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TOPOGRAPHY OF GLYCOPROTEINS IN THE CHICK SYNAPTOSOMAL PLASMA MEMBRANE

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Summary

Chick brain synaptosomes or synaptic subfractions were treated with neuraminidase (EC 3.2.1.18) and/or galactose oxidase (EC 1.1.3.9) preparations in which proteolytic activity was inhibited with phenylmethanesulfonyl fluoride followed, after washing, by reductive incorporation of sodium boro[^{3}H]hydride to identify galactose residues exposed on the synaptosomal external surface. Control experiments to demonstrate restriction of labeling to the external surface involved comparing the radioactivity in synaptoplasmic, soluble polypeptides isolated after labeling with labeled, isolated synaptoplasm and examining incorporation into fractions incubated without enzymes. Intactness of the synaptic plasma membrane after labeling was shown by trypsin digestion studies. Polypeptides were separated on sodium dodecyl sulfate polyacrylamide gels and were detected by a liquid scintillation counting procedure. Eleven major radioactive peaks were found after galactose oxidase treatment and reduction of isolated synaptic membranes. When intact synaptosomes were labeled, the same components were detected. When isolated synaptic membranes or intact synaptosomes were treated with neuraminidase before galactose oxidase treatment, three additional components were labeled. These results suggest that (a) chick synaptic membranes have a complex mixture of glycoproteins, (b) all major chick synaptic membrane glycoproteins labeled by galactose oxidase have most or all carbohydrate groups exposed at the exterior surface of the synaptosome, (c) all major, externally-disposed polypeptides of these synaptic membranes are glycoproteins.

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Introduction

Many data show that membrane surface glycoproteins play important roles in ion transport and intercellular adhesion and recognition [1—5]. In the brain, glycoproteins are particularly common on the surface of the neuronal plasma membrane and in synapses [6,7]. It has been suggested that they may play a role in behavioral adaptation [8]. Short-term memory could be formed through the attachment or modification of carbohydrates to synaptic membrane proteins.

One major difficulty in studying synaptosomal plasma membrane glycoproteins was to prepare material with little contamination. However, methods developed recently in several laboratories [9—12] have facilitated the study of this membrane. With the isolation of highly enriched material and the development of procedures to identify glycoproteins [13—16] information on the glycoproteins of the synaptic plasma membrane has been gradually revealed.

A procedure currently used to localize membrane glycoproteins is the oxidation of terminal galactosyl or *N*-acetyl galactosaminyl residues of a glycoprotein to the corresponding C-6 aldehydes with galactose oxidase followed by reduction with tritiated sodium borohydride. Galactose oxidase has a molecular weight of 42000 [17] and has been shown not to penetrate some cell membranes [14]. It is, therefore, a useful tool to identify the glycoproteins on surfaces of intact cells [14—16].

Neuraminidase can cleave sialic acid residues from glycoproteins. It was found that neuraminidase treatment will make non-terminal galactosyl residues of glycoproteins more accessible to galactose oxidase. Some glycoproteins on the surface of membranes are not accessible to galactose oxidase unless the membranes are pretreated with neuraminidase [14—16]. We have used galactose oxidase and neuraminidase to localize the glycoproteins of intact chick synaptosomes and synaptic plasma membranes.

As of this writing, no data have been reported on the galactose oxidase labeling of intact synaptosomes. Our results show that major, externally-disposed polypeptides in the chick synaptic plasma membrane are glycoproteins with galactose-containing moieties on the external surface.

Materials and Methods

Subcellular fractionation

Male White Leghorn chicks (6—8-days old) were used in these experiments. Fifty chicks were decapitated for each preparation. Cerebral hemispheres and optic lobes were obtained by freehand dissection and pooled (approximately 40 g total wet weight). A synaptosomal preparation was obtained from the interface between 7 and 14% Ficoll 400 (Pharmacia) in the Ficoll centrifugation gradient. A purified synaptic plasma membrane fraction was isolated from the interface between 0.8 and 0.95 M sucrose in a sucrose gradient. These methods have been described in detail previously [9]. The synaptoplasmic soluble proteins were isolated according to the method of Chiu and Babitch [18].

