Journal of Neurochemistry
Raven Press, New York

0022-3042/83/1201-1538/$03.50/0

Characterization of Tubulin in Mouse Brain Myelin

*†Béatrice de Néchaud, *Annie Wolff, *Claude Jeantet, and †Jean-Marie Bourre

Laboratoire de Biochimie Cellulaire, Collège de France; and †Unité de Neurotoxicologie, INSERM U 26, Hôpital Fernand Widal, Paris, France

Abstract: Analysis of mouse brain myelin by sodium dodecyl sulfate-polyacrylamide gel electrophoresis showed that in the high-molecular-weight range it contained, besides the Wolfgram protein doublet, proteins comigrating with actin and with both subunits of tubulin. The occurrence of these α and β subunits was confirmed by peptide mapping in myelin analyzed by two-dimensional electrophoresis. This tubulin did not arise from an artifactual binding of soluble brain tubulin to the myelin fraction: addition of exogenously labeled tubulin to brain homogenates proved that during myelin isolation by the procedure of Norton and Poduslo (1973) the contaminating tubulin was washed out. On the other hand, the distribution of tubulin isoforms in myelin was investigated by isoelectric focusing and compared with the distribution of the 21 isoforms listed for the whole brain soluble tubulin. It was shown that many isoforms were found in myelin (three isoforms for the α subunit and nine for the β subunit), and that some isoforms were represented both in myelin and in soluble tubulin, but in different relative proportions. **Key Words:** Tubulin—Myelin—Wolfgram doublet. **de Néchaud B. et al.** Characterization of tubulin in mouse brain myelin. *J. Neurochem.* **41**, 1538–1544 (1983).

Previous results (Gozes and Richter-Landsberg, 1978) had suggested that tubulin may be a component of rat brain myelin, a possibility questioned by Waehneldt and Malotka (1980) in their studies on myelin from various species.

On the other hand, it was established that the microheterogeneity of brain tubulin increased during development and especially that some acidic isoforms of the β subunit of tubulin appeared only after the first postnatal week in rat and mouse brain (Gozes and Littauer, 1978; Dahl and Weibel, 1979; Denoulet et al., 1982; Wolff et al., 1982).

We were then prompted to investigate the occurrence of tubulin in mouse brain myelin and to attempt an identification of the isoforms of this potential tubulin to evaluate the relationship between the increase of tubulin microheterogeneity and myelination. In a preliminary report α and β subunits of tubulin were both revealed in mouse brain myelin analyzed by electrophoresis (de Néchaud et al., 1983). We present now a detailed analysis implying that several of the listed isoforms of tubulin participate in myelin completion.

MATERIALS AND METHODS

Materials

Acrylamide and *N,N'*-methylenebisacrylamide (bisacrylamide) for isoelectric focusing (IEF) were from Eastman Kodak, recrystallized as described by Loening (1967); otherwise, they were from Biorad. Ultrapure urea was from Schwarz-Mann. Tris, sodium dodecyl sulfate (SDS), L-glycine, sucrose, and TCA were from Merck. GTP, dithiothreitol (DTT), EGTA, EDTA, Nonidet P40, β-mercaptoethanol, L-histidine, and L-glutamic acid were from Sigma. 4-Morpholinoethane sulfonic acid (MES) was from Calbochem. Ampholytes were from LKB. Vinblastine sulfate was a gift from Eli Lilly. [^{35}S]L-methionine was from Amersham. *Staphylococcus aureus* V_8 protease was from Miles. *Papaya latex* papain (type IV) was from Sigma.

Myelin isolation

Myelin was isolated from adult mouse forebrains by a sequence of discontinuous gradient centrifugations and osmotic shocks, as described by Norton and Poduslo (1973). Its purity was checked by electron microscopy (Baumann et al., 1973), lipid analysis (Baumann et al., 1973; Bourre et al., 1977), and protein electrophoresis

Received January 1, 1983; revised March 9, 1983; accepted April 22, 1983.

Address correspondence and reprint requests to Dr. de Néchaud at Unité de Neurotoxicologie, INSERM U 26, Hôpital Fernand Widal, 200, rue du Faubourg-Saint-Denis, 75475 Paris, Cedex 10, France.

Abbreviations used: Bisacrylamide, *N,N'*-Methylenebisacrylamide; DTT, Dithiothreitol; IEF, Isoelectric focusing; MES, 4-Morpholinoethane sulfonic acid; SDS, Sodium dodecyl sulfate; TCA, Trichloroacetic acid.

