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Composition of Myelin Proteins in Murine Genetic Myelin Dysgenesis: The Quaking Mutant (37888)

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(Introduced by C. E. Anderson)

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Quaking mice manifest a disorder of myelin formation which is transmitted as an autosomal recessive trait (1). Several biochemical (2) studies have indicated that the cerebroside content of the CNS of the Quaking mutant approximates 20% of that in littermate control mice at 20 days of age and does not increase significantly thereafter. Since cerebrosides are enriched in myelin, this developmental pattern has suggested that the mutant disorder can be described as a failure of myelin maturation (2). This hypothesis is supported by the finding of lower contents of long-chain and alpha-hydroxy fatty acids in cerebrosides and sulfatides of Quaking brain (3–7) as the pattern found resembles that of immature normal brain. The myelin proteins of adult Quaking brain differ in composition from normals in a deficiency of proteolipid protein (8–10) and this finding, too, suggests a developmental arrest in the process of myelination in view of the studies showing that proteolipid protein is the last of the major myelin proteins to appear during myelination (8, 11, 12). In our study of myelin dysgenesis, we have examined the yield and protein composition of myelin during active myelination (20 days postpartum) in Quaking brain with comparison both to littermate controls and to the similar but more devastating and nonallelic genetic myelin dysgenesis of the Jimpy mutant. Particular attention has been paid to the high molecular-weight or Wolfgram proteins (13) found on SDS—polyacrylamide gel electrophoresis of murine myelin proteins.

Materials and Methods. Mice, heterozygous for the Quaking (qk) mutation, were obtained from Jackson Memorial Laboratory (Bar Harbor, ME) and bred locally. Mutant offspring, homozygous for Quaking, were identified at 11–12 days postpartum by the axial body tremor which is characteristic of the mutation. Control and Quaking mice were sacrificed at 20 days and at adult ages (2–3 months) by cervical dislocation. Additional control mice were sacrificed at 30 days of age. Brains were removed immediately and frozen at −20°. An additional 30 brains of 20-day-old Quaking mice were obtained from Jackson Memorial Laboratory. A total of 112 control brains and 75 Quaking brains were used in this study, with three to six control brains and 6–12 Quaking brains pooled for each experiment.

Myelin was prepared from control and Quaking brains by a modification of the procedure of Waehneldt and Mandel (14). The tissue was homogenized on 0.32 *M* sucrose and centrifuged at 5900*g* for 15 min in the cold. The supernatant was discarded and the pellet was rehomogenized in 0.32 *M* sucrose and centrifuged as described. This step was repeated. The pellet was then homogenized in cold 0.88 *M* sucrose (the concentration of the sucrose became approx 0.83 *M*) and centrifuged at 64,700*g* for 2 hr. The surface layer was removed, subjected to osmotic shock by dilution with 15 vol dis-

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tilled water, and centrifuged at 6800*g* for 15 min. This step was repeated twice.

The myelin pellet was lyophilized and then extracted with diethyl ether–ethanol (3:2, v/v). Myelin proteins were solubilized at room temperature in a solution of 2.5% SDS, 1.0% Na_2CO_3, and 10.0% mercaptoethanol for 2–3 hr (15) and then dialyzed overnight at 4° against a solution of 0.1% sodium dodecyl sulfate (SDS), 0.01 *M* sodium phosphate buffer at pH 7.2, 1.6 *M* urea, and 0.05% dithiothreitol. The system for separation of the proteins (16) employed gels containing 10% acrylamide, 0.25% *N,N'* methylene bisacrylamide, 0.05% TEMED (*N,N,N',N'*-tetramethylethylene-diamine), and 0.75% ammonium persulfate. Gels were removed from the glass tubes, fixed overnight in methanol:water:glacial acetic acid (45:45:10) and stained in 0.25% Coomassie blue in 20% trichloracetic acid (TCA), and destained in 20% TCA. The stained gels were photographed and scanned at 640 nm with a Gilford Model 240 spectrophotometer. Myelin proteins for identification of bands were prepared according to Gonzalez-Sastre (17).

Myelin purity was estimated by the solubility of the fractions in chloroform–methanol (2:1) and the demonstration of negligible (<1% of total homogenate) succinic dehydrogenase activity (18) in this fraction. The molar ratio of cholesterol (19):phospholipid (20):galactolipid (21) in the isolated myelin was 1.05:1.00:0.46 and the protein content (22) was 22–25% of the dry weight of lyophilized myelin.

