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Immunoblot Identification of 13.5 Kilodalton Myelin Basic Protein in Goldfish Brain Myelin

*B. I. Roots, †D. Agrawal, *G. Weir, and †H. C. Agrawal

**Department of Zoology and Erindale College, University of Toronto, Mississauga, Ontario, Canada; and †Department of Pediatrics and Neurology, Washington University School of Medicine, St. Louis, Missouri, U.S.A.*

Abstract: Myelin isolated from goldfish brain shows a multilamellar structure with a major dense line and two intraperiod lines. Sodium dodecyl sulfate gel electrophoresis revealed that the protein profile of goldfish brain myelin is distinctly different from that of rat brain myelin. No protein migrating to the position of proteolipid protein or DM-20 was seen in goldfish myelin. Goldfish acclimated to 5°, 15°, and 30°C showed no qualitative differences in myelin proteins. The 13.5 kD protein in goldfish brain myelin and brain homogenate was intensely immunostained with the antiserum to human basic protein by the immunoblot technique. In contrast, none of the proteins of goldfish myelin were immunostained with antiproteolipid protein serum; however, both proteolipid protein and DM-20 of rat brain myelin were immunostained. The significance of the synthesis of myelin proteins by astrocytes in the goldfish brain is discussed. **Key Words:** Goldfish—Brain—Myelin—Myelin basic protein—Immunoblot. **Roots B. I. et al.** Immunoblot identification of 13.5 kilodalton myelin basic protein in goldfish brain myelin. *J. Neurochem.* **43**, 1421–1424 (1984).

During acclimation of goldfish (*Carassius auratus* L.) to different environmental temperatures, compensatory changes occur in the lipids of both brain and spinal cord myelin (Selivonchick and Roots, 1976; Selivonchick et al., 1977). The present study was designed to determine whether qualitative changes occur in the individual myelin proteins with acclimation to various temperatures. In addition, proteins of goldfish myelin were examined to determine whether they share immunochemical similarity with basic protein or proteolipid protein (PLP) of rat brain myelin. A preliminary report has been published recently (Roots et al., 1983).

MATERIALS AND METHODS

Chemicals

All reagents used were analytical grade. The sources of chemicals used for sodium dodecyl sulfate (SDS) slab gel electrophoresis and for the identification of proteins by the immunoblot technique have been described in detail by Gilbert et al. (1982).

Animals

Goldfish (*Carassius auratus* L.), 5–6 inches total length, were obtained from Hartz Mountain Pet Supplies, Toronto, Ontario. The fish were kept in holding tanks at 10°C for at least 30 days. They were then acclimated to 5°, 15°, and 30°C for 50 days under a constant photoperiod of 12 h light and 12 h dark and fed Purina Trout Chow. Fish at 30°C were fed twice a day, those at 15°C once a day, and those at 5°C once every other day at *ad libitum*.

Isolation of myelin and assessment of its purity

Myelin from fresh goldfish brains and rat brains was isolated as described by Agrawal et al. (1972). The purity of myelin was assessed by electron microscopy. Aliquots of the preparations were fixed overnight at 4°C in 2% glutaraldehyde in Sorenson's 0.1 *M* phosphate buffer (pH 7.4) and postfixed for 1 h in 1% osmium tetroxide in the same buffer. The samples were dehydrated in a graded series of ethanol and embedded in Spurr's medium. Sections were stained on the grids with 7% uranyl acetate in 70% methanol and Reynold's lead citrate.

Generation of antibodies

The method of generating precipitating antibodies to PLP and myelin basic protein (MBP) in rabbits has been described by Agrawal et al. (1977) and by Hartman et al. (1979), respectively. Antibodies to large basic proteins (LBP) of human, chick, and rat myelin were generated as described (Agrawal et al., 1977; Hartman et al., 1979).

Received February 2, 1984; accepted April 18, 1984.
Address correspondence and reprint requests to Dr. B. I. Roots, Department of Zoology and Erindale College, University of Toronto, Mississauga, Ontario, L5L IC6, Canada.

