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Note

Purification of myelin-associated glycoprotein from calf brain using high-performance liquid chromatography

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Myelin-associated glycoprotein (MAG) is one of the most prominent glycoproteins in central nervous system myelin, first found by Quarles et al. [1]. Immunohistochemical studies demonstrated the glycoprotein to be present in periaxonal regions of myelin sheath and in cytoplasm of myelin-forming oligodendrocytes during development [2,3]. MAG is considered to be involved in interactions between neuron and oligodendrocyte or between oligodendrocytes themselves [2,4]. A biochemical change in MAG is reported to be associated with demyelination in multiple sclerosis [5]. Recently, cDNA clones of MAG were isolated and the primary structure was determined [6,7]. These results indicate the presence of homologous sequences between MAG and the member of immunoglobulin super gene family such as neural cell adhesion molecule.

With respect to the preparation of MAG, Quarles and co-workers [1,3] extracted it with lithium diiodosalicylate (LIS)–phenol from brain myelin and purified it by gel permeation chromatography. However, in some instances we encountered the difficulty that the MAG fraction prepared by their procedure was severely contaminated with other proteins. In this study, reversed-phase high-performance liquid chromatography (HPLC) with an alkaline eluent system was applied to the purification of MAG. A considerable amount of MAG, free from detergents, was rapidly and easily obtained.

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EXPERIMENTAL

Chemicals

LIS, phenol and tetrabutylammonium hydroxide were purchased from Wako (Osaka, Japan). LIS was recrystallized from hot water and phenol was distilled before use. Concanavalin A (Con A) was obtained from Boehringer (Mannheim, F.R.G.). Other reagents and solvents were of analytical-reagent grade.

Extraction of MAG from brain myelin

Calf brain was obtained from a slaughterhouse (Tokyo Metropolitan Slaughterhouse, Shinagawa, Tokyo, Japan). For a typical preparation, 30 g of wet tissues from brain white matter were used to prepare myelin by the method of Norton and Poduslo [8]. About 3 g of lyophilized myelin were obtained and delipidated with 30 volumes of chloroform–methanol (2:1, v/v). The residue was extracted essentially as described previously [1,3] with slight modifications. Delipidated myelin was solubilized with 20 volumes of 0.25 *M* LIS in 50 m*M* Tris–HCl buffer (pH 7.5) containing 0.5 m*M* ethylene glycol tetraacetate (EGTA) and 5 m*M* dithiothreitol (DTT) using a Tephron homogenizer and an ultrasonicator. The solubilizing procedure was repeated three times. The extracts were combined and diluted with an equal volume of distilled water. To the resulting solution was then added an equal volume of 50% phenol. The mixture was stirred at 4°C for 30 min and centrifuged (2000 *g*, 30 min). After the separation of both upper and lower layers (8:2, v/v), the former (aqueous layer) was removed, dialysed twice against distilled water until the phenol odour disappeared and finally lyophilized (LIS–phenol extract).

High-performance liquid chromatography (HPLC)

LIS–phenol extract (24 mg) was dissolved in 1 ml of water containing 5 m*M* DTT by heating in a boiling water-bath for 5 min, and the solution was applied to the HPLC column. The MAG-containing fractions were immediately neutralized with 1 *M* acetic acid. An LC-5A liquid chromatograph (Shimadzu, Kyoto, Japan) with a reversed-phase TSK gel Phenyl-5PW RP column (70 mm × 7.5 mm I.D.) (Toyo Soda, Tokyo, Japan) and a SPD spectrophotometric detector (Shimadzu) were used.

Miscellaneous procedures

Protein was determined according to the method of Bradford [9]. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out according to the method of Laemmli [10]. In some experiments the MAG fraction was separated on a polyacrylamide gel, electrophoretically transferred to a nitrocellulose membrane [11] and stained by the two-step method using Con A–horseradish peroxidase and 4-chloronaphthol, as described previously [12].

RESULTS AND DISCUSSION

The delipidated myelin from bovine brain was solubilized with LIS-containing Tris–HCl buffer and the MAG-containing fraction was separated by the phenol precipitation method as described previously [1], with slight modifications (see Experimental). When the extract with 0.25 *M* LIS was diluted with an equal volume of distilled water, the aqueous and phenol layers were mutually effectively separated after an equal amount of 50% phenol had been added to the mixture. These extraction procedures, however, were found to be insufficient to purify MAG because the extract (the upper aqueous phase) was contaminated by other proteins as judged by SDS-PAGE (Fig. 1, lane A).

