THE ISOLATION OF BRAIN 10 nm FILAMENT POLYPEPTIDES FROM UREA-EXTRACTS OF BRAIN WHITE MATTER

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1. Introduction

Until now it has been apparent that neurofilaments from the mammalian central and peripheral nervous systems are composed of different polypeptides. Brain neurofilaments have been reported to be composed principally of one polypeptide with a molecular weight in the region of 50 000 [1–4] whereas those of peripheral nerve have been found to contain a triplet of polypeptides with molecular weight of approximately 210 000, 155 000 and 70 000 [5–7]. We demonstrated that isolated brain 10 nm filaments contain minor components which comigrate with peripheral nerve neurofilament polypeptides and that sciatic nerve does not contain a component corresponding to the major 50 000 mol. wt polypeptide of the preparations from brain [8]. We have also found that antisera to the 210 000 and 155 000 mol. wt polypeptides from brain specifically stain neurones of the cerebellum and sciatic nerve [9]. Thus it seems likely that neurofilaments of central and peripheral neurones share these three polypeptides and that the 50 000 mol. wt material in preparations from brain may partially originate from glia [2,9,10]; a similar suggestion has now been made in [11].

Because the recovery of protein is low (0.1–0.2%) for conventionally isolated brain 10 nm filaments and the contribution to the total protein of the 210 000, 155 000 and 70 000 mol. wt polypeptides is 2–5% for each [12], we have developed an alternative and simple method for isolating these three components separately. We now describe this procedure which relies upon urea extraction of buffer-insoluble white matter. Since the urea changes the mobility of these polypeptides in sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS–PAGE) we have further shown by partial digest peptide mapping [13] that the putative neurofilament polypeptides are indeed present in the urea extract.

2. Experimental

2.1. *Preparation of protein fractions*

Bovine brain 10 nm filaments were isolated as in [8,12]. Urea-treated brain 10 nm filaments were made by incubating overnight at 0°C a pellet of filaments in 8 M deionized urea (deionized by standing for between 1–24 h with Amberlite MB-1 (BDH); all of the pellet was solubilized by this treatment. The mixture was made 1% v/v in 2-mercaptoethanol and heated at 100°C for 5 min. Urea extracts of white matter were prepared by first homogenizing 50 g bovine brain stem in 50 ml 0.001 M EDTA, 0.1 M sodium phosphate (pH 8.2). The homogenate was centrifuged at 100 000 × g_{av} for 60 min, the insoluble material was mixed with 50 ml 0.001 M EDTA, 8 M urea (pre-deionized as a 10 M solution), 0.02 M sodium phosphate (pH 8.2) and allowed to stand overnight at 4°C. The mixture was centrifuged at 100 000 × g_{av} for 1 h and insoluble material discarded, the supernatant was made 1% v/v mercaptoethanol and heated at 100°C for 5 min. Remaining insoluble material was removed by recentrifugation at 100 000 × g_{av} for 2 h. The urea-extract was the final supernatant.

2.2. *Electrophoresis*

Samples were heated at 100°C for 5 min in 2%

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v/v 2-mercaptoethanol 2% w/v SDS. Electrophoresis was as in [8] and the partial proteolysis peptide mapping technique was essentially as in [13].

2.3. *Elution of polypeptides*

Preparative SDS–PAGE was performed using double thickness (3 mm) slab gels and loading urea-extract across the full width of the gel. Following electrophoresis, the gels were stained in 0.1% w/v Coomassie brilliant blue R in 50% v/v methanol, 7.5% v/v acetic acid, 42.4% v/v water until the bands were just visible (1 min). Gel strips containing polypeptides of interest were sliced out and stored at –20°C until the elution step. The polypeptides were eluted electrophoretically by placing gel strips from about 8 slab gels in a glass tube with a scinter at the bottom end; dialysis tubing was fastened over this end. The tubes were fitted into a conventional disc gel electrophoresis apparatus which was then filled with sterilised (by autoclaving), 0.1% w/v SDS, 0.001 M EDTA, 0.02 M glycine, 0.0025 M Tris (pH 8.3) and electrophoresis carried out at 400 V for 24–36 h. The protein which collected in the dialysis bag was precipitated at 0°C by addition of an equal volume of 20% v/v glacial acetic acid, 80% v/v methanol. The precipitate was recovered by gentle centrifugation (bench centrifuge) redissolved in a minimal volume of 0.5 M sodium hydroxide and reprecipitated with methanol/acetic acid. At this stage the bulk of SDS appears to be removed and the polypeptides can then be transferred to the appropriate solution for subsequent experiments.

