NEUROFILAMENTS FROM MAMMALIAN CENTRAL AND PERIPHERAL NERVE SHARE CERTAIN POLYPEPTIDES

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1. Introduction

Preparations enriched in mammalian 10 nm filaments, commonly referred to as neurofilaments have been made from peripheral nerve [1] and brain [2–5]. Morphologically, isolated filaments from the two sources are indistinguishable and often appear aggregated [1–5]. However, there are considerable discrepancies between the reported polypeptide compositions of brain and peripheral nerve neurofilaments. Three polypeptides with mol. wt 200 000, 150 000 and 69 000 have been identified as the major components of peripheral nerve neurofilaments [1]. Indirect evidence obtained from studies of the slow component of axonal transport has also been used to implicate this same triplet of polypeptides in neurofilament structure [6,7]. Preparations of brain 10 nm filaments contain a major polypeptide with a chain wt 50 000–60 000 which has been ascribed the brain neurofilament subunit [3–5], however it was noted that minor higher molecular weight polypeptides are usually present. The possible presence of glial 10 nm filaments in preparations from brain has been recognised for some time [3,8–10], however, the degree of any contamination by these filaments is difficult to assess since their constituent protein, glial fibrillary acidic protein, appears to comigrate electrophoretically with the major brain neurofilament polypeptide [4]. We have now directly compared the polypeptide composition of preparations enriched in brain 10 nm filaments with sciatic nerve neurofilaments in two species and have found that the triplet of polypeptides attributed to peripheral nerve neurofilaments appear to be present also in brain 10 nm filament preparations, and that the brain 50 000 mol. wt polypeptide is entirely absent from peripheral nerve neurofilaments.

Abbreviations: SDS–PAGE, sodium dodecyl sulphate–polyacrylamide gel electrophoresis; EDTA, ethylene diamine tetra acetic acid; mol. wt, molecular weight

2. Experimental

2.1. *Preparation of brain 10 nm filaments*

Brain 10 nm filaments were prepared from rat and rabbit white matter. Briefly, tissue was homogenised in 0.85 M sucrose, 0.03 M KCl, 0.001 M EDTA, 0.02 M sodium phosphate (pH 6.5) and myelinated axons recovered as a floating pad following centrifugation for 15 min at 11 000 g_{av}. The myelinated axons were rehomogenised in this buffer and the centrifugation step repeated (usually twice) until no pellet was produced. The myelinated axons were subjected to overnight osmotic shock (0.001 M EDTA, 0.01 M sodium phosphate, pH 6.5) to release the axonal contents and the readily sedimentable material from the latter (containing much aggregated fibrous material) was separated from the released myelin and soluble fraction by repeated sedimentation from 1.7 M sucrose, 0.001 M EDTA, 0.01 M Tris (pH 8.6) in a swing-out rotor (30 min, 80 000 g_{av}) (details to be published elsewhere). The final pellet was used as the preparation of brain 10 nm filaments.

2.2. *Preparation of peripheral nerve proteins*

Sciatic nerves were dissected, desheathed and demyelinated axonal material isolated as in [1].

