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Myelin Basic Proteins in Myelin Subfractions from Normal and Quaking Mice

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The relative proportions of four myelin basic proteins (preL, L, preS, S) were determined in myelin subfractions prepared from the forebrains of quaking and littermate control mice. The distribution pattern of each protein was similar in both mutant and control fractions. The S component was the only basic protein present in low amounts in myelin from the mutant.

Myelin basic protein is thought to play an important role in the stabilization of the compact multilamellar structure of the myelin sheath (see reviews by Carnegie & Dunkley, 1975; Braun, 1977; Rumsby & Crang, 1977). The protein has been isolated from both central and peripheral neural tissue, and that from the central nervous system has been characterized in some detail. One noteworthy feature of the protein is its high degree of heterogeneity, which results (a) from post-translational modifications such as methylation, deamidation and phosphorylation and (b) from the presence, in some species of rodents, of an additional small basic protein (S) that differs from the large molecule (L) by an internal deletion of a 40-residue section of the polypeptide chain (for a comprehensive review see Martenson, 1979). Barbarese *et al.* (1977), by using immunological and peptide-mapping techniques, have identified two further basic protein components in mouse brain myelin; these were termed prelarge (preL) and presmall basic proteins (preS). The preL protein (mol.wt. 21 500) was shown to be similar in amino acid sequence to the L protein (mol.wt. 18 500), but with an additional 25–35-residue sequence located at, or near, the *N*-terminus of the molecule. The relationship between the preS and S proteins (mol.wts. 17 000 and 14 000 respectively) appeared the same as that between the preL and L proteins.

The present paper describes the distribution of the preL, L, preS and S proteins in subfractions of myelin isolated from the forebrains of quaking myelin-deficient (Sidman *et al.*, 1964) and littermate control mice. Previous studies have shown that the small amount of myelin isolated from the mutant's brain contains low amounts of both S and proteolipid proteins (Greenfield *et al.*, 1971; Gregson & Oxberry, 1972), and that these abnormalities are present throughout the entire population of myelin membranes (Matthieu *et al.*, 1978; Waehneldt *et al.*, 1979). The experiments reported in the present paper were

undertaken to provide additional information about the multiple forms of myelin basic protein in the myelin sheath.

Experimental

Quaking (*qk/qk*) and littermate control mice (+/+ or +/*qk*) of strain C57B1/6J (aged about 2 months) were obtained from the Centre de Sélection et d'Élevage d'Animaux de Laboratoire, Orléans-la-Source, France. Myelin was isolated from freshly dissected forebrains by using the procedure described by Waehneldt *et al.* (1977) and was immediately subfractionated by zonal centrifugation (Waehneldt, 1978) on linear 0.1–1.0 M-sucrose gradients. The fractions (10 ml) recovered from the rotor were assayed for their sucrose molarities (by refractometry) and turbidity (A_{300} , corrected for sucrose molarity), and were pooled to yield five or six myelin subfractions (see Fig. 1). These were washed three times with water and were stored at -70°C until analysis. In total, four separate experiments were conducted; two each with tissue pooled from 20 control animals, and two with tissue pooled from 54 and 65 mutants.

Myelin subfractions were solubilized as previously described (Fagg *et al.*, 1979) and proteins were separated by electrophoresis in linear 1–30% (w/v) polyacrylamide gradient micro-gels (Rüchel *et al.*, 1974). Gels were stained with Coomassie Brilliant Blue R250 and were scanned densitometrically (Zimmer & Neuhoﬀ, 1975) at 520 nm and 600 nm in a Zeiss (ZK4) gel scanner. Proteins were quantified at 520 nm by using a Servogor (RE544) pen recorder and integrator. Myelin basic proteins were identified (a) by comparison with published densitometric scans (Barbarese *et al.*, 1977, 1978) and (b) by using differences in the absorption spectra of Coomassie Blue complexed to the basic proteins and to 'other' myelin proteins. Thus on gel scans the ratio of peak heights of basic proteins to 'other' myelin proteins was greater at 520 nm than at 600 nm (by a factor of about 1.7-fold, when using S and proteolipid proteins as

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standards for basic and 'other' proteins respectively). A similar effect has been noted for the protein stain Fast Green (Cammer & Norton, 1976).