Galactose oxidase-sodium borohydride labeling of glycoproteins in isolated synaptic plasma membranes

Isolated synaptic plasma membranes were suspended in 0.1 M sodium phosphate buffer, pH 7.0, to a concentration of 1 mg protein per ml (1 ml for each experiment). 5 μl 0.2 M phenylmethanesulfonyl fluoride (Sigma) in ethanol was added to inhibit proteolytic activity. Then 100 μl of 0.1 M sodium phosphate buffer, pH 7.0, containing 0 (control) or 8.5 units of galactose oxidase (85 and 104 units/mg solid, Sigma) were added and the incubation was carried out at 20°C for 1 h. The mixture was then diluted with 10 vols. of phosphate buffer and centrifuged at $106500 \times g_{av}$ for 45 min. The membranes were resuspended in 1 ml of 0.1 M phosphate buffer, pH 8.0. 10 μl of NaB^3H_4 (276 mCi/mmol, New England Nuclear) freshly prepared in 0.01 M NaOH was added to give a final concentration of 2.0 mM. After 30 min incubation at 20°C, the membrane suspension was diluted with 10 vols. of phosphate buffer and recentrifuged. The membranes were washed twice with the same buffer to remove residual NaB^3H_4.

Neuraminidase treatment of isolated synaptic plasma membranes

Isolated synaptic membranes were suspended in 0.1 M sodium phosphate buffer, pH 6.0, to a concentration of 1.0 mg protein per ml (1 ml for each experiment). 5 μl of 0.2 M phenylmethanesulfonyl fluoride in ethanol was added to inhibit proteases. 100 μl of neuraminidase solution (500 units/ml, Calbiochem) was added to the suspension and the incubation was carried out at 30°C for 30 min. The mixture was then diluted with 10 vols. of phosphate buffer, pH 6.0, and centrifuged at $106500 \times g_{av}$ for 45 min. The membranes were washed again with the same buffer, resuspended in 0.1 M sodium phosphate buffer, pH 7.0, and treated with galactose oxidase as described above.

Galactose oxidase-sodium borohydride labeling of glycoproteins in intact synaptosomes

Synaptosomes were washed twice in saline-phosphate buffer (0.15 M NaCl/50 mM sodium phosphate, pH 7.0) and suspended in the same buffer to a concentration of 1.1 mg protein per ml (50 ml for each experiment). An aliquot of 0.2 M phenylmethanesulfonyl fluoride in ethanol was added to give a final concentration of 1 mM. 2 ml of 50 mM sodium phosphate buffer, pH 7.0, containing 0 (control) or 200 units of galactose oxidase was added and the incubation was carried out at 20°C for 1 h. Synaptosomes were then centrifuged at $17000 \times g_{av}$ for 17 min and resuspended in 20 ml of saline-phosphate buffer, pH 8.0. 200 μl of NaB^3H_4 (276 mCi/mmol) was added to give a final concentration of 2.5 mM and the mixture was incubated at 20°C for 30 min. It was then diluted with 5 vols. of saline-phosphate buffer, pH 8.0, recentrifuged and washed again with the same buffer. This procedure eliminated proteolytic and cross-linking artifacts observed previously [15] as indicated by the identity of Coomassie Blue-staining membrane polypeptides from reacted and unreacted synaptosomes. The labeled synaptosomes were then subjected to hypotonic lysis to continue purification of synaptic plasma membranes and synaptoplasmic soluble proteins as described previously [9].

Neuraminidase treatment of intact synaptosomes

Synaptosomes were suspended in saline-phosphate buffer (0.15 M NaCl/ 50 mM $NaPO_4$, pH 6.5) to a concentration of 1.0 mg protein per ml (20 ml for each experiment). An aliquot of 0.2 M phenylmethanesulfonyl fluoride in ethanol was added to give a final concentration of 1 mM. 1 ml of neuraminidase solution (500 units/ml, Calbiochem) was added to the suspension and the incubation was carried out at 30°C for 30 min. The mixture was then diluted with 5 vols. of saline-phosphate buffer, pH 6.5, and centrifuged at 17000 $\times g_{av}$ for 17 min. The synaptosomes were then treated with galactose oxidase as described above.

Trypsin digestion of intact synaptosomes and synaptosomal subfractions

These incubations were performed as described previously [18].

Sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis

Samples for electrophoresis were incubated 16 h at 37°C in sample buffer (1% SDS/10% glycerol/10 mM Tris · HCl/1 mM EDTA/1% β-mercaptoethanol, pH 8.0) before electrophoresis. Bromphenol Blue was added to the electrophoresis samples as the tracking dye. Electrophoresis was performed on 5.6% tube gels which were subsequently stained and destained as described by Fairbanks et al. [13]. The gels were sliced into 1 mm slices with a gel slicer (BioRad). Slices were solubilized in 0.3 ml TS-1 tissue solubilizer (Research Products International Corp.) and 30 μl of water and 5 ml of Instafluor (Packard) were added for counting in a Packard Tricarb liquid scintillation counter.

Results

Galactose oxidase labeling of isolated synaptic plasma membranes

It is important to show that the proteolytic activity of a galactose oxidase sample is eliminated when it is used in a labeling experiment. In preliminary

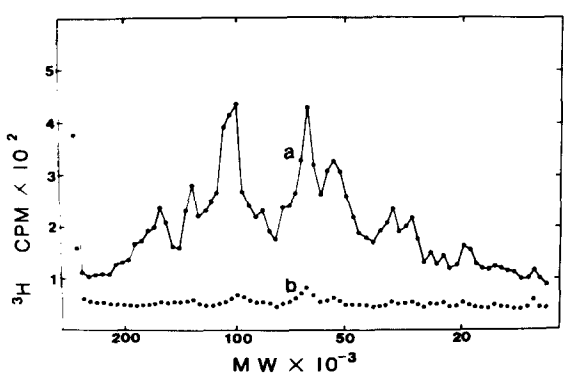

Fig. 1. Labeling of isolated synaptic membranes by galactose oxidase and NaB^3H_4. Synaptic membranes (1 mg protein in 1 ml of 0.1 M phosphate buffer, pH 7.0) were treated with 8.5 units of galactose oxidase at 20°C for 1 h. After centrifugation, the membranes were resuspended in 1 ml of 0.1 M phosphate buffer, pH 8.0, and were reduced with 2 mM NaB^3H_4 at 20°C for 30 min. The membranes were then washed, electrophoresed, and counted as described in Materials and Methods. a, Galactose oxidase-treated; b, control without galactose oxidase.

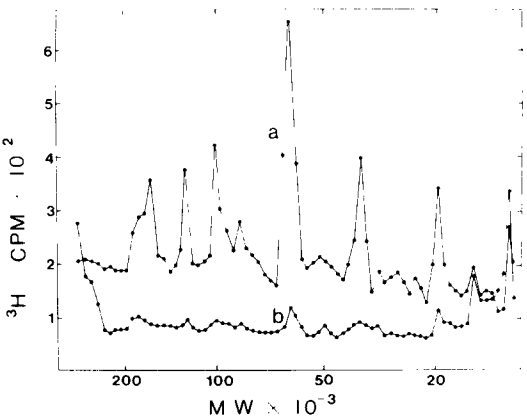

Fig. 2. Labeling of intact synaptosomes by galactose oxidase and NaB^3H_4. 50 ml of synaptosomes (1.1 mg protein/ml) in 0.15 M NaCl/50 mM sodium phosphate, pH 7.0, were treated with 200 units of galactose oxidase at 20°C for 1 h. After centrifugation, synaptosomes were resuspended in 20 ml of saline-phosphate buffer, pH 8.0, and were reduced with 2.5 mM NaB^3H_4 at 20°C for 30 min. After washing, the labeled synaptosomes were lysed and synaptic membranes were isolated as described in Materials and Methods. a, Galactose oxidase-treated; b, control without galactose oxidase.

studies, we pretreated the galactose oxidase by heating the diluted enzyme at 50°C for 30 min as described by other workers [15,19]. We found that the contamination by proteolytic enzymes was not eliminated. Major polypeptides with apparent molecular weights higher than 130000 almost all disappeared from the polyacrylamide gel patterns of galactose oxidase treated samples, but not from control samples incubated without galactose oxidase (data not shown). Thus, heating the enzyme at 50°C for 30 min did not inhibit proteolytic activity. We finally solved this problem by adding a small amount of protease inhibitor (phenylmethanesulfonyl fluoride) which does not affect the activity of galactose oxidase.

Eleven components were labeled when isolated synaptic plasma membranes were labeled by galactose oxidase oxidation and NaB^3H_4 reduction. They had apparent molecular weights of 160000, 130000, 100000, 82000, 60000, 52000, 34000, 29000, 26000, 24000, and 19000 (Fig. 1). We did not prereduce the membranes with $NaBH_4$ because control experiments with NaB^3H_4 only had essentially background radioactivity. Wang and Mahler also did not prereduce their synaptic membrane preparation [20]. About 5% of the radioactivity stayed at the origin of the polyacrylamide gels. There was little radioactivity associated with the dye front.

