Comment

ISOLATION AND PURIFICATION OF MYELIN PROTEOLIPID PROTEIN USING HIGH SPEED GEL FILTRATION IN SODIUM DODECYL SULFATE

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Accepted February 7, 1985

Small and preparative gel filtration columns were studied for high pressure liquid chromatography of myelin proteins in sodium dodecyl sulfate. The preparative column proved useful for isolating and purifying proteolipid protein almost free (0.3-0.5%) of myelin basic protein as demonstrated by SDS-PAGE, MBP RIA, and immunoblotting. The small columns were not as useful as SDS-PAGE for analytical purposes.

INTRODUCTION

TSK-Gel SW type (Toyo Soda, Tokyo Japan) has been shown to have high separation efficiency for proteins in sodium dodecyl sulfate (SDS) at high speed (1-3). We have a need to generate large amounts of very pure myelin proteolipid protein (PLP) to improve our radioimmunoassay for that protein (4) and current methods were long and cumbersome (5-7). The applicability of gel filtration high pressure liquid chromatography (HPLC) in SDS as a method of protein preparation has been examined.

EXPERIMENTAL PROCEDURE

HPLC Apparatus and Operation. We used a Beckman model 110A HPLC apparatus with an LKB Uvicord S uv monitor and a Hewlett-Packard 3390A integrator and recorder. For exploratory work we used two Altex Spherogel TSK 3000SW columns in series each 7.5 mm ID × 30 cm, particle size 10 µm. A .020 ml or 0.5 ml sample loop was used with these columns. For preparative work an Altex 21.5 mm ID × 60 cm Spherogel TSK-G 3000SW column was used with a Spherogel TSK-G guard column. A 2.0 ml sample loop was used with this column.
Varying concentrations of sodium phosphate containing 0.1% SDS were used to elute delipidated (ether-ethanol) human myelin or chloroform-methanol extracted myelin PLP proteins from the two small columns.

The small columns were operated at room temperature at a flow rate of 0.6 ml/min while the preparative column was run at 4.0 ml/min also at room temperature. In either case the buffer contained sodium phosphate (of varying molarities in pilot experiments, but usually 0.1 M) pH 7.2 with 0.1% SDS.

Myelin Preparation. The enriched myelin fraction was prepared from autopsied human white matter according to the method described by Agrawal (8). Myelin was partially delipidated with ether-ethanol 3:2 (v/v) by vortexing 10 mg lyophilized myelin with 20 ml solvent followed by centrifugation at 10,000 g for ten minutes. This was repeated thrice followed by an ether wash. The protein (DHM) was dried under a stream of nitrogen.

Delipidated human myelin (DHM) was solubilized by homogenization in 0.05 M sodium phosphate pH 7.2 containing 2% (w/v) sodium dodecyl sulfate (SDS) and 1% (v/v) 2-mercaptoethanol to a final concentration of 2-4 mg protein/ml. The protein solution was then heated for 30 minutes at 56°C and rehomogenized. After heating in a boiling water bath for 3 minutes, the protein solution was centrifuged at 3000 g in a table top centrifuge and filtered through a 0.22 micron filter. Total protein was measured with appropriate control solutions by the Lowry procedure (9).

SDS Polyacrylamide Gel Electrophoresis (SDS-PAGE) and Immunoblot. SDS-PAGE was performed in a Bio Rad slab gel apparatus with the buffer system described by Neville (10). Immunoblotting was performed according to Towbin (11).

Radioimmunoassay (RIA) for MBP. The RIA for MBP was performed according to Cohen et al. (12).