Results and Discussion

After zonal centrifugation on linear sucrose gradients, myelin from quaking- and control-mouse forebrains exhibited Gaussian-like distributions (Fig. 1) that peaked at 0.73M and 0.67M respectively (means for two experiments each). The range of particle densities was somewhat broader in the case of

Fig. 1. Density distribution of myelin particles isolated from the forebrains of quaking and littermate control mice. Myelin preparations were centrifuged on linear 0.1–1.0 M-sucrose gradients and the fractions recovered were assayed for sucrose molarity and turbidity (A_{300}). Values shown are percentages of the total absorbance (summed for all fractions). Only that part of the gradient from 0.4 to 0.9 M-sucrose is shown. Fractions were pooled as shown at the top of the Figure to give five (control; ●) or six ('quaking'; ○) myelin subfractions for electrophoretic analysis. In each case, subfraction 3 contained myelin at the peak sucrose molarity. The arrows indicate the pooling of fractions for the gradients from 'quaking' (lower line of arrows) and control-mouse myelin preparations (upper line of arrows).

the mutant's myelin, and additionally included a shoulder on the low-density side of the distribution profile. The high flotation density of quaking-mouse myelin has been noted in previous studies by Greenfield *et al.* (1971) and Gregson & Oxberry (1972).

Fig. 2 shows densitometric scans of Coomassie Blue-stained myelin proteins separated by micro-gel electrophoresis; the characteristically low amounts of S and proteolipid proteins, and the high proportion of high-molecular-weight proteins in the mutant's myelin are apparent. A quantitative analysis of the four basic proteins (on the basis of dye staining) in the five subfractions prepared from control forebrains (Fig. 3) indicated that the content of the S protein decreased from the low-density (fraction 1) to the high-density side (fraction 5) of the myelin particle distribution. Similar, but less marked, patterns were observed for preS and L proteins, whereas the amount of preL protein was essentially constant in all fractions. In previous subfractionation studies on rat brain myelin, the basic protein has

Fig. 2. Densitometric scans (A_{520}) of Coomassie Blue-stained myelin proteins in micro-polyacrylamide gels. Gels were cast in 10 μ l capillaries and loaded with 0.5–1.0 μ g of protein (origins at left of Figure) for electrophoresis. The gel scans shown are of myelin subfraction 3 from control- and quaking-mouse forebrains (see Fig. 1). Abbreviations used: P, proteolipid protein; I, intermediate protein; L, S, preL and preS, the four myelin basic proteins defined in the text.

Fig. 3. Distribution of basic proteins in myelin subfractions from the forebrains of quaking and littermate control mice

The basic protein composition of each fraction is expressed as a percentage of the total dye bound to micro-gels; values shown are means. Abbreviations used: L, S, preL and preS, the four myelin basic proteins defined in the text.

generally been considered to be a single entity (Matthieu *et al.*, 1973; Zimmerman *et al.*, 1975; Benjamins *et al.*, 1976). The present experiments indicate, however, that the four basic proteins are not distributed identically in different myelin particles, and that the distribution pattern of total myelin basic protein predominantly reflects that of the S component.

Recent observations suggest that, of the myelin basic proteins, it is the S component that is most affected by the 'quaking' mutation (Matthieu *et al.*, 1978; Fagg, 1979), although in these studies L and preS proteins were not resolved electrophoretically and were quantified together as 'large basic protein'. The present data both confirm and extend these observations. Considering only myelin subfractions 1–5 from the mutant, the distribution pattern of each of the basic protein components was similar to that described for controls, but with low amounts of S protein in all fractions (Fig. 3). The amounts of preS and L proteins were not markedly different between mutant and control subfractions; however, the relationship between preS and preL proteins was reversed, such that, in the mutant, the amount of preL protein was greater than that of preS protein. Subfraction 0 (representing the shoulder on the quaking-mouse-myelin density profile; see Fig. 1) was anomalous, and we are unable to explain the nature of this material.

Barbarese *et al.* (1978, 1979) have suggested that during ontogenetic development, gene expression changes from the synthesis of mainly preL protein initially to primarily S protein at a later time. Possibly

the basic proteins are normally incorporated into the cytoplasmic surfaces of preformed membranes at a fairly late stage in the formation of the compact myelin sheath; hence, under circumstances in which the production of new membranes ceases, the most recently synthesized basic proteins will not be utilized and the existing myelin sheath will exhibit an immature type of basic protein composition. In the 'jimpy' mutant, myelin formation ceases early in development and the central nervous system is almost totally devoid of compacted membranes (Sidman *et al.*, 1964); on the other hand, the 'quaking' mutation induces an arrest in myelinogenesis after some multilamellar myelin has already been formed (Wisniewski & Morell, 1971). In neither mutant does the synthesis of the basic proteins appear to be a limiting factor in myelin production (Carson *et al.*, 1975; Brostoff *et al.*, 1977). Thus studies of the 'myelin' fraction from the 'jimpy' mutant indicate that preL protein is the major basic protein component to be incorporated into the earliest myelin membranes (Barbarese *et al.*, 1979), whereas at a slightly later developmental stage (the quaking mouse; the present study) the myelin sheath contains essentially adult proportions of preL, L and preS proteins, but a low amount of the S component of myelin basic protein.

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