Results. After centrifugation on 0.88 *M* sucrose, myelin from adult control mice appears almost entirely as a compact floating layer with a minor portion observed as a diffuse layer 3–6 mm below the surface. In contrast, myelin from brains of 20-day-old and adult Quaking mice is found predominantly in the region of the denser and more diffuse myelin of control mice. Observations from this lab and others (6, 8, 9) suggest that the denser myelin of Quaking mice (20-day-old to adult ages) may be analogous to the myelin of immature (12-day) control mice.

The recovery of myelin protein from the brains of control and Quaking mice is summarized in Table I. The content of CNS myelin protein from control mice triples between 20 and 30 days of age and more than doubles again between 30 days and adult ages. This pattern of myelin protein accretion in murine brain is similar to that observed previously in our laboratory in other strains. In contrast with the normal 6- to 7-fold increase in myelin protein content between 20 days and adult ages, Quaking mice demonstrate no increase during this period. This deficiency of myelin protein is of the same order as that found by analysis of myelin-associated lipids of brains (2, 23).

Proteins solubilized from preparations of myelin from control mice resolved in order of decreasing electrophoretic mobility as follows: (1) the smaller and larger basic proteins (BP_1 and BP_2) (24); (2) proteolipid protein (25); and (3) several minor bands of high molecular-weight proteins (8, 13). Between 20 days and adult ages there is an increase in the ratio of proteolipid proteins to the basic proteins. This developmental pattern has also been observed by Morell *et al.* (26) and in our laboratory (11) in mice and by Savolainen *et al.* (12) in humans.

TABLE I. Recovery of Myelin Protein from Murine Brain.[a]

Postpartum age	Normal	Quaking
20 Days	301 ± 128 (7)	55 ± 18 (7) *
30 Days	905 ± 425 (4)	—
Adult (2–3 months)	2303 ± 570 (7)	57 ± 14 (3) *

[a] Values are expressed as micrograms of myelin protein recovered per brain and represent the means ± standard deviations for the number of experiments given in parentheses. Statistically significant differences from control brains are denoted by * ($P \leq 0.01$). Control brains were obtained from littermate animals without neurologic dysfunction. Student's *t* test was used in assessing significance of data. The myelin subfraction was isolated from groups of murine brains using a modification of the method of Waehneldt and Mandel (14) and protein content determined according to Lowry *et al.* (22).

The major proteins of the myelin subfractions were identified by comparison to authentic purified protein standards including the addition of standards to the myelin protein fractions obtained from mouse brain. The myelin proteins from the Quaking mutant showed a reduction of the basic proteins and of the proteolipid protein with an increase in the relative concentrations of the high molecular-weight proteins (Fig. 1). At the ages between 20 days and 3 months postpartum, the basic protein was relatively preserved when compared to the decrease in proteolipid (Fig. 1). In the Quaking mutant, we repeatedly found the faster-migrating of the more prominent of the high molecular-weight proteins to be decreased (Fig. 1).

When care was taken to avoid delay in processing or freezing the tissues in order to avoid autolytic degradation of the basic proteins (27) (note peaks before that of BP_1 in Fig. 1), no difference in the relative amounts of the two basic proteins was found in comparing control and Quaking brain.

Discussion. The myelin content of Quaking brain is identical at 20 days postpartum and at maturity, and the myelin recovered represents approximately 16% and 2%, respectively, of the myelin content of control brains at these ages. This pattern during