Galactose oxidase labeling of intact synaptosomes

Fig. 2 shows the labeling patterns of synaptic plasma membranes after labeling of intact chick synaptosomes with galactose oxidase and a control experiment incubated without galactose oxidase. The same eleven polypeptides were labeled whether intact synaptosomes or isolated membranes were the substrate, indicating that no galactose-containing glycoproteins which were accessible to galactose oxidase had carbohydrate moieties only on the internal membrane surface. Much radioactivity was associated with six polypeptides with apparent molecular weights of 160000, 130000, 100000, 60000, 34000 and 19000. Polypeptides with apparent molecular weights of 82000,

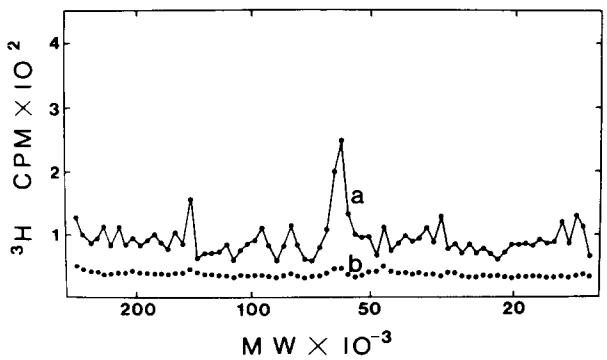

Fig. 3. Galactose oxidase labeling of isolated synaptoplasmic, soluble proteins. The synaptoplasmic polypeptide fraction was isolated as described previously [18]. The protein concentration was adjusted to 0.2 mg/ml and the pH was adjusted to 7.0. Phenylmethanesulfonyl fluoride in ethanol (0.2 M) was added to give a final concentration of 1 mM. Galactose oxidase in 50 mM NaH_2PO_4, pH 7.0, was added to the suspension to give a concentration of 0.6 units/ml. The incubation was carried out at 20°C for 1 h. An aliquot of NaB^3H_4 was then added to give a final concentration of 2 mM and the incubation was continued for 30 min at 20°C. The soluble proteins were isolated and electrophoresed as described before [18]. a, Galactose oxidase- and NaB^3H_4-treated; b, control, NaB^3H_4-treated only.

52000, 29000, 26000 and 24000 were labeled to a lesser extent. The component at 15000 probably contains a bond(s) capable of reduction by NaB^3H_4 because it was labeled in control experiments when NaB^3H_4 was added alone. Kelly and Cotman found 45—50% of the total incorporation at the dye front in the polyacrylamide gel pattern of polypeptides from rat synaptic junctions labeled with galactose oxidase and NaB^3H_4 [19]. They postulated that this extensive galactose oxidase-dependent labeling at the dye front was due to intrinsic glycopeptides and glycolipids. We found less than 10% of the total radioactivity at the dye front after labeling of intact synaptosomes and almost none after labeling of isolated synaptic plasma membranes (see above). Since synaptosomal preparations are contaminated by other subcellular particles with proteolytic activity, e.g. lysosomes [9], while isolated synaptic plasma membranes are relatively pure, we cannot rule out the possibility that proteolytic activity was present from both added galactose oxidase and other brain proteases.

The synaptoplasmic soluble fractions from galactose oxidase-treated and untreated (control) synaptosomes were also examined. Only background incorporation of ^3H in these fractions was observed indicating that labeling was restricted to external components. A few minor ^3H peaks appeared in both experiments, but they were never greater than peaks from membranes incubated without galactose oxidase. When synaptosomes were lysed and synaptoplasmic soluble polypeptides were labeled with galactose oxidase, many radioactive peaks were detected on the gel of soluble polypeptides (Fig. 3a). A control experiment without galactose oxidase (NaB^3H_4 only) shows essentially background incorporation (Fig. 3b). These experiments indicate the impenetrability of intact synaptosomes by galactose oxidase, because the synaptoplasmic, soluble polypeptides were not labeled prior to lysis of synaptosomes.

