COMPARISON OF THE PROTEINS OF 10 nm FILAMENTS FROM RABBIT SCIATIC NERVE AND SPINAL CORD BY ELECTROPHORESIS IN TWO DIMENSIONS

Henryk CZOSNEK[+] and David SOIFER[+,*]

[+]*Dept. Pathological Neurobiology, Institute for Basic Research in Mental Retardation, Staten Island, NY 10314* and *[*]Dept. Anatomy and Cell Biology, SUNY Downstate Medical Center, Brooklyn, NY 11203, USA*

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1. Introduction

It has become evident that neurofilaments are formed of three proteins, termed the 'triplet' by Hoffman and Lasek, who first identified these proteins with neurofilaments [1]. The triplet polypeptides vary in molecular weight from one species to another but have M_r: 68 000–75 000 (P68); 140 000–165 000 (P150); and 180 000–220 000 (P200) [2,3]. Procedures developed for the isolation of neurofilaments from axons generally rely upon flotation of myelinated axon fragments and then liberation of the filaments from the myelin either by osmotic shock or by detergent extraction [4–8]. Alternative procedures for preparing filaments depend upon their being washed away from axon fragments [8,9] or upon subcellular fractionation procedures [10]. Most of these procedures yield triplet and something else – the 'something else' varies from one procedure to another as applied to the central nervous system (CNS) and varies depending upon whether the filaments were isolated from CNS or from peripheral nerve [8].

A simple and rapid procedure developed for the preparation of filaments from spinal cord [6,7], has been adapted to the isolation of filaments from rabbit sciatic nerve. The polypeptides of peripheral and central filaments were compared by gel electrophoresis in both one- and two-dimensional systems.

2. Materials and methods

2.1. *Preparation of intermediate filaments*

The spinal cord and sciatic nerves of adult (2–5 months old) New Zealand White rabbits were quickly removed after sacrifice and transferred to ice-cold 0.5 M sucrose, 50 mM Tris (pH 7.6), 25 mM KCl, 10 mM $MgCl_2$ (buffer A). The meninges were removed from the spinal cord which was then minced and homogenized with a teflon–glass homogenizer in 7 ml buffer A/g tissue wet wt. The sciatic nerves were desheathed, thoroughly minced with razor blades and homogenized in 2 ml buffer A (per pair of nerves) with a conical ground glass homogenizer. Filaments were obtained as in [6,7] for spinal cord and brain.

Address correspondence to: Dr David Soifer, Institute for Basic Research in Mental Retardation, 1050 Forest Hill Road, Staten Island, NY 10314, USA

2.2. *Electrophoresis of proteins on polyacrylamide gels*

For analysis on one-dimensional SDS–polyacrylamide gels, samples were made 2% (w/v) SDS, 1% (w/v) β-mercaptoethanol, 10% glycerol, 0.001% bromophenol blue and 20 mM Tris-phosphate (pH 7.6) and heated for 5 min at 95°C. The electrophoresis was carried out on 0.75 mm thick 5–20% acrylamide gradient slab gels employing the discontinuous buffer system of Laemmli [11]. The gels were stained with Coomassie blue R-250 and destained with 10% acetic acid–20% methanol. For two dimensional analysis, samples were prepared and analyzed in the first dimension according to [12], utilizing ampholines from LKB to form the pH gradient. In the second dimension, the isoelectric focussing tube gels were applied on 0.75 mm thick 5–20% acrylamide gradient slab gels and samples were run using the buffer system in [11]. The gels were stained and destained as above.

2.3. *Protein determination*

Protein was estimated using Coomassie blue G-250 [13].

3. Results

The proteins of filament preparations from spinal cord and sciatic nerve are each resolved into 4 prominent bands following SDS gel electrophoresis (fig.1). Three of the bands of the sciatic nerve protein migrate to the same positions as 3 of the bands of the spinal cord filament protein, with app. M_r ($\times 10^{-3}$): 200 (P200), 150 (P150) and 68 (P68) [7]. In contrast to the filaments from CNS, which include a fourth polypeptide at M_r 50 000 [7], which is probably derived from astrocytes [5,15], the sciatic nerve filaments are characterized by the presence of a polypeptide with an app. M_r 60 000.