(Jacque et al., 1972). After the final washes the pellet was treated as follows. For slab gel electrophoresis, it was either suspended in pH 6.4 buffer (0.1 *M* MES; 1 m*M* EGTA; 0.5 m*M* $MgCl_2$; 0.1 m*M* EDTA; 1 m*M* GTP; 1 m*M* β-mercaptoethanol) or lyophilized and delipidated in ether:ethanol (3:2, vol/vol), and suspended in the same pH 6.4 buffer. Concentrated sample buffer was then added to obtain final concentrations of 0.08 *M* Tris-HCl, pH 6.8; 2% SDS; 0.1 *M* DTT; 10% glycerol; and 0.001% bromophenol blue and the samples were heated in boiling water for 2 min. For IEF the last pellet was homogenized in pH 6.4 buffer to which were added sample buffer: 2% Nonidet P 40, 2% ampholytes, 5% (wt/vol, final) β-mercaptoethanol, and urea (9.5 *M* final) (O'Farrell, 1975).

SDS-polyacrylamide slab gel electrophoresis

The method used for slab gel electrophoresis was adapted from Laemmli (1970). A 1-mm thick slab gel composed of a stacking gel (4.5% acrylamide; 0.12% bisacrylamide; 0.125 *M* Tris-HCl, pH 6.8; 0.1% SDS) and a separating gel (8% acrylamide, 0.09% bisacrylamide or 12% acrylamide, 0.32% bisacrylamide; 0.375 *M* Tris-HCl, pH 8.8; 0.1% SDS) was run at a constant current of 7 mA until the bromophenol blue front reached the bottom of the gel. Running buffer was 0.05 *M* Tris, 0.38 *M* L-glycine, and 0.1% SDS.

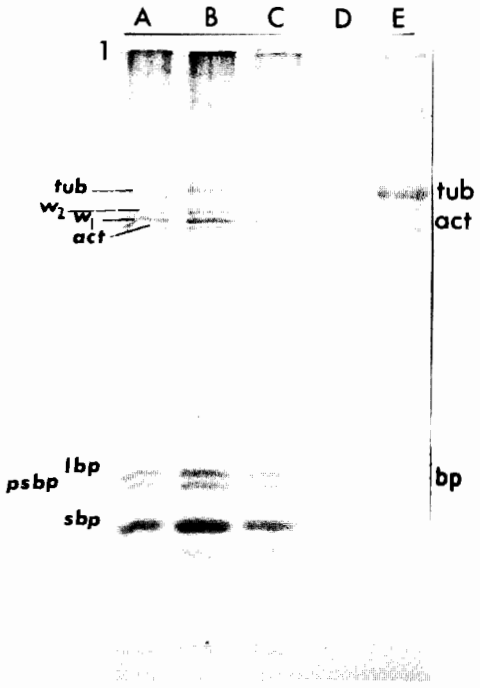

FIG. 1. Electrophoretogram in SDS polyacrylamide gel (12% acrylamide, 0.32% bisacrylamide). **A–C:** Delipidated myelin prepared in pH 6.4 buffer (C), supplemented with 1% SDS (A) or with 1% Sarkosyl (B). These myelin samples contained 15 μg of protein. Protein concentrations were determined by the method of Bramhall et al. (1969). **D:** Purified human basic protein (bp, gift of Dr. Claude Jacque). **E:** Tubulin-enriched fraction obtained by one-cycle microtubule self assembly from mouse brain soluble extract (Denoulet et al., 1982). 1, Limit between the stacking gel and the separating gel. Distance between 1 and the tracking dye front was 14.5 cm. tub., Tubulin; W_1 and W_2, fast- and slow-migrating Wolfgram doublet components; act, actin; lbp, large basic protein; psbp, presmall basic protein; sbp, small basic protein. Nomenclature according to Waehneldt and Malotka (1980). Coomassie Blue stain.

Contamination of myelin by radioactively labeled tubulin

C1300 mouse neuroblastoma was cultured for 24 h in the presence of [^{35}S]L-methionine (Eddé et al., 1982). The cells were harvested, sonicated, and centrifuged at 12,000 *g* for 10 min at 4°C. The supernatant (gift of B. Eddé) was precipitated by 1 m*M* vinblastine and centrifuged at 100,000 *g* for 45 min. The vinblastine precipitate, containing mostly tubulin and some actin as seen on slab gel electrophoretograms, was dissolved in the pH 6.4 buffer (see Myelin Isolation) and added during homogenization in 0.32 *M* sucrose to the mouse forebrains (6×10^5 cpm for 3 g of forebrain). Myelin was then isolated as previously described. At each step throughout the isolation procedure, the amount of radioactivity was estimated either directly in the trichloroacetic acid (TCA)-insoluble fractions, or by autoradiography of electrophoretograms of the isolation fractions.

