COMMON GLYCOPROTEINS OF SYNAPTIC VESICLES AND THE SYNAPTOSOMAL PLASMA MEMBRANE

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1. Introduction

Many secretory processes occur by exocytosis: temporary or extended fusion of the granule membrane with the limiting plasma membrane of the cell [1–5]. In the peripheral nervous system, the transmitter noradrenaline appears to be released by this mechanism [6]. The fate of the vesicle membrane after release of the vesicle contents is not known; it could be secreted, permanently integrated into the cell membrane, or resorbed by pinocytosis into the cell. In general it is believed that the vesicle or granule membranes are resorbed into the cell [1–3, 7]. However exocytosis, by definition involves fusion of the two membranes; but it is not known how stable this fusion is. We have investigated this problem by comparing the glycoproteins of synaptic vesicles and synaptosomal plasma membranes from adult rat brain.

2. Methods

2.1. *Separation of synaptosomal plasma membranes and synaptic vesicles*

Synaptosomal plasma membranes were isolated as previously described [8]. Enzymatically and chemically they appear to be over 85% pure plasma membrane [8] and over 75% pure neuronal plasma membrane [9]. Synaptic vesicles were isolated as described in detail elsewhere [10] by Millipore filtration of crude synaptic fractions prepared in parallel with the synaptosomal plasma membranes. Enzymatically and chemically the synaptic vesicles appear to be over 85% pure.

2.2. *Extraction of synaptic vesicles and synaptosomal plasma membranes*

Pellets of synaptic vesicles and synaptosomal plasma membranes (around 10 mg protein) were homogenized in 0.05% sodium dodecyl sulphate (SDS) in 10 mM Tris-HCl pH 7.0 (0.1–0.2 mg SDS/mg protein). After centrifugation at 78,000 *g* for 2 hr, the supernatant was carefully removed (0.05% SDS extract). The insoluble material was homogenized in 0.5% Triton X-100 in 10 mM Tris-HCl pH 7.0 (3 mg Triton X-100/mg protein) and recentrifuged. The supernatant (0.5% Triton extract) was carefully removed, and the insoluble material rehomogenized in 0.2% SDS in 10 mM Tris-HCl pH 7.0 (1.0–1.5 mg SDS/mg initial protein). After centrifugation, the supernatant (0.2% SDS extract) was carefully removed. This process solubilized over 95% of the protein in the original synaptic vesicle and synaptosomal plasma membrane pellets.

2.3. *Polyacrylamide gel electrophoresis*

Discontinuous polyacrylamide gel electrophoresis in the presence of SDS was performed by the method of Grossfeld and Shooter [11] using 12% polyacrylamide gels according to Waehneldt and Mandel [12]. Before electrophoresis, all samples were dialysed against 0.2% SDS and 40 mM dithiothreitol was added. Gels were stained with a modified periodic acid-Schiff (PAS) reaction [13]. In some experiments gels were stained according to Glossman and Neville [14]. No changes in glycoprotein profile were observed with the latter techniques. Gels were scanned

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using a Vernon densitometer with a green filtre (Kodak Wratten no. 66).

3. Results

As shown in fig. 1, the glycoprotein profiles of the synaptosomal plasma membranes were relatively simple, in agreement with previous results [15]. No PAS-staining material was extracted with 0.05% SDS, the 0.5% Triton extract contained 4 and the 0,2% SDS extract 3 main glycoprotein bands. Mixing experiments showed that two glycoproteins (SPM-2 and SPM-3) were found in both extracts. The glycoprotein profiles of the synaptic vesicle extracts were simpler (fig. 2) since the 0.5% Triton extract contains 2 main glycoproteins. Only one prominent glycoprotein band was observed in the 0.2% SDS extract. Mixing experiments showed that the faster-migrating glycoprotein band (V-2) observed in the 0.5% Triton

