arise during purification procedures.

The size of the protein portion of the solubilized STX receptor was previously determined to be 316,000 ± 63,000 (20). The M_r values of the solubilized receptor and the α and β polypeptides are too imprecise to permit determination of the subunit stoichiometry of the solubilized STX receptor but they do place limits on it. There can be only one of the large α subunits and one or more of the smaller β subunits per soluble STX receptor.

Our results suggest that the purified STX receptor contains the STX binding site in native form and the scorpion toxin binding site in altered form. Solubilized STX receptor does not bind scorpion toxin (10), so the scorpion toxin binding site must be in an inactive or low-affinity state. STX is thought to act by binding to and occluding the ion-conducting pore of the Na channel (22, 23). Scorpion toxin is thought to bind to a component of the Na channel involved in voltage-dependent activation and inactivation because the voltage dependence of binding parallels that of activation (24). Thus, the purified STX receptor preparation may contain both the ion-conducting pore and the gating apparatus of the Na channel. Confirmation of this requires reconstitution of the purified receptor into phospholipid bilayer vesicles and comparison of its functional properties with those of intact Na channels.

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