Isoelectric focusing and two-dimensional gel electrophoresis

For IEF we used the O'Farrell procedure (1975) modified by Wolff et al. (1982). The modifications included

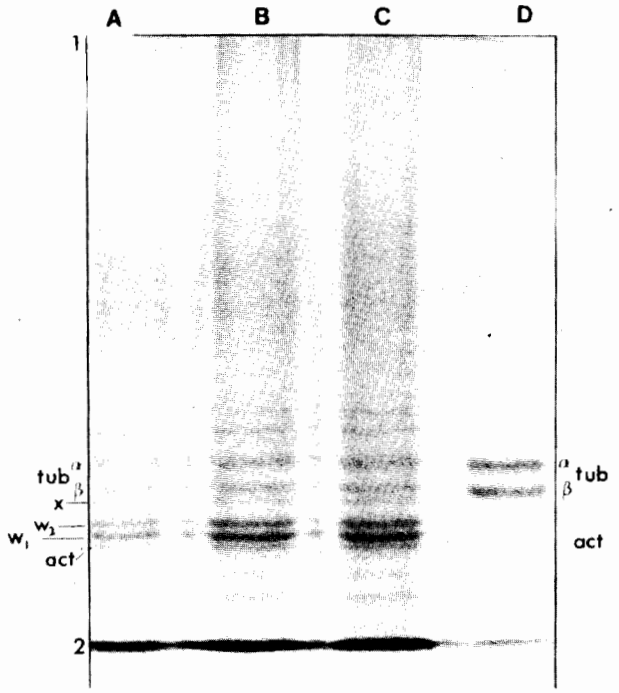

FIG. 2. Electrophoretogram in SDS polyacrylamide gel (8% acrylamide, 0.09% bisacrylamide). **A–C:** Delipidated myelin prepared in pH 6.4 buffer (A), supplemented with 1% SDS (B) or with 1% Sarkosyl (C). Protein content was as described in the legend to Fig. 1. **D:** Tubulin-enriched fraction from mouse brain soluble extract (cf. legend to Fig. 1). Distance between 1 (upper limit of separating gel) and 2 (bromophenol blue front) was 6.5 cm. α, α Subunit; β, β subunit of tubulin (tub.); x, unidentified component; W_2, slow-migrating Wolfgram component; W_1, fast-migrating Wolfgram component; act, actin. Coomassie Blue stain.

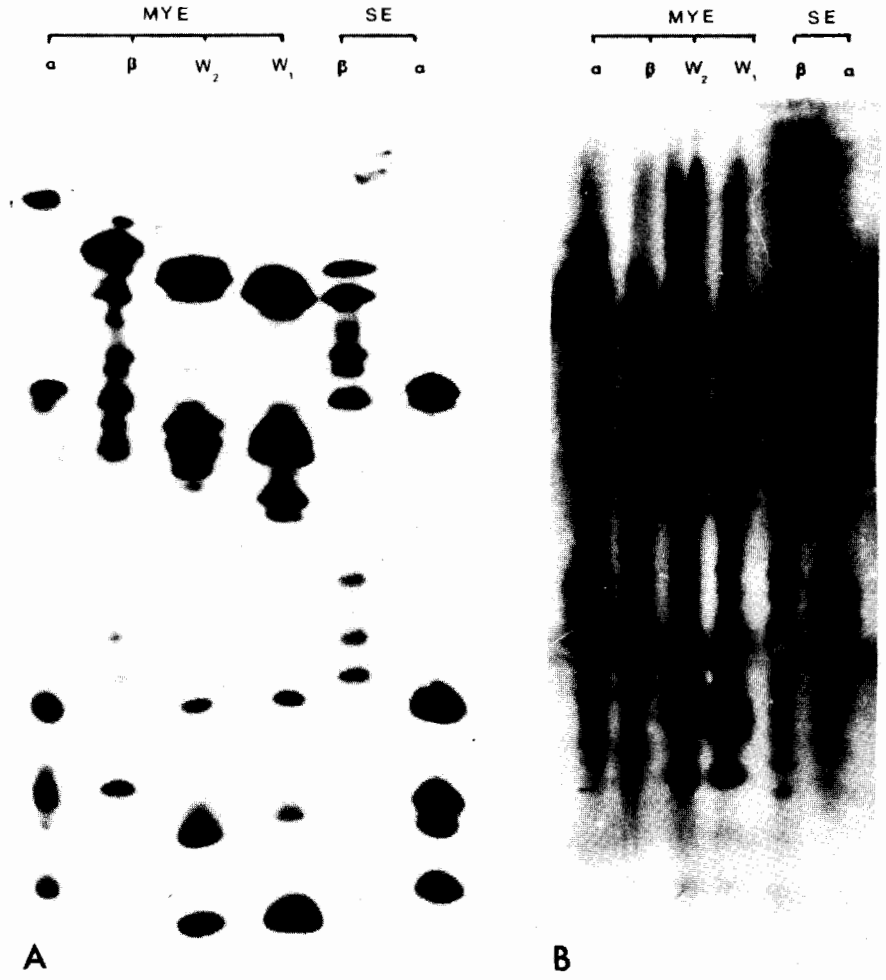

FIG. 3. Peptide mapping of α and β tubulin subunits (α,β) from mouse brain myelin (MYE) and tubulin-enriched soluble extract (SE), and of W_1 and W_2 Wolfgram components. The proteins were separated by electrophoresis as described in the legend to Fig. 2, cut out from the gels, and proteolyzed by *S. aureus* protease (**A**, 12.5 ng of protease per slot) or by papain (**B**, 5.0 ng of papain per slot). SDS-polyacrylamide separating gel (15% acrylamide; 0.0875% bisacrylamide). Silver stain.

the length of the gels (18 cm), the pH range of the ampholytes (4–6), the electrode reservoir solutions (10 m*M* L-histidine in cathode reservoir, 10 m*M* L-glutamic acid in anode reservoir), and the prefocusing step.