3. Results and discussion

The total complement of polypeptides present in the urea extract of bovine white matter is compared in fig.1 with that of bovine brain 10 nm filaments. The triplet of 210 000, 155 000 and 70 000 mol. wt

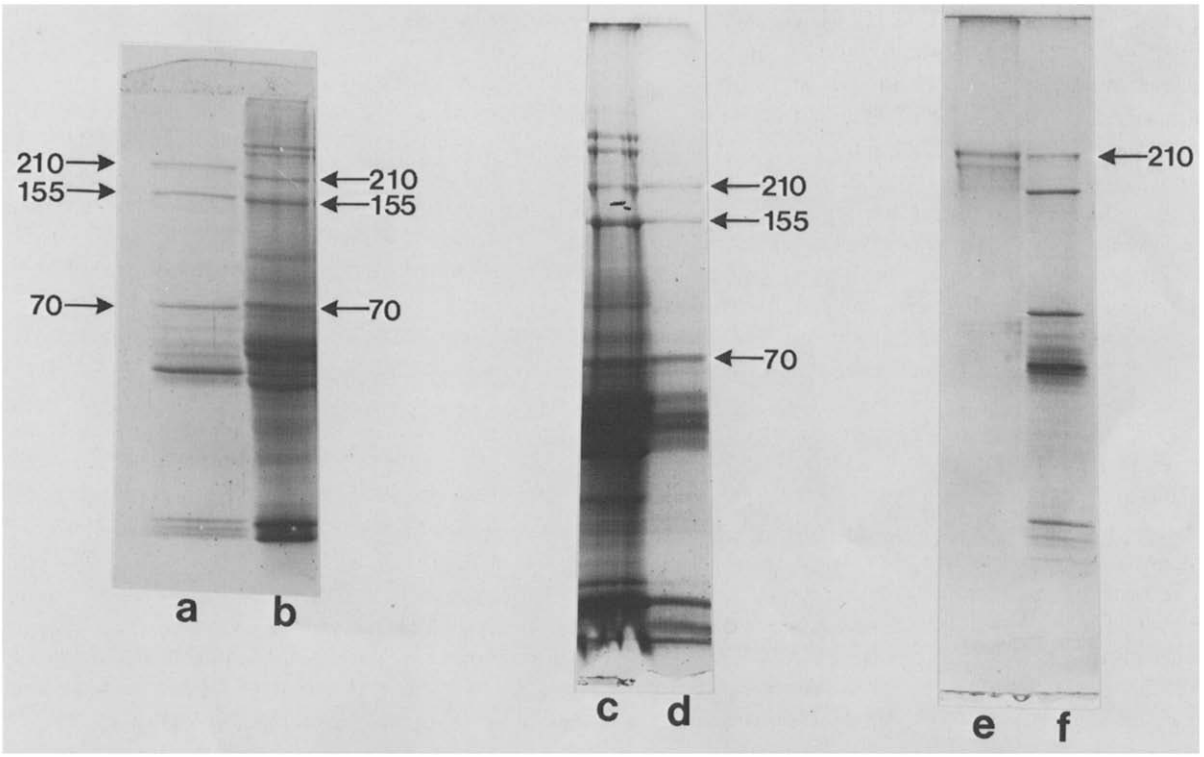

Fig.1. SDS–polyacrylamide gel electrophoresis of (a,f) bovine brain 10 nm filaments; (b,c) urea-extract of insoluble material from bovine brain white matter; (d) bovine brain 10 nm filaments treated with urea; (e) eluted 210 000 mol. wt bovine brain 10 nm filament polypeptide. Polypeptides are identified by mol. wt × 10^{-3}.

polypeptides in the brain 10 nm filament preparation (fig.1a) appears to be present in the urea extract (fig.1b), however, the mobility of each of the three bands seems altered by the urea treatment. Treatment of brain 10 nm filaments with 8 M urea demonstrated that the urea does indeed change the mobility of these polypeptides on SDS–PAGE (fig.1c,d). In order further to establish that this triplet in the urea extract is identical with that in isolated brain 10 nm filaments, the individual bands were compared by the partial proteolysis peptide mapping procedure [13]. Each of the triplet polypeptides from urea-treated brain 10 nm filaments were compared with the co-migrating polypeptides from urea-extracts of white matter. In each case the partial digest peptide maps were identical and this is illustrated in fig.2 for the pair of 210 000 mol. wt polypeptides.

The separated polypeptides in preparative SDS–PAGE of the urea-extract were found to be extremely susceptible to degradation during elution. To minimize this problem, we found it necessary to use only sterilized buffers for electrophoretic elution. Nevertheless, we have often observed that the eluted polypeptides still show signs of degradation as can be seen for the 210 000 mol. wt component illustrated in fig.1e,f. The acidified methanol precipitation step for removal of SDS did not appear to lead to any additional degradation.

