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Note

Use of deoxycholate in gel filtration chromatography of synaptosomal membrane glycoproteins

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Important neuronal specializations include a plasma membrane which is capable of generating and propagating nerve impulses and a unique intracellular structural entity, the synapse. Pinched-off nerve endings (synaptosomes) have proven to be useful in elucidating morphological[1] and compositional[2] characteristics of the synaptic region. Synaptosomes possess an intrinsic, membrane-associated sialidase (neuraminidase) which is capable of hydrolyzing sialic acid residues from native sialoglycoproteins and sialoglycolipids (gangliosides)[3,4]. In experiments designed to determine differences in endogenous protein-directed sialidase activity, and its inhibition by Ca^{2+}, new information was collected concerning the use of deoxycholic acid as a dissociative agent in gel filtration studies of synaptosomal glycoproteins, the details of which are reported here.

EXPERIMENTAL

Methods

Bovine brain synaptosomal plasma membranes were prepared by a zonal rotor technique[5]. Membrane samples were incubated at pH 3.9, 37 °C for 90 min so as to activate endogenous sialidase activity[6]. Sialic acid residues remaining bound to membrane constituents after this incubation were labeled by a periodate–$NaB[^3H]_4$ procedure[7]. Membranes were suspended in 1% (w/v) deoxycholate and 5 m*M* Tris–HCl, pH 8.4, at a final concentration of 1 mg protein per ml, and were incubated at 37 °C for 15 min[8]. Undissolved material was removed by centrifugation at 100,000 *g* for 2 h. Supernatants were collected and 1 ml was chromatographed at 4 °C on a column of Sepharose 4B (28 × 1.2 cm), previously equilibrated with 1% (w/v) deoxycholate, 5 m*M* Tris-HCl, pH 8.4. From each 2-ml fraction, a 100-μl aliquot was examined for radioactivity in 6 ml of Aquasol. The Sepharose 4B column was calibrated by chromatographing a mixture of standard proteins, labeled individually by

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reductive methylation using H[^{14}C]HO and $NaCNBH_3$ (refs. 9, 10). Pooled fractions from the experimental samples were dialyzed for three days, precipitated by the addition of one-tenth volume of 4% acetic acid, washed twice with ethanol to remove any remaining deoxycholate and were dissolved in 4 *M* urea, 1% β-mercaptoethanol and 1% sodium dodecyl sulfate by heating at 100 °C for 5 min. Polyacrylamide slab gels (7.5% running gel; 3% stacking gel; 1.5 mm thick; 100 mm long) were run at 8 mA per gel employing the discontinuous buffer system of Laemmli[11]. After electrophoresis, gels were stained with Coomassie Blue, and destained at room temperature in the presence of a mixed-bed ion-exchange resin. Absorption at 595 nm was monitored with a Schoeffel SD3000 Spectrodensitometer.

RESULTS AND DISCUSSION

The results of the gel chromatography studies are given in Fig. 1. Monitoring of the eluting fractions for protein by absorption at 280 nm was not possible due to the high absorption of the deoxycholate–Tris solution at this wavelength, presumably due to impurities in the deoxycholate used (Sigma, St. Louis, MO, U.S.A.). Two distinct peaks of radioactive sialoglycoproteins were observed in each sample, one eluting at the void volume, determined with Blue Dextran 2000 (termed fraction I). A second peak, termed fraction II, was retained by the column and eluted at a position corresponding to a molecular weight range of 500,000 to 300,000.