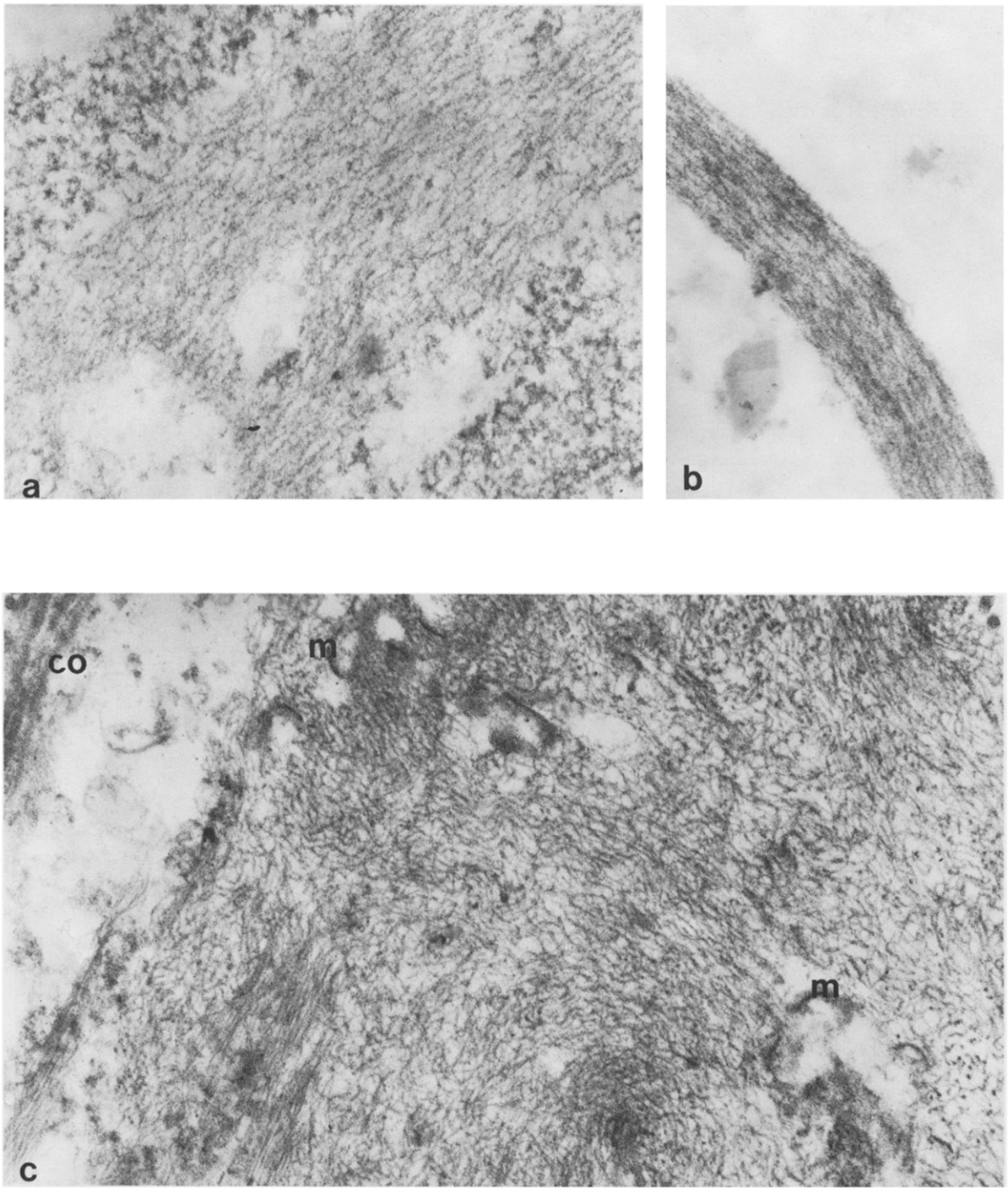

Fig.1. Electron micrographs of: (a) loosely-packed brain 10 nm filaments; (b) part of a tight bundle of brain 10 nm filaments; (c) rat sciatic nerve neurofilaments. All 49 100 ×. m, membranes; CO, collagen.

2.3. *SDS–PAGE*

Samples were heated at 100°C for 5 min in 2% w/v SDS, 2% v/v 2-mercaptoethanol (for brain proteins) or 2% w/v SDS, 0.1 M dithiothreitol (for peripheral nerve proteins). Electrophoresis was performed using a slab gel apparatus with the acrylamide solutions and buffers in [11], one exception was that the separating gel consisted of a gradient of 5–15% w/v total acrylamide.

2.4. *Electron microscopy*

Pelleted material was fixed in 2.5% w/v glutaraldehyde, 0.1 M sodium cacodylate (pH 7.2) for 2 h, rinsed overnight in 0.1 M sodium cacodylate (pH 7.2), post-fixed in 1% w/v osmium tetroxide for 2 h, rinsed in water and dehydrated in graded alcohols. All procedures except the step in 100% v/v alcohol were carried out at 4°C. The material was then infiltrated and embedded in Spurrs resin. Sections were cut using an LKB III microtome. These were then stained with uranyl acetate and lead citrate, and examined with a Phillips 301 microscope.

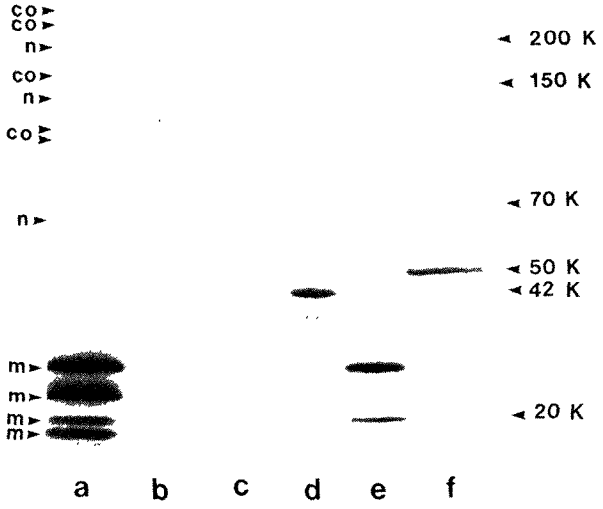

Fig.2. SDS–PAGE: (a) myelinated rat sciatic nerve; (b) rat sciatic nerve demyelinated axonal material; (c) rat brain 10 nm filament preparation; (d) rabbit skeletal muscle myofibrils (main bands, myosin heavy chain mol. wt 200 000 and actin mol. wt 42 000); (e) myelinated rabbit sciatic nerve; (f) rabbit brain 10 nm filament preparation. Mol. wt values are shown on the right hand side. CO, collagen polypeptides; m, myelin associated polypeptides; n, neurofilament polypeptides.