The major labeled synaptoplasmic polypeptide had an apparent molecular weight of 60000. The relationship between this polypeptide and the synaptic

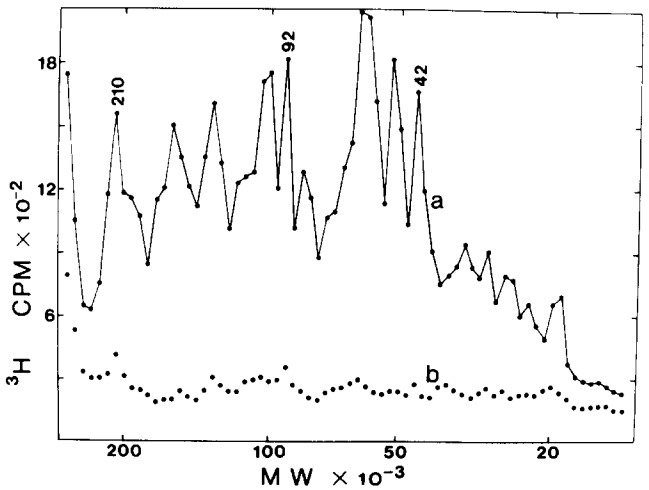

Fig. 4. Effect of neuraminidase on the galactose oxidase labeling pattern of isolated synaptic plasma membrane glycoproteins. Synaptic membranes (1 mg protein in 1 ml of 0.1 M sodium phosphate buffer, pH 6.0) were treated with 50 units of neuraminidase at 30°C for 30 min. After washing, the membranes were treated with galactose oxidase and NaB^3H_4 as in Fig. 1. a, Neuraminidase treatment followed by galactose oxidase and NaB^3H_4 labeling; b, control, neuraminidase treatment followed by NaB^3H_4 labeling only.

plasma membrane glycoprotein of the same apparent molecular weight has not been determined, but we cannot exclude the possibility that some membrane polypeptides may be partially extracted into the soluble fraction during lysis of synaptosomes. Galactose oxidase itself probably is not labeled under these conditions because the peak at its molecular weight (42000) is very minor. However, we did not examine this point in greater detail by reacting purified galactose oxidase with NaB^3H_4 because in the absence of normal substrate artifactual self-labeling seems a possibility.

Labeling pattern of isolated synaptic plasma membranes after neuraminidase treatment

Neuraminidase preparations frequently contain proteolytic activity. In our hands high molecular weight components (greater than 100000) almost disappeared from the gels when synaptic plasma membranes were treated with this enzyme in the absence of a protease inhibitor (data not shown). With the addition of phenylmethanesulfonyl fluoride, proteolytic activity was inhibited without affecting the activity of neuraminidase and the Coomassie Blue staining patterns of synaptic plasma membrane polypeptides were not changed by neuraminidase (or galactose oxidase) treatment. This agreed with the finding that such treatment with these enzymes did not change the polypeptide pattern of erythrocyte membranes [16].

After incubation with neuraminidase, the overall incorporation of ^3H into glycoproteins by the galactose oxidase labeling procedure was higher than without neuraminidase treatment. Three new radioactive components with apparent molecular weights of 210000, 92000 and 42000 were detected in addition to those eleven components detected previously (Fig. 4). This suggests that some external synaptic plasma membrane glycoproteins cannot be oxidized by galac-

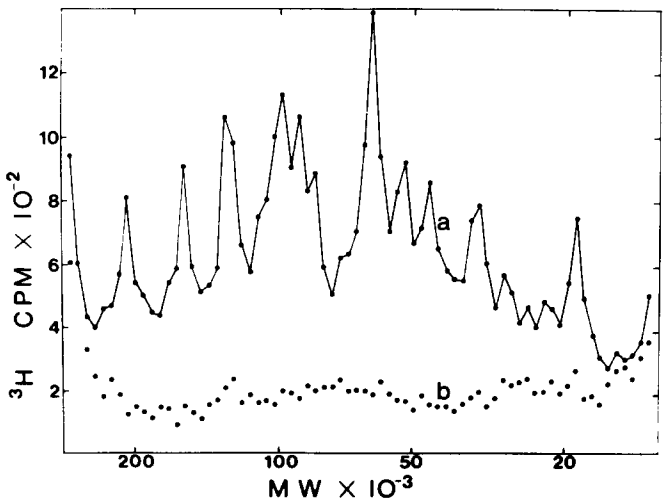

Fig. 5. Labeling of intact synaptosomes by galactose oxidase after neuraminidase treatment. 20 ml of synaptosomes (1 mg protein per ml) in 0.15 M NaCl/50 mM NaH_2PO_4, pH 6.5, were treated with 500 units of neuraminidase at 30°C for 30 min. After centrifugation, the synaptosomes were resuspended in 0.15 M NaCl/50 mM NaH_2PO_4, pH 7.0, and labeled with galactose oxidase and NaB^3H_4 as in Fig. 2. a, Neuraminidase treatment followed by galactose oxidase and NaB^3H_4 labeling; b, control, neuraminidase treatment followed by NaB^3H_4 labeling only.