For the second dimension, as described by O'Farrell, the equilibrated IEF gels were fixed by agarose on the top of 1-mm thick gel composed of a 2-cm long stacking gel (5% acrylamide, 0.13% bisacrylamide) and a 24-cm long separating gel (8% acrylamide, 0.09% bisacrylamide). Running and gel buffers as well as running conditions were the same as those described in the section on SDS polyacrylamide slab gel electrophoresis.

Peptide mapping

Slices from the IEF gels, bands from monodimensional electrophoretograms, and spots from the two-dimensional electrophoretograms were cut out. They were then digested and analyzed as described by Cleveland et al. (1977). Sometimes we used a lower amount of bisacrylamide in the separating gel (0.0875% instead of 0.4%), resulting in a better spread of the peptide distribution (for instance, compare Fig. 3 with Fig. 6).

Gel stain

Slab gels, IEF, and two-dimensional gels were stained for 1 h with 0.25% (wt/vol) Coomassie Brilliant Blue in 50% (vol/vol) methanol and 7% acetic acid. They were destained in 45% methanol, 5% acetic acid. For peptide mapping, we used silver stain (Oakley et al., 1980).

RESULTS

Analysis of the mouse brain myelin on SDS-polyacrylamide slab gels led to electrophoretic profiles very similar to the ones published by Waehneldt and Malotka (1980). We did not find differences in these profiles when the buffer in which myelin was prepared was supplemented by SDS or by Sarkosyl (Figs. 1 and 2). As previously stated (Gozes and Richter-Landsberg, 1978; Waehneldt and Malotka, 1980), besides the Wolfgram protein doublet, there were proteins comigrating with tubulin and with actin. Moreover, the resolution in the 8% acrylamide slab gel (Fig. 2) allowed us to separate α and β subunits of tubulin, in the soluble brain tubulin as well as in myelin. W_2, the slow-migrating Wolfgram doublet component, was clearly distinct from the β subunit of tubulin by its migration (Figs. 1 and 2). The peptide mapping of the protein bands resolved by slab gel electrophoresis (as in Fig. 2) was ob-