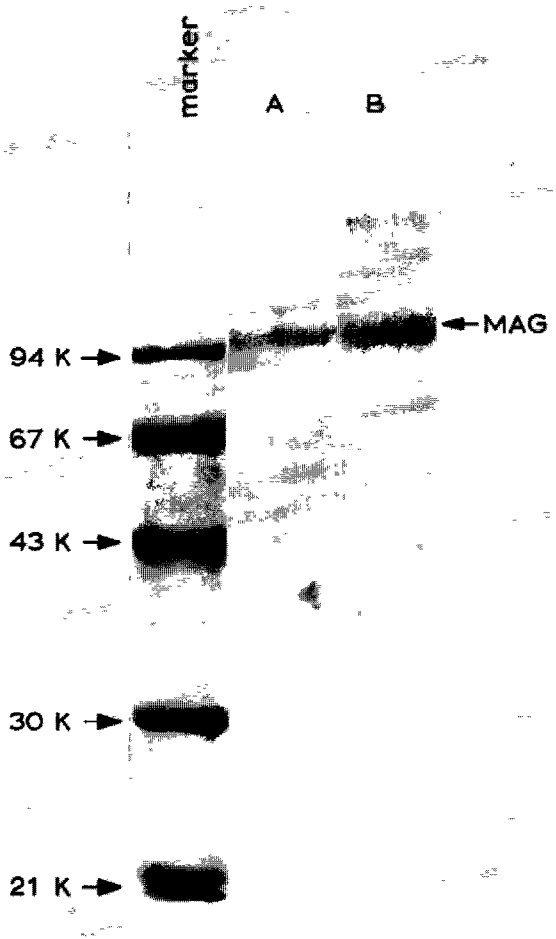

Fig. 1. SDS-PAGE of the MAG fractions. The bands were stained with Coomassie Brilliant Blue (A) LIS–phenol extract of delipidated myelin; (B) MAG fraction eluted from HPLC column.

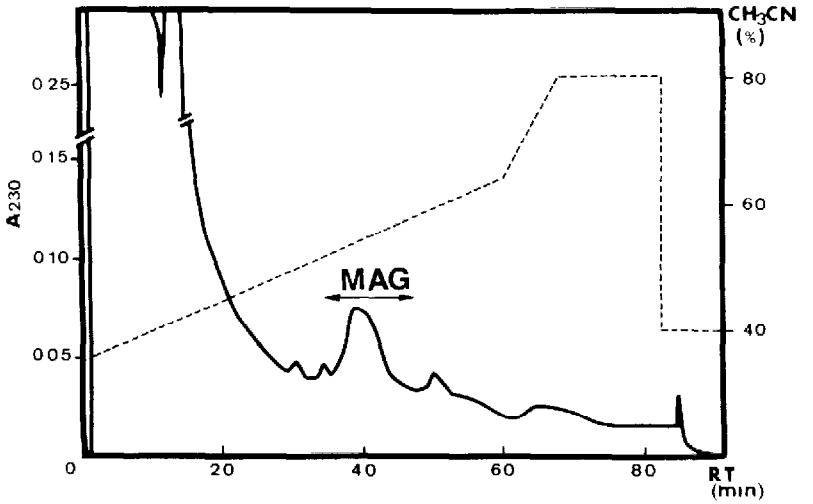

Fig 2. Chromatogram of the LIS–phenol extract of myelin. A TSK gel Phenyl-5PW RP column (75 mm×7.5 mm I.D.) was used at 23°C. Flow-rate, 1 5 ml/min. Eluent, 5 m*M* borate buffer containing 20 m*M* tetrabutylammonium hydroxide (pH 9 5) and acetonitrile. The dashed line shows the percentage of acetonitrile in the mobile phase.

TABLE I

YIELD OF MAG FROM CALF BRAIN

Sample	Yield
Calf brain	30 g wet weight
Myelin	3.3 g dry weight
	1 1 g protein
LIS–phenol extract	24 mg dry weight
	4.4 mg protein
HPLC-purified MAG fraction	2.3 mg protein

An attempt was made to develop a facile method for the further purification of MAG using reversed-phase HPLC. The HPLC elution pattern of MAG is shown in Fig. 2. Using a reversed-phase column, MAG was efficiently separated with an alkaline eluent containing a relatively high concentration of an ion-pair reagent. In contrast, no MAG was eluted with an acidic eluent such as a trifluoroacetic acid-containing solution. Fig. 1, lane B, shows the electrophoretic pattern of the MAG fraction obtained from HPLC separation. Essentially a single band with an apparent relative molecular mass of 100 000 was observed. Only this band exhibited binding activity to Con A [13] and monoclonal antibody HNK-1 [14] within the LIS–phenol-extracted proteins from myelin (data not shown). This band is considered to correspond to MAG. MAG

prepared in this study by means of reversed-phase HPLC was more homogeneous and free from any detergent such as LIS or SDS, in contrast to the gel permeation chromatographic method reported previously [3]. The yield of MAG was 2.3 mg from 30 g of brain (Table I), and it can be used in neuroscience studies because of its high purity.

The method described is applicable to the purification of membrane-bound hydrophobic proteins such as MAG with considerable yields.

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