tose oxidase until terminal sialic acid residues of the carbohydrate moieties are removed by neuraminidase.

Labeling of intact synaptosomes after neuraminidase treatment

The overall incorporation of ^3H by galactose oxidase and NaB^3H_4 into synaptic membranes of intact synaptosomes after neuraminidase treatment was less than after labeling of isolated synaptic membranes, but the labeling patterns were very similar. Apparently the same fourteen components were labeled (Fig. 5) suggesting that the carbohydrate groups of all fourteen are externally disposed. Natural and neuraminidase-induced microheterogeneity of the glycoproteins do not seem to be substantial problems in these experiments. Peak widths were similar to those observed earlier after lactoperoxidase-catalyzed iodination [18] and they did not seem to broaden when the galactose oxidase incubation was preceeded by neuraminidase treatment (cf. Figs. 1 and 2 with Figs. 4 and 5). Technical factors incidental to the electrophoretic procedure probably play an equally important role with glycoprotein microheterogeneity in determining peak breadth.

The synaptoplasmic soluble polypeptides from the labeling of intact synaptosomes after neuraminidase treatment were also examined. The results showed similar labeling patterns in both experimental and control incubations indicating no labeling of internal components before lysis. It is not surprising that the synaptosomes were still intact after these incubations, because the proteolytic activity was greatly inhibited by phenylmethanesulfonyl fluoride.