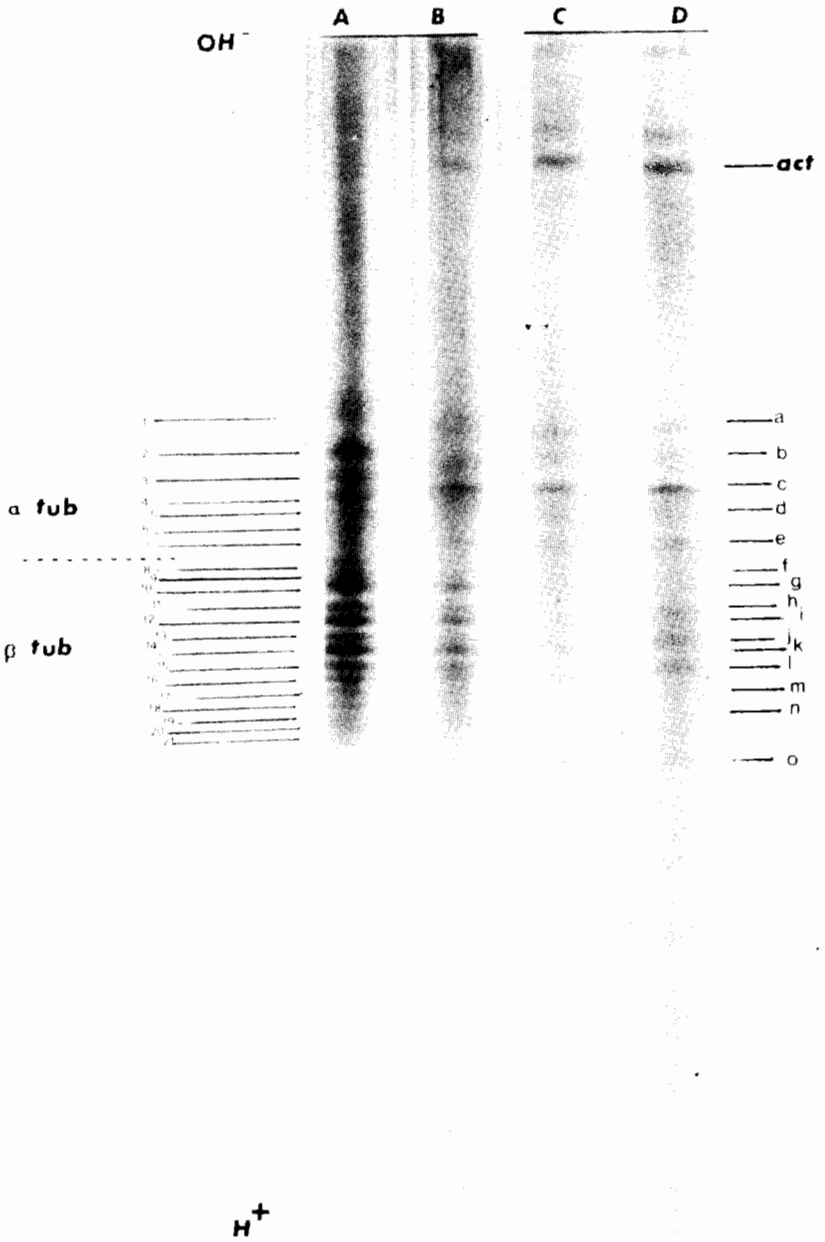

FIG. 4. Isoelectric focusing pattern (modified procedure of Wolff et al., 1982) of adult mouse brain purified soluble tubulin **(A)** prepared as described by Weingarten et al. (1975), of adult brain myelin **(C, D)**, and of a mixture of both **(B)**. act, Actin. For the purified soluble tubulin (A), the seven isoforms of the α subunit of tubulin (α tub) and the 14 isoforms of the β subunit (β tub) are numbered according to Wolff et al. (1982), whereas the 15 focusing bands of the myelin preparation (D) are lettered. Coomassie Blue stain.

tained after limited proteolysis (Cleveland et al., 1977) by two different enzymes (Fig. 3). It allowed us to identify the two tubulin subunits from myelin by reference to those from brain soluble extract although the pattern of the β subunit from the myelin included additional peptides, resulting probably from proteins comigrating with this subunit. The peptide map proved also the difference between each of the tubulin subunits and the Wolfgram doublet and, further, the strong resemblance between W_1 and W_2, previously noticed by Nussbaum et al. (1977) in immunochemical studies.

To ascertain that tubulin found in the myelin preparations did not arise from an artifactual binding of cytoplasmic tubulin during the isolation procedure we contaminated the brain homogenate with exogenously labeled tubulin and followed the distribution of the radioactivity throughout the myelin isolation. The initial amount of radioactivity was 610 $\times 10^3$ cpm; 138 $\times 10^3$ cpm (23%) was found in the interface of the first sucrose gradient, 17 $\times 10^3$ cpm (3%) in the pellet after the sucrose washout, and 1.6 $\times 10^3$ cpm (0.3%) in the pellet after the two consecutive osmotic shocks. In the interface of the second sucrose gradient 7 $\times 10^2$ cpm (0.1%) remained, and in the pellet after the following sucrose

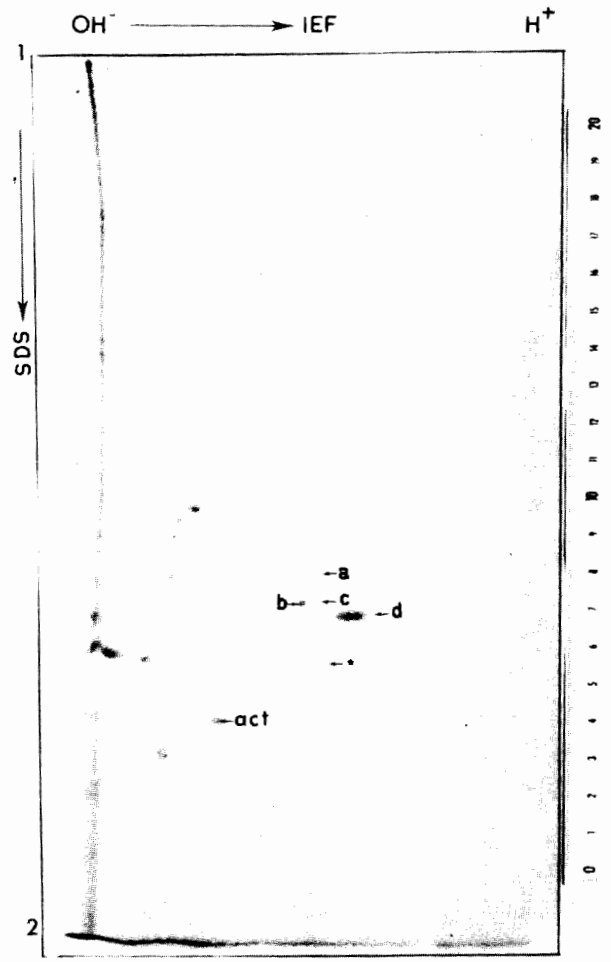

FIG. 5. Two-dimensional electrophoretogram of mouse brain myelin. The IEF gel was adjusted to the top of the SDS polyacrylamide slab gel by cutting 6 cm at its acidic end. Distance between the upper limit of the separating gel (1) and the dye front (2) was 24 cm. act, Actin; a–d, spots in the tubulin area. Coomassie Blue stain.