Since the only practicable method available for isolating the individual triplet polypeptides is elution

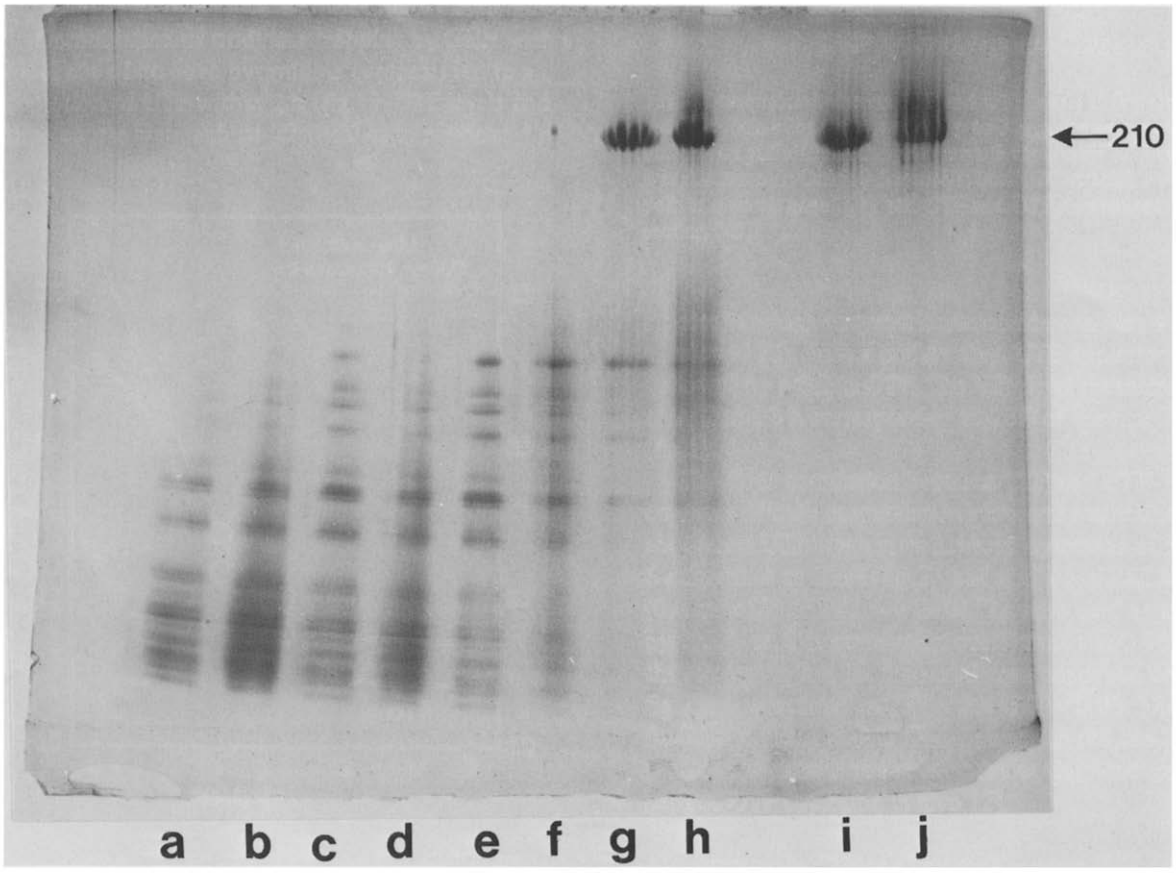

Fig.2. Partial proteolysis peptide maps of the 210 000 mol. wt polypeptide from urea-treated bovine brain 10 nm filaments and from the urea-extract of insoluble material from bovine brain white matter. (a,c,e,g,i) 210 000 mol. wt polypeptide from urea treated filaments; (b,d,f,h,j) 210 000 mol. wt polypeptide from urea extract. Various amounts of papain (1.6–2.5 ng) were used in channels (a–h). The 210 000 mol. wt polypeptide is identified by mol. wt × 10^{-3}.

from preparative SDS gels, it is, therefore, not essential to purify the 10 nm filaments as a prior step as these polypeptides are more abundant in simple urea-extracts of insoluble material from brain white matter. We have estimated the relative yields for the triplet in urea-extracts and in conventionally isolated brain 10 nm filaments by comparison of the quantity of material from each type of preparation required to give the same intensity of staining of these bands in SDS gels. By this method of estimating relative yield, the urea-extracts contain, as a conservative estimate, 20-times more protein for each of the triplet polypeptides. Thus for experiments in which only pure brain neurofilament polypeptides are required, urea-extraction would appear to be the method of choice and we have found this to be especially useful for producing antisera to these components.

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