3. Results and discussion

Preparations of brain 10 nm filaments consisted predominantly of aggregates with a small amount of amorphous and membraneous material present. The aggregates were of two forms, 10 nm filaments in a loosely-packed meshwork and in tightly-packed bundles (fig.1a,b). These results are similar to [2,3,9] where two types of aggregate of brain 10 nm filaments are reported. Demyelinated axonal material was found to contain large numbers of loosely-packed aggregates of 10 nm filaments, presumably neurofilaments, along with a considerable number of collagen fibres. Contamination with other cell debris was evident (fig.1c). We have not found in these preparations tightly-packed bundles of the type found in preparations of brain 10 nm filaments.

Brain 10 nm filaments and sciatic nerve proteins from both rat and rabbit were analyzed and compared using SDS–PAGE. Rat and rabbit brain 10 nm filaments isolated by us contain a major component with a mol. wt ~50 000 with other prominent bands with chain wt ~200 000, 150 000, 70 000 and 20 000 (fig.2c,f); all of these polypeptides have been consistently observed in every preparation (5) and have always been found in many preparations of bovine brain 10 nm filaments (in preparation). A number of other minor components can also be detected on the gels. Axonal proteins from rat sciatic nerve were found to be a more heterogeneous mixture than the brain filament proteins (fig.2b). It can be seen that in addition to polypeptides which comigrate with α- and β-tubulin and actin 3 peripheral nerve proteins have identical electrophoretic mobilities with the 200 000, 150 000 and 70 000 mol. wt polypeptides of rat brain 10 nm filaments (fig.2b,c). These same proteins correspond to the polypeptide triplet identified by others as neurofilament proteins in peripheral nerve [1,6,7]. Strikingly, the sciatic nerve does not contain a polypeptide which comigrates with the brain 10 nm filament 50 000 mol. wt component. Up to 5 high molecular weight polypeptides have been

identified [1,12] as collagen components, the corresponding bands on our gels have been attributed to collagen (labelled Co in fig.2) on the basis of red–purple staining with Coomassie brilliant blue R [12], whereas the above neurofilament polypeptides stained blue. Four small mol. wt polypeptides (labelled M in fig.2) appear to be myelin-associated proteins [1], these are the major components of whole desheathed myelinated nerve (fig.2a) and are mostly lost when the myelin has been stripped off the axons (fig.2a,b).

Comparison of rabbit brain 10 nm filament polypeptides with those from whole myelinated rabbit sciatic nerve revealed the same relationship between the sciatic neurofilament polypeptide triplet and comigrating brain polypeptides, again there was no 50 000 mol. wt polypeptide in sciatic nerve corresponding to that of the brain filaments.

Until now it has appeared that brain and peripheral nerve neurofilaments are biochemically distinct although morphologically indistinguishable [1–7]. Our findings have now demonstrated that preparations of brain 10 nm filaments which are often assumed to be neurofilaments [2–5] contain at least 3 polypeptides with identical molecular weights to a triplet of polypeptides found in peripheral nerve and which have been attributed to neurofilaments [1,6,7]. We wish to propose, therefore, that this triplet found in isolated brain 10 nm filaments represents brain neurofilament polypeptides. The origin of the 50 000 mol. wt component of brain filaments remains unclear since obviously a polypeptide of this size is absent in peripheral nerve which is nevertheless rich in neurofilaments. It has been suggested by some authors that the tightly-packed bundles of filaments present in brain preparations represent glial filaments [3,4,8–10] and thus some or most of the 50 000 mol. wt component might be glial fibrillary acidic protein [9,10]; our data would be consistent with this view since we have not found tightly-packed bundles of 10 nm filaments of the type found in preparations from brain in isolated axonal proteins from sciatic nerve. Alternatively, the 50 000 mol. wt species in brain filaments might be partially the result of proteolysis during the lengthy isolation procedure employed; neurofilaments from the giant axon of *Myxicola infundibulum* are extremely susceptible to proteolysis [13]. A third possibility remains, that brain neurofilaments are not identical with peripheral nerve neurofilaments and that the 50 000 mol. wt polypeptide is unique to those from brain, perhaps confering some special functional properties. However, our results have demonstrated that neurofilaments from the central and peripheral nervous systems probably share 3 common polypeptides, thus both types of neurofilaments are likely to have the same basic structure. A similar triplet of polypeptides has been observed in association with tubulin during the first cycle of in vitro microtubule polymerization and appears to be associated with the 10 nm fibres which are formed at the same time as the microtubules when the soluble protein fraction from brain is heated [14,15]. We have shown that these three polypeptides are identical with the 200 000, 150 000 and 70 000 mol. wt components of isolated brain 10 nm filaments (in preparation) further suggesting that these polypeptides may have a structural role in neurofilaments.

Acknowledgements

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