FIG. 6. Peptide mapping of α and β subunits (α,β) of purified mouse brain soluble tubulin, of mouse brain actin (Ac), of some cytoskeletal proteins of C1300 mouse neuroblastoma (numbered 1, 2, 3, 4, 5, 6 according to Eddé et al., 1982), and of several components of mouse brain myelin (e and l are the two IEF bands lettered on gel D of Fig. 4; a, b, c, and d are the spots on the two-dimensional electrophoretogram, Fig. 5). The amount of *S. aureus* protease was 1.0 ng per slot. The separating gel was 15% acrylamide, 0.4% bisacrylamide. Silver stain. Notice that there are no additional peptides in the tubulin β subunit from myelin isolated by two-dimensional electrophoresis, in contrast with Fig. 3.

washout, the amount of radioactivity was undetectable. Thus the trapping of soluble tubulin occurring during the initial step of myelin isolation was eliminated in the next steps. A similar conclusion was drawn from this type of myelin cross-contamination by Gozes and Richter-Landsberg (1978).

The IEF analysis of myelin preparation showed several bands in the isoelectric point range of tubulin (Fig. 4). Five of them fitted within the α subunit isoform range and nine within the β isoform range, excluding the o-lettered band, which is more acidic than the 21st isoform. On the two-dimensional electrophoretograms two spots ranked among tubulin subunits by their isoelectric points and by their apparent molecular weights (spots a and d on Fig. 5). There were also two proteins focusing as α isoforms but running faster in the second dimension (spots b and c on Fig. 5), and another protein with coincident isoelectric point but smaller apparent molecular weight (star on Fig. 4). Spots a, b, c, and d were excised from two-dimensional electrophoretograms and submitted to partial proteolysis (Cleveland et al., 1977). The peptidic patterns (Fig. 6) provided identification of the protein from spot a with the α subunit of tubulin and of that from spot d with the β subunit. Both spots b and c showed the same pattern, different from both tubulin subunits and from vimentin (row numbered 5 on Fig. 6) but identical to the one published by Eddé et al. (1982) for a still unidentified component of C1300 neuroblastoma cells (spot 4 in their publication and row 4 on Fig. 6).

To enumerate the isoforms of tubulin in myelin, we used the procedure of Denoulet et al. (1982). This procedure avoids overlapping between two closely adjacent IEF bands during the second dimension run and sorts by their molecular weights the potential proteins focusing at the same isoelec-

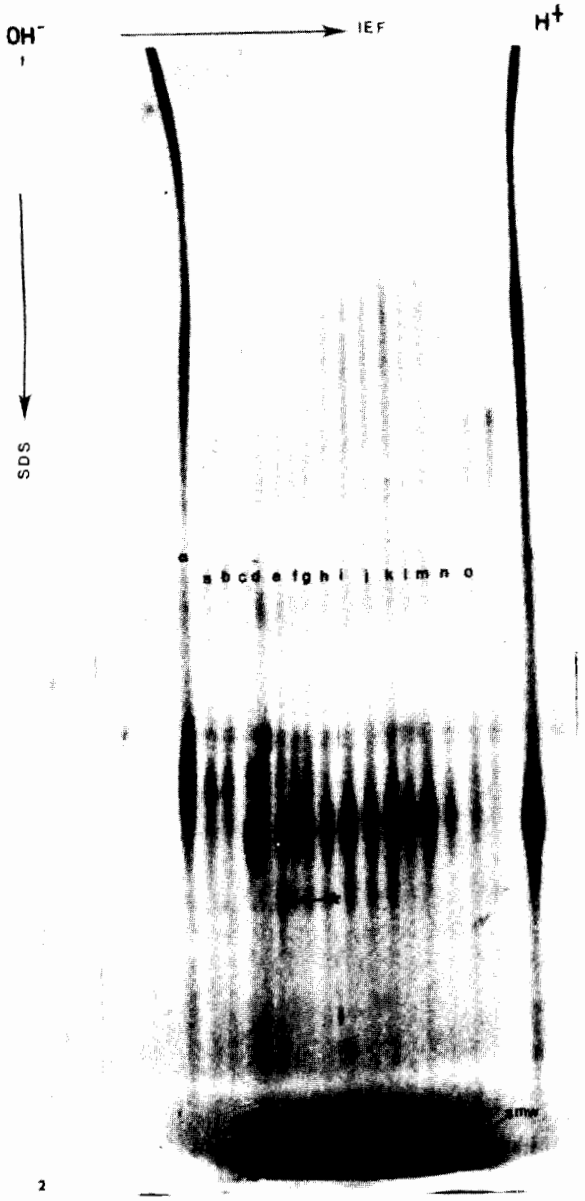

FIG. 7. Two-dimensional electrophoretogram of mouse brain myelin after slicing and spacing of the IEF gel (procedure of Denoulet et al., 1982). α, α Tubulin isoform no. 5 of purified brain soluble tubulin; a–o, bands cut out from stained IEF gel of myelin; β, β tubulin isoform no. 15 of purified brain soluble tubulin. For lettering and numbering, refer to fig. 4. *, Refer to fig. 5. smw, Small molecular-weight component. Silver stain.