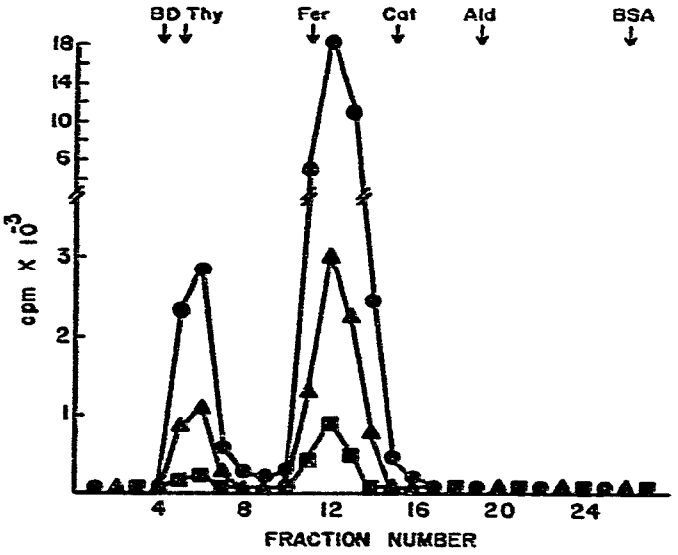

Fig. 1. Gel filtration of synaptosomal plasma membrane proteins on Sepharose 4B. One group of membranes was held on ice in 1 m*M* $HgCl_2$ (●) or incubated at pH 3.9, 37 °C for 90 min (ref. 6) in the presence (▲) or absence (■) of 5 m*M* Ca^{++}. After collection by centrifugation (15,000 *g*, 20 min) and twice washing with 50 m*M* EDTA, surface sialic acid residues were labeled by a periodate–NaB[^{3}H]$_4$ procedure[7]. Dissolving and eluting buffer was deoxycholate–Tris–HCl, pH 8.4. The eluting positions of molecular weight markers are given at the top of the figure: BD = Blue Dextran 2000, Thy = thyroglobin, Fer = ferritin, Cat = catalase. Ald = aldolase and BSA = bovine serum albumin. The material eluting first was termed fraction I; the second peak was termed fraction II.

A comparison of the two fractions, however, demonstrated that the polypeptide composition of each was not distinctly different. Rechromatography of an aliquot taken from fraction II again yielded two peaks of radioactivity, eluting in the same positions with similar relative peak heights. Spectrodensitometric analysis at 595 nm of precipitated proteins separated by polyacrylamide gel electrophoresis revealed a similar polypeptide profile to be present in each fraction. The results of an analysis of the control sample fractions is given in Fig. 2.

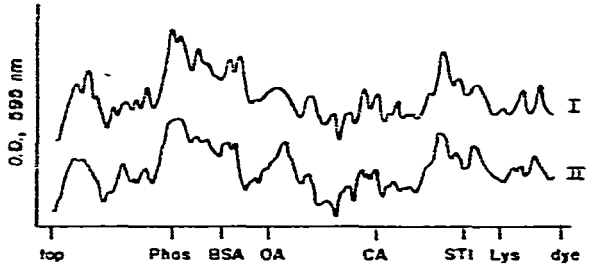

Fig. 2. Profile of absorbance at 595 mn of polypeptides of control fractions I and II (●, Fig. 1), separated by polyacrylamide slab gel electrophoresis employing a discontinuous sodium dodecyl sulfate buffer system[11], and stained with Coomassie Blue. Upper, fraction I; lower, fraction II. Profiles of separated fractions I and II polypeptides from incubated samples showed similar patterns. The positions of molecular weight markers are indicated at the bottom of the figure: top = 3%/7.5% gel interface, Phos = phosphorylase B, BSA = bovine serum albumin, OA = ovalbumin, CA = carbonic anhydrase, STI = soybean trypsin inhibitor, Lys = lysozyme and Dye = bromphenol blue.

It appears that gel filtration chromatography on Sepharose 4B in deoxycholate solutions is inadequate for separation of synaptic membrane proteins. Results very similar to those reported here were obtained by another investigator when attempting to fractionate proteins solubilized in sodium dodecylsulfate on Sepharose 4B columns[12]. It was concluded that while dodecyl sulfate may solubilize membrane proteins, it does not fully dissociate them. Membrane proteins in SDS solutions, in contrast to water-soluble proteins in dodecyl sulfate, seemed to exist in equilibrium between a dissociated form and one of random association. The results obtained in the present studies suggest that a similar situation exists for synaptosomal membrane proteins dissolved in deoxycholate. This finding is important in that deoxycholate has been employed as a solubilization agent for molecular and ultrastructural studies of the synaptic area[13,14]. Deoxycholate-solubilized material has also been used in lectin affinity chromatographic separations of glycoproteins from synaptosomal preparations[8,15]. Investigations on the effects of various detergents on lectin affinity separations have been reported by others[16,17].

ACKNOWLEDGEMENT

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