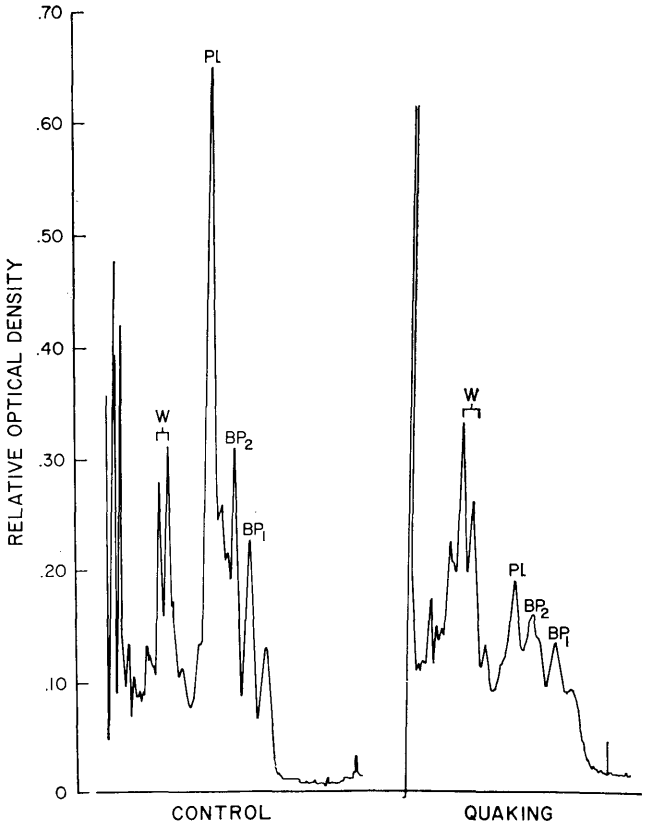

FIG. 1. Densitometry of gels containing myelin proteins. Gels were charged with comparable amounts of protein (100 μg). Labels: W, Wolfgram or high molecular-weight proteins: Pl, proteolipid protein; BP_1, smaller basic protein; BP_2, larger basic protein. Peak migrating before BP_1 is product of postmortem catheptic degradation of basic protein (27). Quaking mutant and littermate controls were 20 days postpartum. The procedures for isolation of myelin, and for solubilization and electrophoretic resolution of the myelin proteins are as described in the text.

development supports the hypothesis of a failure in maturation of the myelin sheath in the Quaking mutant which had previously been suggested by our studies of brain cerebroside content and composition during development (2) and that of Greenfield *et al.* (8) of the myelin protein composition of the adult Quaking mouse. In agreement with the latter, we have demonstrated in both immature and mature myelin of the Quaking mutant a selective deficiency of not only the proteolipid protein but also of the faster-migrating of the major Wolfgram proteins. We have also observed a decrease in this faster-migrating Wolfgram protein in two other murine hereditary disorders of myelin formation, the Jimpy and MSD mutants. This finding in three different myelin-deficient mutants indicates a close association of this glycoprotein with myelin and suggests the possibility of a pathogenetic relationship of this finding of the disorder.

The Jimpy mutant is a similar genetic disorder of myelin formation occurring in the mouse. It differs from the Quaking in manifesting a sex-linked recessive pattern of transmission and in being a more devastating disorder viewed clinically (1), histologically (1, 5), and biochemically (2, 28, 29). Using the same methods, our yield of myelin protein from Jimpy brain was 31.2% of that obtained from the Quaking at 20 days postpartum, a result offering another means of comparing these mutants. No comparison of mature brains is possible since the Jimpy mutant has a shortened life span. The proteolipid and basic proteins were seen to be diminished more in the Jimpy than in the Quaking.

The proteolipid proteins of myelin seem to be ultimate or penultimate additions to the myelin in its maturation to a mature compact sheath (8, 11, 12). Thus, it seems reasonable to interpret the alterations in myelin protein composition found in the Quaking mutant as reflecting a failure in myelin maturation. Our findings could be a biochemical effect of hypomyelination of any cause or result from disturbed synthesis or maintenance of the proteolipid proteins. An interesting additional possibility susceptible to experimental test is of an altered functional group on the basic protein necessary to protein–protein or protein–lipid binding essential to formation of the stable myelin membrane. Theoretically, such a point mutation could affect deletion or substitution of a few amino acids without changing the molecular weight sufficiently to observe a difference with the techniques employed in this study. Finally, the alteration in one of the high molecular-weight proteins raises the possibility of a crucial role of this molecule either in signaling (inducing) the formation of stable compact myelin or in maintaining the stable multilammellar structure.

Summary. Myelin was isolated by sucrose density-gradient centrifugation from brains of Quaking mutants and littermate controls at the developmental ages of most active myelination. The myelin proteins were solubilized in sodium dodecyl sulfate (SDS) and separated by electrophoresis in SDS–polyacrylamide gels. The yield of myelin from the Quaking mutant at ages varying from 3 weeks to 3 months postpartum was constant and did not show the progressive increase found in normal brain. There was a marked reduction of proteolipid and lesser decrease of basic protein in the myelin subfraction from the Quaking mutant. The proportion of high molecular-weight (Wolfgram) proteins of the Quaking mutant was increased though a consistent diminution of one of these was observed. Alteration or deficient synthesis of a myelin protein could be the primary genetic error in the dysmyelination manifest by this mutant.

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