tric point. Figure 7 showed the result obtained by slicing an IEF gel (similar to gel D, Fig. 4) and spacing the slices. Myelin contained three α isoforms (bands a, b, and d) and nine β isoforms (bands f, g, h, i, j, k, l, m, and n). The two unidentified proteins referred as spots b and c on Fig. 5 were found, respectively, in bands c and e (Fig. 4). Band e harbored an additional small molecular-weight component, pointed out by a star on Fig. 5. Band o corresponded to a very small molecular-weight component (smw on Fig. 7).

Two examples of peptide mapping of individual IEF bands are shown on Fig. 6. The pattern given by band 1 evoked a β isoform of tubulin (compare to rows β, 3, 6, and d) although band e exhibited a less clear pattern, obviously because this band joined two proteins focusing at the same point.

Assimilation of the tubulin isoforms enumerated in myelin with the isoforms in purified mouse brain soluble tubulin listed by Wolff et al. (1982) was somewhat difficult. Coincidence between isoelectric points was not easy to demonstrate (see, for example, gel B on Fig. 4). However, among the reference β isoforms, there was a triplet (isoforms 8, 9, 10) followed by two doublets (isoforms 11 and 12, 13 and 14). On the basis of this peculiar spacing, we propose to assimilate band g with isoform 10, h with 11, i with 12, j with 13, k with 14, l with 15. We noticed also that the distribution of these bands was rather different in myelin. In soluble tubulin, among the doublets, 12 was stronger than 11, and 14 than 13; it seems to be the reverse in myelin in all the preparations tested.

DISCUSSION

Our results with SDS-polyacrylamide slab gel electrophoresis are inconsistent with those of Reig et al. (1982), who assimilated the W_2 Wolfgram component with a tubulin-like protein. We confirm the presence in mouse brain myelin of the two Wolfgram components, W_1 and W_2 (Waehneldt and Malotka, 1980), differing from each other and from the subunits of tubulin by their apparent molecular weights. Moreover, both peptide patterns generated by limited proteolysis with papain or with *S. aureus* protease reveal the dissimilarity between W_2 and tubulin and the similarity between W_2 and W_1. W_2 and W_1 also share common antigenic determinants (Nussbaum et al., 1977), and their restricted localization in the oligodendroglial cells and in the myelin sheaths (Roussel et al., 1977) prevents any confusion with tubulin.

The demonstration of the occurrence of tubulin in brain myelin was first achieved by Gozes and Richter-Landsberg (1978) and related to the well-documented association of tubulin with membranes (Feit and Barondes, 1970; Walters and Matus, 1975; Bhattacharyya and Wolff, 1976; Gozes and Littauer, 1979; Strocchi et al., 1981). In our study, mouse brain myelin was analyzed by high-resolution techniques, and the distribution of its isotubulins was established by reference to purified whole brain soluble tubulin; there is less microheterogeneity in myelin (three α isotubulins and nine β isotubulins) and a different relative proportion among the common isoforms. Similar conclusions were drawn

by Gozes and Littauer (1979) when they compared the tubulin associated with the presynaptic membrane with the cytoplasmic (soluble) tubulin. What then is the role of isotubulins in myelin? Are they associated with myelin as they would be with any brain membrane? Do they play a more specific role during myelination or after, during the interchanges between the myelinated axons and their surroundings? Do they only reflect the isotubulin pattern of the oligodendrocyte or are they synthesized by this glial cell especially for the myelin fraction? As a matter of fact, dissimilarities in the distribution of isotubulins from neuronal and glial cells were reported by Gozes et al. (1979) and by Moura Neto et al. (1982; 1983).

Acknowledgments: We thank Philippe Denoulet, Bernard Eddé, Claude Jacque, and Vivaldo Moura Neto for their generous help and critical discussions. This investigation was supported by grants from Collège de France, INSERM CRL 79.4.324.6 and CNRS.

REFERENCES

Baumann N., Bourre J. M., Jacque C., and Harpin M. L. (1973) Lipid composition of quaking mouse myelin: Comparison with normal mouse myelin in the adult and during development. *J. Neurochem.* **20**, 753–759.

Bhattacharyya B. and Wolff J. (1976) Polymerisation of membrane tubulin. *Nature* **264**, 576–577.

Bourre J. M., Cassagne C., Larrouquere-Regnier S., and Darriet D. (1977) Occurrence of alkanes in brain myelin. Comparison between normal and quaking mouse. *J. Neurochem.* **29**, 645–648.

Bramhall S., Noack N., Wu M., and Loewenberg J. R. (1969) A simple colorimetric method for determination of protein. *Anal. Biochem.* **31**, 146–148.

