Proteins from Sciatic-Nerve Myelin in Quaking and Jimpy Mice

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Myelin from two neurological mutants in mice was isolated from sciatic nerves and its protein composition analysed. In Quaking mice, two intrinsic myelin proteins P_1 and P_2 were drastically decreased, whereas the major myelin protein P_0 was unaffected. A normal protein composition was found in sciatic myelin from Jimpy mice.

The Quaking mutation in mice is characterized by a severe myelin deficit secondary to an arrest of myelinogenesis. A great number of morphological and biochemical studies have shown that the myelin sheath formed in the brain of Quaking mutants is abnormal (for a review, see Hogan, 1977). Although the central nervous system of the Quaking mice was studied extensively, only a little attention was devoted to myelin in the peripheral nervous system. Although less affected than central nervous system, peripheral nervous system from Quaking mice is also hypomyelinated (Samorajski *et al.*, 1970; Watanabe & Bingle, 1972; Appeldoorn *et al.*, 1975; Suzuki & Zagoren, 1976, 1977). The lipid content of peripheral nervous system from Quaking mice was reported to be decreased and indicated defective myelin formation (Kishimoto, 1971; Friedrich & Hauser, 1973). The protein composition of isolated myelin from peripheral nervous system is very different from that in central nervous system. Only one protein, the encephalitogenic myelin basic protein, is present in both peripheral nervous system and central nervous system, and is referred to as P_1 protein (Brostoff & Eylar, 1972). In this present study, I was interested in analysing the protein composition of peripheral-nervous-system myelin from myelin-deficient mice to see whether proteins unique to peripheral-nervous-system myelin were affected by the disorder. In central-nervous-system myelin from Quaking mutants of mice, we reported previously that the large myelin basic protein was not decreased, in contrast with the small basic protein, proteolipid protein and the major myelin glycoprotein (Matthieu *et al.*, 1978). In addition to peripheral-nervous-system myelin proteins from Quaking mice, those from another neurological mutant, the Jimpy mouse, were also analysed. In spite of an almost absence of myelination in the central nervous system, the peripheral nervous system from Jimpy mutants seems to be normally myelinated and their lipid content normal (for a review, see Hogan, 1977).

Experimental

The mutants and littermate controls of strain C57Bl/6J-Qk and C6CBA-A^{w-j} were originally obtained from the Jackson Laboratory (Bar Harbor, ME, U.S.A.) and bred in our laboratory. Pooled sciatic nerves stored frozen at -70° were ground in liquid N_2 (Greenfield *et al.*, 1973) and homogenized in 0.32M-sucrose. Myelin was isolated by the method of Norton & Poduslo (1973). For a typical experiment, sciatic nerves from 30 Quaking (Qk/Qk) and littermate control mice (+/+, +/Qk) 25 days of age, or 16 Jimpy (Jp/Y) and littermate control (+/Y) mice 17 days of age were used. Total proteins were measured in the myelin suspension by the method of Lowry *et al.* (1951). Myelin fractions were partially delipidated with diethyl ether/ethanol (Greenfield *et al.*, 1971), and dried under N_2 . Proteins were solubilized and separated in the presence of sodium dodecyl sulphate on 15% polyacrylamide gels as previously reported (Matthieu *et al.*, 1975). The gels were stained with 1% Fast Green and scanned on a spectrophotometer at 580nm (Morell *et al.*, 1972). The relative amounts of protein in the different bands are expressed as percentages of the total dye-binding capacity (Matthieu *et al.*, 1973). For optimal comparison of the different protein bands, both disc and slab gels were used.

Results and Discussion

The amount of myelin isolated from sciatic nerves of Quaking mutants was only 50% that from littermate controls. Peripheral-nervous-system myelin proteins from Quaking mice (Fig. 1) showed important differences when compared with the profile of control myelin. Among the high-molecular-weight proteins, a band labelled H_3 was decreased. This indicates that band H_3 is probably an intrinsic myelin component since it is decreased in the hypomyelinating disorder. Proteins P_1 (identical with central-nervous-system myelin basic protein) and P_2

(a basic protein unique to peripheral nervous system) (Brostoff *et al.*, 1975) were drastically decreased in Quaking mice. Protein P₄ (also called X) is a doublet and only the fast-migrating component was decreased in Quaking mice. The relative amounts of the different proteins confirmed the important decrease of proteins P₁ and P₂, one-third of that in control animals, whereas the major protein of peripheral-nervous-system myelin, protein P₀, was unaffected (Table 1). The other minor components were relatively increased. The ratios of protein P₀ to P₁ and of protein P₀ to P₂ confirmed the selective loss of these two basic proteins P₁ and P₂. These results contrast with the protein composition of peripheral-nervous-system myelin in Jimpy mice which showed no anomaly. The ratio of protein P₁ to P₂ was found to be extremely constant in all these experiments with animals of strain C57B1/6J (Table 1). This ratio, extremely variable among different species (Greenfield *et al.*, 1973), was found in the present study to be very constant in the same strain even in Quaking mutants,