Abbreviations used: kD, Kilodalton; LBP, Large (18.5 kD) basic protein; MBP, Myelin basic protein; PLP, Proteolipid protein; SBP, Small (14 kD) basic protein; SDS, Sodium dodecyl sulfate; WP, Wolfgram protein.

Removal of lipids from myelin, and SDS-slab gel electrophoresis

Lipids were removed from lyophilized myelin (5–10 mg) by successive extraction at 4°C with ether-ethanol (3:2 vol/vol) and ether. The proteins were dissolved in Tris-glycine sample buffer containing 62.5 m*M* Tris-HCl (pH 7.4), 1% SDS, 8% sucrose, and 1.5% 2-mercaptoethanol and heated in a boiling water bath for 2 min. Goldfish brain was homogenized in 2% SDS containing 8 *M* urea. An equal volume of the sample buffer containing 125 m*M* Tris-HCl (pH 6.8), 3% 2-mercaptoethanol, and 16% sucrose was added to the brain homogenates and the mixture was heated in a boiling water bath for 2 min. Protein concentration was determined according to the technique of Lowry et al. (1951). Myelin proteins (55–75 μg) and proteins in the brain homogenate (100 μg) were separated in a 12% acrylamide-SDS slab gel in a Tris-glycine buffer system described by Laemmli (1970). The proteins in the gel were stained with Coomassie Blue R-250, and excess dye was removed with decreasing concentrations of methanol (Agrawal et al., 1972).

Identification of myelin proteins by the immunoblot technique

Myelin proteins (75 μg) and proteins in brain homogenate (100 μg) were separated by SDS-gel electrophoresis and then transferred to nitrocellulose sheets by the technique of Towbin et al. (1979). The nitrocellulose sheets were treated with 4% gelatin to block the unoccupied protein binding sites and then treated with antiserum to MBP and PLP. The nitrocellulose sheets were then treated with horseradish peroxidase-conjugated goat anti-rabbit IgG followed by diaminobenzidine and hydrogen peroxide as described by Towbin et al. (1979).

RESULTS

Morphology of myelin isolated from goldfish brain

Myelin isolated from goldfish brain was examined by electron microscopy; it exhibited characteristic multilamellar structure with a distinct major dense line and two intraperiod lines (Fig. 1).

SDS-slab gel electrophoresis of proteins of goldfish and rat brain myelin

Proteins of rat brain and goldfish brain myelin were separated by SDS-slab gel electrophoresis under identical conditions. The protein profiles are shown in Fig. 2. Most of the proteins of rat brain myelin (Fig. 2, lane 1) did not appear to migrate to the position of proteins of goldfish brain myelin (Fig. 2, lanes 2, 3, and 4). The goldfish brain myelin contained a unique protein (apparent molecular weight of 36,000) first described and designated X by Elam (1974). The goldfish brain myelin was also characterized by the presence of two proteins migrating above and below PLP and a 13.5 kD protein migrating just below the position of the small basic protein of rat brain myelin (Fig. 2, lanes 2, 3, and 4). A similar protein profile of goldfish brain myelin has been reported by Jeserich (1983). There were no qualitative differences in the myelin proteins of the fishes maintained at different temperatues (Fig. 2, lane 2, 5°C; lane 3, 15°C; and lane 4, 30°C).