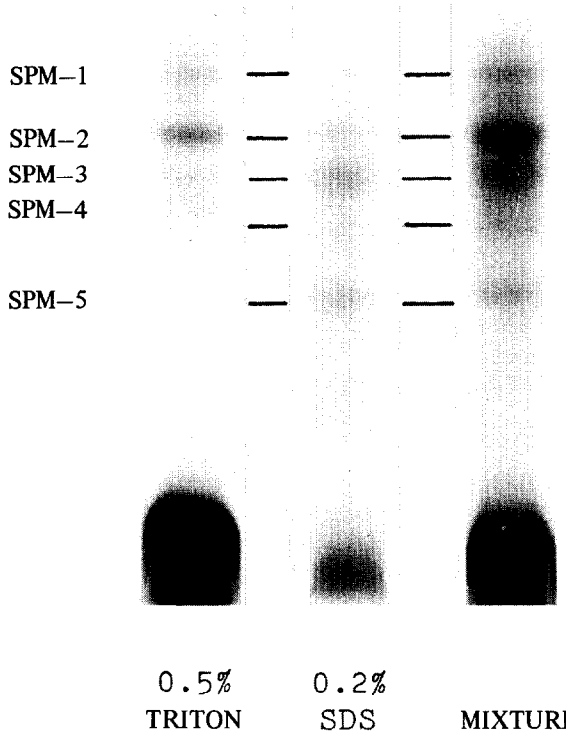

Fig. 1. Sequential detergent extracts of synaptosomal plasma membrane analysed by electrophoresis in 12% acrylamide gels. Gels were stained by the PAS-reaction as described under Methods.

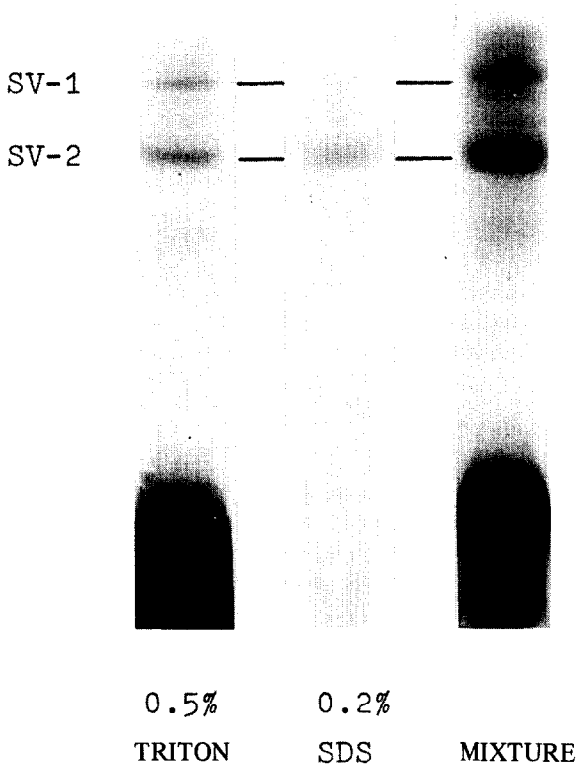

Fig. 2. Sequential detergent extracts of synaptic vesicles analysed as described in the legend to fig. 1.

extract corresponded to the single band found in the 0.2% SDS extract. The material migrating with the buffer discontinuity found in all gels was due to PAS-staining lipids. When the corresponding extracts from synaptic vesicles and synaptosomal plasma membranes were mixed (fig. 3), glycoproteins SV-1 and SV-2, the major glycoproteins and proteins of rat brain synaptic vesicles [10], corresponded electrophoretically to glycoproteins SPM-1 and SPM-3 of the synaptosomal plasma membranes preparations.

4. Discussion

Two banal explanations can be advanced for these findings. The first is simply that the electrophoretically identical bands represent different molecular species. It was for this reason that we have studied glycoproteins, since the simplicity of the glycoprotein profiles makes interpretation of the results easier: and the observation that the electrophoretically identical