The P200 of sciatic nerve may occur as a single band or as a doublet on SDS gels (fig.1, lanes 2,3). This dimorphism is also apparent in the P200 from rabbit spinal cord and optic nerve [7,14].

Application of the two-dimensional system of analysis [12] to sciatic nerve filaments confirms the similarity of the neurofilament polypeptides of peripheral and central nerve (fig.2,3). Not only do the triplet polypeptides of peripheral and central nerves display the same behavior in the two-dimensional system, they also show the same degree of heterogeneity. The P68 is slightly more acidic than the P150, and is often resolved as a pair of adjacent spots (fig.2,3). The P150 is resolved as a doublet of overlapping spots (fig.2). The P200 is more alkaline than the other triplet polypeptides and focusses over several tenths of a pH unit. On overloaded gels, more basic accumulations of all three proteins are detected.

The similarity between triplet proteins of the sciatic nerve filaments and spinal cord filaments,

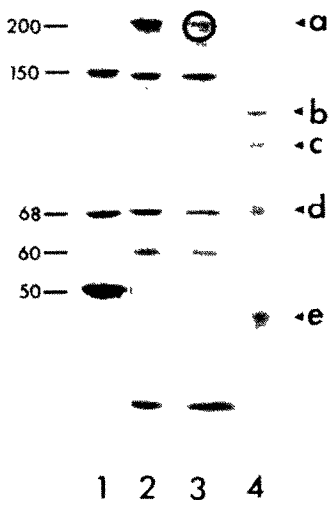

Fig.1. SDS gel analysis of spinal cord (1) and sciatic nerve (2,3) filaments. Apparent molecular weights ($\times 10^{-3}$) of filament proteins indicated at left. Doublet form of P200 encircled in (3). (4) Are molecular weight standards ($\times 10^{-3}$): (a) myosin 200; (b) β-galactosidase 130; (c) phosphorylase *b* 94; (d) bovine serum albumin 68; (e) ovalbumin 43.

Fig.2. Two-dimensional analysis of filaments from sciatic nerve (a) and spinal cord (b). Molecular weights of main filament proteins are $\times 10^{-3}$. Arrows mark P68* the alkaline peptide of P60 and multiple forms of P150.