Cleveland D. W., Fischer S. G., Kirschner M. W., and Laemmli U. K. (1977) Peptide mapping by limited proteolysis in sodium dodecyl sulfate and analysis by gel electrophoresis. *J. Biol. Chem.* **252**, 1102–1106.

Dahl J. L. and Weibel V. J. (1979) Changes in tubulin microheterogeneity during postnatal development of rat brain. *Biochem. Biophys. Res. Commun.* **86**, 822–828.

de Néchaud B., Jeantet C., and Bourre J. M. (1983) Présence des sous-unités α et β de la tubuline dans la myéline de cerveau de la souris. *C. R. Acad Sci. Paris* **296**, 11–13.

Denoulet P., Eddé B., Jeantet C., and Gros F. (1982) Evolution of tubulin heterogeneity during mouse brain development. *Biochimie* **64**, 165–172.

Eddé B., Portier M. M., Sahuquillo C., Jeantet C., and Gros F. (1982) Changes in some cytoskeletal proteins during neuroblastoma cell differenciation. *Biochimie* **64**, 141–151.

Feit H. and Barondes S. (1970) Colchicine-binding activity in particulate fractions of mouse brain. *J. Neurochem.* **17**, 1355–1364.

Gozes I. and Littauer U. Z. (1978) Tubulin microheterogeneity increases with rat brain maturation. *Nature* **276**, 411–413.

Gozes I. and Littauer U. Z. (1979) The α-subunit of tubulin is preferentially associated with brain presynaptic membrane. *FEBS Lett.* **99**, 86–90.

Gozes I. and Richter-Landsberg C. (1978) Identification of tubulin associated with rat brain myelin. *FEBS Lett.* **95**, 169–172.

Gozes I., Saya D., and Littauer U. Z. (1979) Tubulin microheterogeneity in neuroblastoma and glioma cell lines differs from that of the brain. *Brain Res.* **171**, 171–175.

Jacque C., Louis C., Guede C., and Baumann N. (1972) Proteines myéliniques de la Souris. Étude du mutant quaking. *C. R. Acad. Sci. Paris* **274**, 126–139.

Laemmli U. K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. *Nature* **227**, 680–685.

Loening U. E. (1967) The fractionation of high molecular weight ribonucleic acid by polyacrylamide gel electrophoresis. *Biochem. J.* **102**, 251–257.

Moura Neto V., Jeantet C., Glowinski J., and Prochiantz A. (1982) Studies on tubulin microheterogeneity in cultured glial and neuronal cells. *Biol. Cell* **45**, 145.

Moura Neto V., Mallot M., Jeantet C., and Prochiantz A. (1983) Microheterogeneity of tubulin proteins in neuronal and glial cells from the mouse brain in culture. *The Embo Journal* **2**, 1243–1248.

Norton W. T. and Poduslo S. E. (1973) Myelination in rat brain. Method of myelin isolation. *J. Neurochem.* **21**, 749–757.

Nussbaum J. L., Delaunoy J. P., and Mandel P. (1977) Some immunochemical characteristics of W_1 and W_2 Wolfgram proteins isolated from rat brain myelin. *J. Neurochem.* **28**, 183–191.

Oakley B. R., Kirsch D. R., and Morris N. R. (1980) A simplified ultrasensitive silver stain for detecting proteins in polyacrylamide gels. *Anal. Biochem.* **105**, 361–363.

O'Farrell P. H. (1975) High resolution two-dimensional electrophoresis of proteins. *J. Biol. Chem.* **250**, 4007–4021.

Reig J. A., Ramos J. M., Cozar M., Aguilar J. S., Criado M., and Monreal J. (1982) Purification and chemical characterization of a W_2 protein from brain myelin. *J. Neurochem.* **39**, 507–511.

Roussel G., Delaunoy J. P., Nussbaum J. L., and Mandel P. (1977) Immunohistochemical localization of Wolfgram proteins in nervous tissue of rat brain. *Neuroscience* **2**, 307–313.

Strocchi P., Brown B. A., Young J. D., Bonventre J. A., and Gilbert J. M. (1981) The characterization of tubulin in CNS membrane fractions. *J. Neurochem.* **37**, 1295–1307.

Waehneldt T. V. and Malotka J. (1980) Comparative electrophoretic study of the Wolfgram proteins in myelin from several mammalia. *Brain Res.* **189**, 582–587.

Walters B. B. and Matus A. I. (1975) Tubulin in postsynaptic junctional lattice. *Nature* **257**, 496–498.

Weingarten M. D., Lockwood A. H., Hwo S. Y., and Kirschner M. W. (1975) A protein factor essential for microtubule assembly. *Proc. Natl. Acad. Sci. USA* **72**, 1858–1862.

Wolff A., Denoulet P., and Jeantet C. (1982) High level of tubulin microheterogeneity in the mouse brain. *Neurosci. Lett.* **31**, 323–328.