Fig. 1. Densitometric scans of Fast Green-stained proteins on polyacrylamide gels
Each gel contained 100 μ g of protein. The different protein bands were labelled according to the nomenclature of Greenfield *et al.* (1973) and Wiggins *et al.* (1975).

Table 1. Protein composition of peripheral-nervous-system myelin

The different protein bands separated by electrophoresis and scanned as shown on Fig. 1 were quantified and their values expressed as percentages of the dye-binding capacity (means of two separate experiments). HMW represents all the high-molecular-weight proteins (including H₃) from the top of the gel to the P₀ protein (see Fig. 1). Quaking controls, +/+, +/Qk; Quaking mutants, Qk/Qk; Jimpy controls, +/Y; Jimpy mutants, Jp/Y. The results are means of two experiments (individual results in parentheses).

Component	Percentage of dye-binding capacity			
	Quaking		Jimpy	
	Controls	Mutants	Controls	Mutants
HMW	19.4 (18.6, 20.2)	28.3 (26.9, 29.7)	24.7 (22.6, 26.8)	20.2 (19.9, 20.5)
P ₀	34.3 (32.9, 35.7)	34.0 (32.4, 35.6)	30.3 (29.8, 30.8)	33.3 (30.6, 36.0)
P ₃	6.4 (5.3, 7.5)	10.3 (9.4, 11.2)	4.6 (2.7, 6.5)	6.8 (6.5, 7.1)
P ₄	9.8 (9.4, 10.2)	9.0 (8.5, 9.5)	10.1 (7.9, 12.3)	11.7 (10.6, 12.8)
P ₁	9.4 (8.4, 10.4)	3.4 (3.2, 3.6)	9.4 (8.8, 10.0)	8.9 (8.5, 9.3)
P ₂	9.2 (8.1, 10.3)	3.2 (2.9, 3.5)	9.5 (9.3, 9.7)	7.7 (6.4, 9.0)
P ₅	2.1 (1.3, 2.9)	3.3 (3.0, 3.6)	2.5 (2.2, 2.8)	2.2 (1.7, 2.7)
P ₆	2.0 (1.6, 2.4)	2.2 (2.1, 2.3)	1.6 (1.5, 1.7)	1.3 (0.7, 1.9)
Rest	7.4 (6.1, 8.7)	6.4 (5.8, 7.0)	7.4 (5.4, 9.4)	8.0 (7.2, 8.8)
Ratio				
P ₀ /P ₁	3.7	10.0	3.2	3.7
P ₀ /P ₂	3.7	10.6	3.2	4.3
P ₁ /P ₂	1.0	1.0	1.0	1.2

where proteins P₁ and P₂ were affected to the same extent. This is particularly interesting since protein P₁ is a myelin protein expressed in both central nervous system and peripheral nervous system and protein P₂ is expressed apparently only in peripheral nervous system. While this study was in progress, a preliminary report was presented, indicating a slight decrease (15%) of proteins P₁ and P₂ with a relative increase of protein P₀ in sciatic nerves from Quaking mice (Greenfield *et al.*, 1978).

In Quaking mice, peripheral nerves present a defect of myelination less severe than in the brain. An ultrastructural study of peripheral nerves from Quaking mice demonstrated the presence of atypical Schmidt-Lanterman incisures, major dense lines of compacted myelin sheath opened to the cytoplasmic island and intraperiod lines widely separated (Suzuki & Zagoren, 1976, 1977). The regions of abnormal myelin alternate with others where myelin lamellae are normally compacted. It is tempting to attribute these anomalies to the defect of P₁ and P₂ proteins, which are assumed to be topographically disposed at the major dense lines (Braun, 1977). P₀ protein, a glycoprotein (Everly *et al.*, 1973; Wood & Dawson, 1973), is presumably located at the intraperiod line (Braun, 1977). Therefore, one would expect no abnormality of the apposition between myelin lamellae since, as I report here, the protein-P₀ content was normal. This assumption is refuted by ultrastructural data, which show that intraperiod lines are widely separated (Suzuki & Zagoren, 1976). Further speculation will have to wait for experimental data on the molecular architecture of normal peripheral-nervous-system myelin.

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