Identification of proteins of goldfish brain myelin by the immunoblot technique

Proteins of goldfish brain homogenate, brain myelin, and rat brain myelin were transferred from SDS gel to the nitrocellulose sheet and treated with antiserum to human MBP by the immunoblot technique. A protein with molecular mass of 13.5 kD in the goldfish brain myelin (Fig. 3B, lane 4) and brain homogenate (Fig. 3B, lane 5) was immunostained. Similar results were obtained when proteins of goldfish brain myelin were treated with anti-rat LBP and anti-chick LBP sera (data not shown). Further, one high-molecular-weight protein in goldfish brain myelin and a few high-molecular-weight proteins in brain homogenate were very faintly immunostained with anti-MBP serum. The origin of these high-molecular-weight proteins and their relationship to MBP is not known. When proteins of goldfish brain myelin and rat brain myelin were treated with anti-

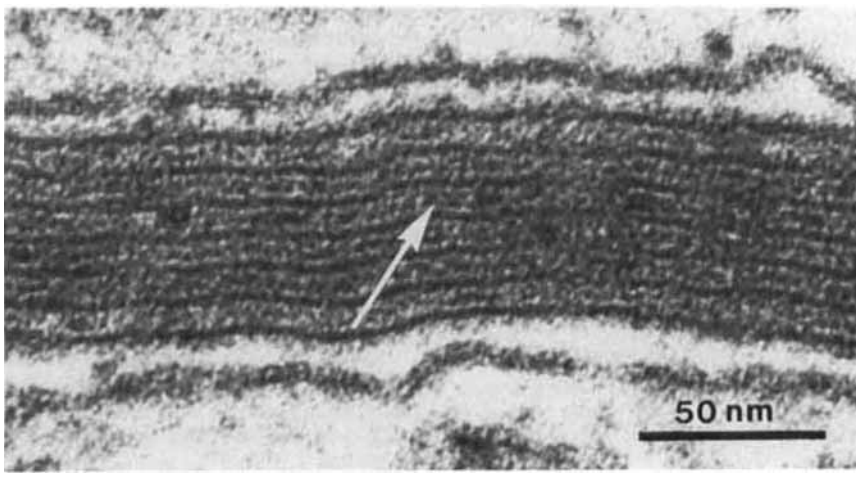

FIG. 1. Electron micrograph from goldfish brain myelin showing the double intraperiod line (arrow). Bar = 50 nm.

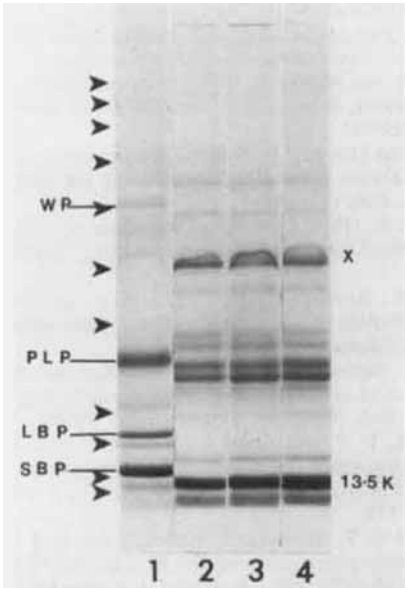

FIG. 2. *SDS-slab gel electrophoresis of proteins of rat brain* and goldfish brain myelin. Proteins of rat brain myelin (55 μg) and goldfish brain myelin (55 μg) were separated in a 12% (wt/vol) SDS slab gel. Electrophoresis was carried out first at 20 mA for 90 min, and then at 40 mA for 4 h. The proteins in the gels were stained with Coomassie Blue R-250. Lane 1, rat brain myelin. Lanes 2–4, brain myelin of goldfish acclimated to 5°, 15°, and 30°C, respectively. Arrows indicate mobilities of molecular weight standards in descending order: β-galactosidase (136,000), phosphorylase (94,000) conalbumin (76,000), catalase (60,000), ovalbumin (43,000) glyceraldehyde 3-phosphate dehydrogenase (36,000), chymotrypsinogen (25,700), soybean trypsin inhibitor (21,000), myoglobin (17,200), lysozyme (13,700), and cytochrome *c* (11,700). Identical results were obtained when *three separate preparations of goldfish brain myelin proteins* were subjected to gel electrophoresis. The apparent molecular weight of the protein designated X in lane 4 was found to be 36,000; this protein was not stained with antisera to MBP or PLP.