Trypsin digestion of treated synaptosomes and synaptic subfractions

Control experiments demonstrating the intactness and impenetrability of the

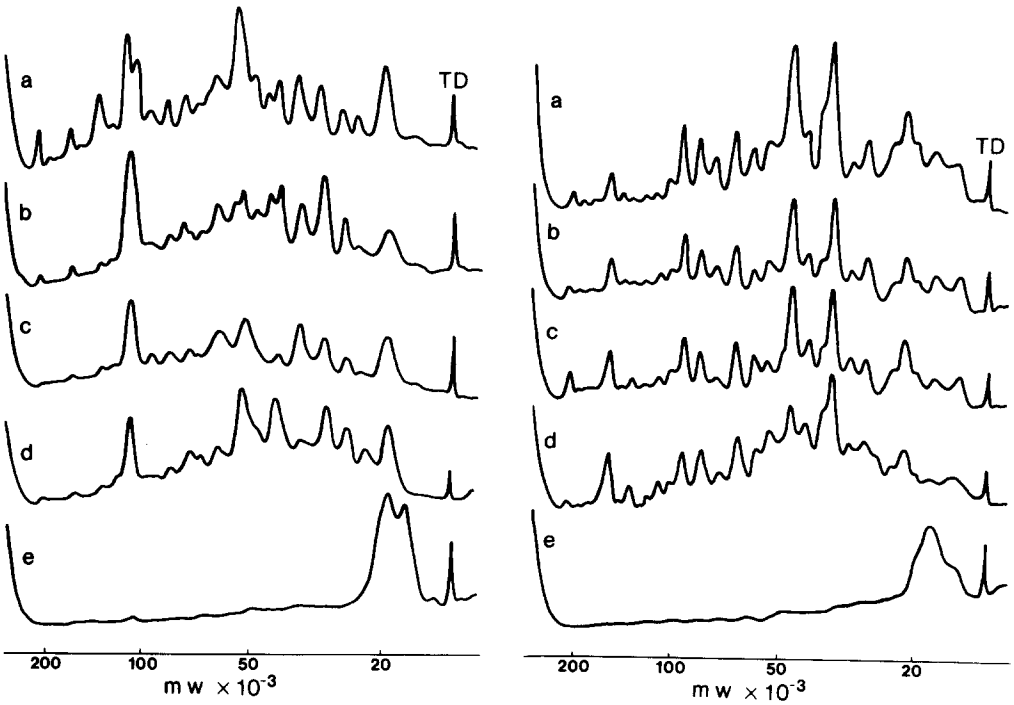

Fig. 6. Coomassie Blue staining patterns of synaptic plasma membrane polypeptides isolated from synaptosomes digested with trypsin after various surface labeling treatments. Synaptosomes were (a) lysed for isolation of synaptic plasma membranes (untreated control), (b) surface labeled with iodine by the procedure of Chiu and Babitch [18], (c) surface labeled with galactose oxidase and $NaB[^3H]_4$ as described in Materials and Methods, and (d) surface labeled by neuraminidase incubation followed by galactose oxidase and $NaB[^3H]_4$ as described in the Experimental section. All surface labeled synaptosomes were then washed and incubated with 0.2 mg trypsin/mg synaptosomal protein as described in ref. 18. After trypsin treatment the synaptosomes were washed and lysed. Synaptic plasma membranes were prepared from all lysates and were electrophoresed on polyacrylamide gels as described in Materials and Methods. Curve e represents the electrophoretic pattern of isolated synaptic membrane polypeptides after trypsin digestion.

Fig. 7. Coomassie Blue staining patterns of synaptoplasmic, soluble polypeptides isolated from synaptosomes digested with trypsin after various surface labeling treatments. Curves (a)—(d) represent soluble polypeptides isolated from synaptosomes treated as described in Fig. 6 (a—d). Curve e is the electrophoretic pattern of isolated synaptoplasmic polypeptides digested by trypsin.

labeling reagents are the sine qua non of this type of topographical study. We determined that the neuraminidase and galactose oxidase reactions did not increase synaptosomal permeability by digesting labeled synaptosomes with trypsin and then examining the patterns of membrane and synaptoplasmic polypeptides on polyacrylamide gels. The results (Figs. 6 and 7) showed that synaptosomes remained intact after the labeling reactions as we had shown previously for labeling via lactoperoxidase-catalyzed iodination.

Trypsin digestion produced rather similar membrane polypeptide patterns whether the synaptosomes first had been labeled with lactoperoxidase (Fig. 6, curve b), galactose oxidase and sodium boro[^{3}H]hydride (Fig. 6, curve c) or neuraminidase followed by galactose oxidase and sodium boro[^{3}H]hydride (Fig. 6, curve d). In all cases the largest effects of trypsin digestion were on the high (greater than 90000) molecular weight membrane polypeptides, in agree-

ment with earlier findings [18]. However, none of these combined treatments caused significant changes in the polypeptide profiles of synaptoplasmic, soluble material (Fig. 7, curves b—d) despite the sensitivity of these polypeptides to trypsin (Fig. 7, curve e). These data suggest that the synaptosomes were not made permeable by any of these labeling incubations and that the large labeling reagents employed did not cross the membranes to react with internal components.

Discussion

Several groups of workers have labeled isolated synaptic membranes in their studies of glycoproteins in synaptic membranes [19—21]. Data on the labeling of intact synaptosomes by galactose oxidase had been lacking until now. The results here demonstrate that all of the major glycoproteins labeled by this enzyme have most or all of their carbohydrate group(s) exposed at the exterior surface because the same polypeptides are labeled by galactose oxidase whether isolated synaptic plasma membranes or intact synaptosomes were the substrate. Some glycoproteins (with apparent molecular weights of 160000, 130000, 60000, 34000 and 19000) had higher reactivity toward galactose oxidase oxidation and NaB^3H_4 reduction in the labeling of intact synaptosomes. These proteins might be relatively more accessible to galactose oxidase in intact synaptosomes than in isolated synaptic plasma membranes because the conformations of proteins in the membrane could change during lysis and membrane isolation. Then the glycoproteins in the membrane would change their relative reactivity to galactose oxidase. Because the number of major peaks remains constant, the changes in reactivity are probably among glycoproteins rather than because some glycoproteins are selectively lost during purification of the membranes.

Glycopeptides with apparent molecular weights of 100000 and 52000 seemed to incorporate more label when isolated membranes were incubated than when intact synaptosomes were the substrate. This might indicate that some galactose residues in these glycoproteins were internally disposed in situ. However, other explanations are possible, e.g. lysis-induced membrane structural rearrangements increasing carbohydrate accessibility, so we cannot say that this membrane contains internally-disposed galactose residues.