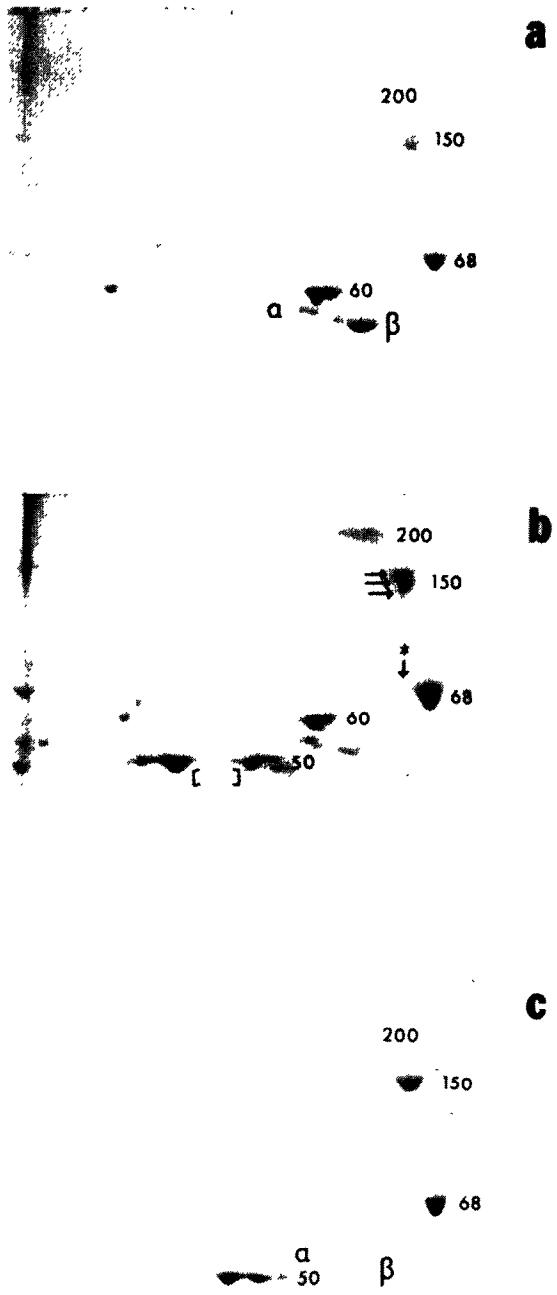

Fig.3. Portions of gels of mixtures of: (a) sciatic nerve filaments and tubulin; (b) sciatic nerve filaments and spinal cord filaments (in equal amounts); (c) spinal cord filaments and tubulin. P68* and multiple forms of P150 indicated by arrows. The brackets in (b) indicate a discontinuity in the pH gradient which distorted the alkaline region of the gradient. Molecular weights of main filament proteins are $\times 10^{-3}$. α and β-indicate the positions of tubulins.

and the differences between the P60, P50 and tubulins are readily demonstrable when mixtures of the two types of filaments are analyzed by the two dimensional procedure (fig.3b) or when each of these types of filaments is mixed with microtubule protein prior to analysis (fig.3a,c). Although the migration patterns of the P68 and P150 are similar, they are not quite identical; small differences are often resolved when they are mixed. The minor spot at P68 (P68*), which is slightly more alkaline than the primary spot, is further from the primary spot in the sciatic filaments than in the spinal cord filaments. As a result, when a mixture of the two types of filaments is compared (fig.3b), P68* may appear as a shoulder on the primary spot rather than as a distinct spot. The P150 spots, which each resolve as partially overlapping doublets when sciatic nerve or spinal cord filaments are analyzed separately (fig.2), sometimes appear as three overlapping spots on the two-dimensional gels of the mixture of filaments (fig.3b).

The P60 band of peripheral nerve resolves into three spots on the two-dimensional gels (fig.2a, 3a,b). The two largest of these spots migrate just above α-tubulin. A less prominent spot focusses near pH 6.

4. Discussion

The application of isoelectric focussing followed by SDS gel electrophoresis to the analysis of the constituent proteins of filaments isolated from sciatic nerve demonstrates that the triplet polypeptides of peripheral nerve are similar to the triplet of filaments from the CNS. This is consistent with the identification of the triplet proteins as the constituent proteins of the neurofilament and supports the conclusion that both peripheral and central neurofilaments are formed of the same proteins. The apparent differences in the heterogeneity of the proteins are not always demonstrable and it is difficult to be certain, at this time whether they reflect real differences in the proteins. The prominent complex of CNS proteins with M_r ~50 000 is not detectable in sciatic nerve filament preparations [5,8,15]. The sciatic nerve filament fractions include a group of proteins with M_r 60 000. Two of these have nearly the same isoelectric pH values and appear as adjacent spots on two-dimensional gels. Another component of P60 focusses at a more alkaline pH. We are not aware of intermediate filament proteins from other sources which have these properties.

SDS gels of homogenates of sciatic nerve indicate that P68 is present in greater amount than the other two triplet polypeptides [16,17]. In [16] P68 was considered to be the main neurofilament protein. It soon became apparent that there might be considerable serum albumin in the preparation of [16]. Two-dimensional gel analysis of intact rabbit sciatic nerve demonstrated clearly that the most prominent protein of sciatic nerve is serum albumin, which focusses at a much more alkaline pH than does P68 (D. S., unpublished). The relative amounts of each of the triplet proteins seen on two-dimensional gels of sciatic nerve homogenates and of filament fractions are similar to each other. P68 and P160 appear to be present in roughly equivalent amounts. As in the case in CNS, P200 is fairly labile and its concentration relative to the other triplet polypeptides varies from one preparation to another.

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