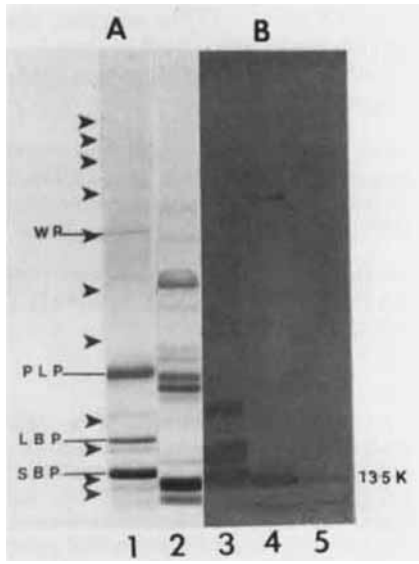

FIG. 3. Immunoblot identification of MBP in goldfish brain myelin. The proteins of rat brain myelin, goldfish myelin, and goldfish brain homogenate were separated by SDS-slab gel electrophoresis and transferred to nitrocellulose sheets and treated with antiserum to human MBP. (A), Proteins stained with Coomassie Blue. (B), Nitrocellulose sheets treated with antiserum to MBP. Lane 1, rat brain myelin (55 μg); lane 2, goldfish brain myelin (55 μg); lane 3, rat brain myelin (55 μg); lane 4, goldfish brain myelin (75 μg); lane 5, goldfish *brain homogenate* (100 μg). Designation of the bands visualized is the same as for Fig. 2.

PLP serum, no proteins of goldfish brain myelin were immunostained (data not shown), whereas PLP and DM-20 (Agrawal et al., 1972) were intensely stained in the rat brain myelin.

DISCUSSION

It is well known that both the basic protein and PLP are synthesized by the oligodendroglial cells in the mammalian CNS (Hartman et al., 1982). The presence of a 13.5 kD basic protein and the absence of PLP from the goldfish brain myelin raises a number of questions. Are oligodendroglial cells responsible for the synthesis of myelin proteins in the goldfish brain? If oligodendrocytes are producing myelin proteins in the goldfish brain, why is PLP absent? Since PLP is absent from goldfish brain myelin, this implies that either the gene for PLP is absent, or the gene for PLP is present but not expressed, or MBP is not produced by oligodendroglial cells but is synthesized by another cell type in the goldfish brain. One of the striking features of teleost brains is that very few oligodendrocytes are present, whereas astrocytes are common in both gray and white matter (Kruger and Maxwell, 1967). Connections between oligodendrocytes and myelin sheaths have not been observed in teleosts, and there is no morphological evidence, as there is in mammals, to implicate them in myelination. It may be that in goldfish brain astrocytes are responsible for myelination. There is some evidence that even in mammals, in some locations (e.g., the inferior olive and the lamina cribrosa region of the optic nerve) astrocytes rather than oligodendrocytes may be the myelinating cells (Walberg, 1963; Wendell-Smith et al., 1966).

It is well known that MBP and P_0 protein in the mammalian peripheral nervous system are produced by Schwann cells (Sternberger et al., 1978; Trapp et al., 1981). Jessen and Mirsky (1980) demonstrated that glial cells in the enteric nervous system of mammals, which like Schwann cells are derived from the neural crest, are rich in glial fibrillary acidic protein and thus resemble astrocytes. It is possible that astrocytes resemble Schwann cells in the type of myelin proteins that they produce. The presence of MBP in goldfish brain myelin (present study) and P_0 protein in shark brain myelin (Tai and Smith, 1984) and in electric ray (*Torpedo marmorata*) and trout (*Salmo gairdneri*) CNS

myelin (Jeserich et al., 1984) suggests that myelin proteins in fish brain are, in all probability, synthesized by another cell type such as astrocytes, rather than by oligodendrocytes.

Note added in proof: T. V. Waehneldt and G. Jeserich (*Brain Research*, in press) have also shown by the immunoblot technique the presence of myelin basic proteins BP1 and BP2 in rainbow trout brain.

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