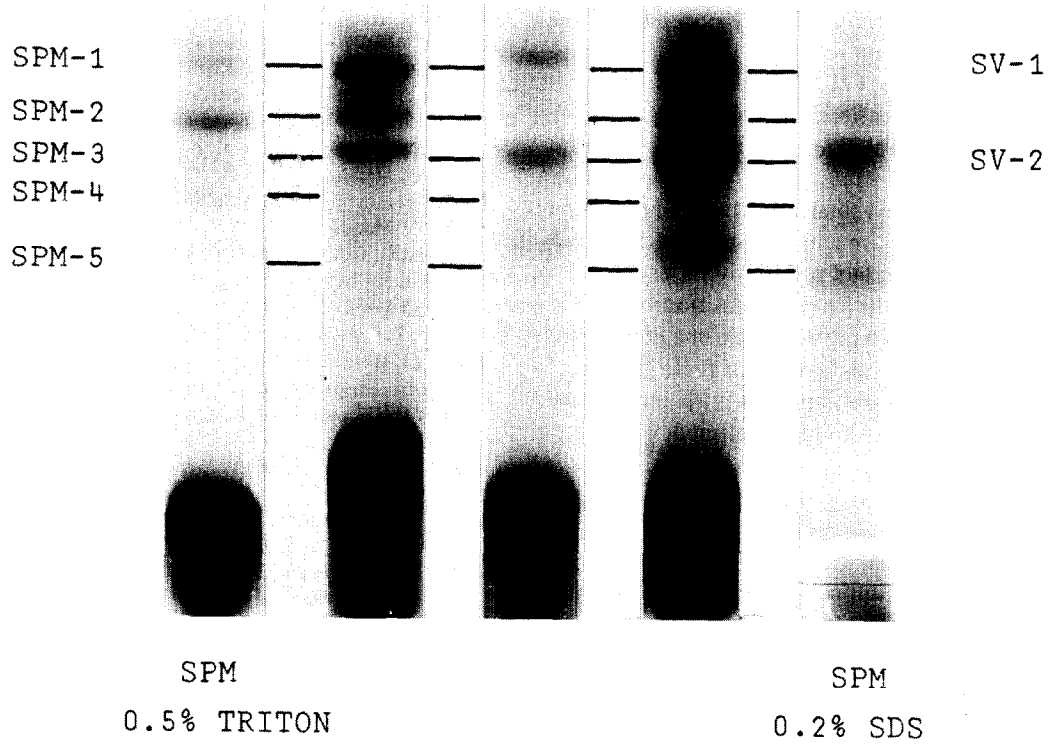

Fig. 3. Mixing experiments with the 0.5% Triton extract of synaptic vesicles. Gels are from the left: 0.5% Triton extract of synaptosomal plasma membranes, the 0.5% Triton extract of synaptosomal plasma membranes plus the 0.5% Triton extract of the synaptic vesicles, the 0.5% Triton extract of the synaptic vesicles, the 0.5% Triton extract of the synaptic vesicles plus the 0.2% SDS extract of the synaptosomal plasma membranes, and the 0.2% SDS extract of the synaptosomal plasma membranes. Extracts were analysed as described under Methods.

protein bands are also glycoproteins adds a criterion of identity. Nevertheless, to rule out this possibility, it will be necessary to isolate and characterize the glycoproteins of the synaptic vesicles and synaptosomal plasma membranes. Such studies are now in progress.

The second possible explanation of these findings is that the synaptosomal plasma membrane preparations were contaminated with synaptic vesicles. In the absence of a marker for the synaptic vesicle membrane, this possibility is difficult to rule out. However electron microscopy [8] gave no evidence that the synaptosomal plasma membrane preparations were contaminated with synaptic vesicles. In addition, after passing the synaptosomal plasma membranes three times through the final sucrose gradient used in their preparation, the glycoprotein profiles were unchanged. This repeated purification should have eliminated contaminating or adsorbed synaptic vesicles. Thus, it seems likely that the SPM-1 and SPM-3 glycoproteins are well integrated into the synaptosomal plasma membrane.

Three other possibilities must be considered. Common glycoproteins could exist in the two membranes without any functional implications, or they could be there to facilitate, rather than as a consequence of, fusion. The third possibility is that the proteins found in the synaptosomal plasma membrane result from the fusion process. Since the exocytosis process implies by definition that the vesicle proteins are incorporated into the corresponding plasma membrane for a more or less extended time, these results do not establish that the vesicle membrane glycoproteins are permanently integrated into the plasma membrane and degraded in parallel with the other constituents of the synaptosomal plasma

membrane. On the contrary, in many systems it seems likely that the vesicles are reformed by pinocytosis inside the cell [1, 2, 16, 17]. Nevertheless, from consideration of the densitometric profiles, glycoproteins SPM-1 and SPM-3 account for 10–20% of the synaptosomal plasma membrane glycoproteins, which suggest that at any given time a substantial proportion of the synaptosomal plasma membrane is derived from the vesicles. We have in fact previously observed marked similarities in the lipid composition of synaptic vesicles and synaptosomal plasma membranes [10, 18]. The possible relationship of these findings to pre-synaptic membrane networks [19] and coated vesicles [20, 21] remains to be established.

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