The polypeptides with molecular weights of 210000, 160000 and 82000 were not labeled in our previous study of the surface-exposed polypeptides of intact synaptosomes using lactoperoxidase-catalyzed iodination [18]. The carbohydrate moiety of these polypeptides might hinder the accessibility of lactoperoxidase to iodinatable groups. Perhaps only their carbohydrate groups are exposed at the synaptosomal external surface. Also, they may be transmembrane glycopeptides because they were iodinated when isolated synaptic membranes were labeled by lactoperoxidase-catalyzed iodination [18]. An alternative explanation is that lysis and membrane isolation causes hidden iodinatiable groups on these glycopeptides to become accessible to lactoperoxidase at the exterior surface.

The component at 210000 appears to contain sialic acids that hinder accessibility of galactose oxidase to galactose or galactosamine residues because it

was labeled by galactose oxidase only after neuraminidase treatment. The polypeptides of apparent molecular weights 92000 and 42000 were iodinated by lactoperoxidase but were labeled by galactose oxidase only after neuraminidase treatment. These, unlike the 210000 dalton polypeptide, have iodinatiable groups exposed at the exterior surface and were not accessible to galactose oxidase before removal of sialic acids.

It is unlikely that the polypeptides we have identified as such are not synaptic plasma membrane polypeptides. Galactose oxidase and neuraminidase were always washed off the synaptosomes prior to reduction with sodium borohydride and their adhering to membranes has not been found previously [14—16]. The labeling pattern of galactose oxidase in synaptoplasm was quite different from the pattern of labeled plasma membranes, and synaptoplasmic incorporation was too low to propose that synaptoplasmic material absorbed to the membrane could have accounted for any synaptic plasma membrane peak. That the same number of peaks was found whether isolated membranes or intact synaptosomes were labeled suggests that the isolated synaptic plasma membranes were not substantially contaminated with material from the glycoprotein-containing, smooth membrane cisternae observed in presynaptic terminals by Wood and McLaughlin [6]. Finally, Zanetta and co-workers [23] have demonstrated that mitochondria do not substantially contribute to the glycoprotein pattern of synaptic plasma membranes.

Wang and Mahler reported that the components labeled by galactose oxidase in rat synaptic membranes have molecular weights of 175000, 137000, 97000, 68000, 54000 and 33000 [20]. Kelly and Cotman found the gel regions from 85000 to 165000 and from 45000 to 52000 to contain concanavalin A-binding glycoproteins [19]. Mena and Moore in their recent study reported that five polypeptides (100500, 59000, 52000, 34000 and 32000) were labeled by galactose oxidase in rat synaptic membranes. After neuraminidase treatment, four more polypeptides (230000, 155000, 57000 and 39500) were labeled by galactose oxidase [21]. Morgan et al. also reported five major glycoproteins (120000, 66000, 43000, 34000 and 23000) in rat synaptic plasma membranes [22]. The glycoproteins reported here all have molecular weights corresponding to those found by other workers, but this study shows a broader spectrum of glycoproteins in chick synaptic plasma membranes. Since Gahmberg reported that the erythrocyte membrane contains at least 20 glycoproteins [16], it is not surprising that the synaptic plasma membranes may also have a complicated distribution of glycoproteins.

Possibly the most important aspect of these data is the support they provide for the suggestion of Gahmberg [16] that all mammalian surface proteins are glycoproteins. We cannot say that our techniques have labeled every externally-disposed polypeptide in the chick synaptic plasma membrane. Still, every major polypeptide which was shown to occur on the external surface by lactoperoxidase-catalyzed iodination [18] is labeled by sodium boro[^{3}H]hydride after neuraminidase and galactose oxidase treatments. Why might all surface polypeptides be glycopeptides? At least three explanations seem possible. First, carbohydrates could be attached to polypeptides to indicate that these polypeptides are to be inserted into the external portion of the cell membrane. In this way, polypeptides without attached sugars would not be added to the cell

surface. A second possibility is that the carbohydrate moiety is needed to anchor part of the polypeptide in the external half of the lipid bilayer. The third possibility is that every externally disposed polypeptide performs some function which requires the presence of carbohydrate groups. Of course, any particular carbohydrate group could serve both passive (indicating and anchoring) and active (functional) roles. Whether or not any or all of these possibilities are true will require further examination of Gahmberg's suggestion.

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