# THE CYANOGEN BROMIDE PEPTIDE MAPS OF NEUROFILAMENT POLYPEPTIDES IN AXONAL PREPARATIONS ISOLATED FROM BOVINE BRAIN ARE DIFFERENT

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## 1. Introduction

It has been conclusively demonstrated [1] that a major fraction of ~50 000 mol. wt protein in axonal preparations isolated from bovine brain according to the basic procedure developed [2,3] is the astroglia-specific GFA protein [4], thus indicating that glio- and neurofilaments copurify in this procedure. GFA protein was purified by immunoaffinity chromatography with GFA antisera [5] and accounted for ~33% of the total protein in the axonal preparation. The major polypeptides not specifically absorbed to the column were at ~70 000 mol. wt and >100 000 mol. wt. In the 50 000 mol. wt range, protein non-absorbed to the column resolved into at least 3 minor bands on SDS–polyacrylamide gel electrophoresis.

We now report a comparative study of the cyanogen bromide peptide maps of the putative neurofilament proteins in the axonal preparation. Neurofilaments isolated from peripheral nerve are predominantly formed of a 68 000 mol. wt polypeptide [6], while studies of the slow component of axonal transport showed that 3 polypeptides with 212 000, 160 000 and 68 000 appeared to be associated with a single structure which was tentatively identified as the 'neurofilament triplet' [7]. The report [8] indicates that the neurofilament triplet is shared by neurofilaments from central and peripheral nerve, while the brain 50 000 mol. wt polypeptide is absent from peripheral nerve neurofilaments. It will be shown here that the polypeptides attributed to the neurofilament have different cyanogen bromide peptide maps.

## 2. Experimental

### 2.1. *Preparation of demyelinated axons from bovine white matter*

In the procedure developed to isolate demyelinated axons from bovine white matter, advantage is taken of the myelin to float the axon away from the other tissue constituents. The axons are then stripped from the myelin by 'osmotic shock' and separated from the myelin by differential centrifugation.

Following a report on the solubility of the mammalian neurofilament at low ionic strength [9], the possibility was considered that neurofilaments (as opposed to glial filaments) were dissolved during this stage of the procedure [10]. To rule out this possibility we compared preparations isolated according to [11] (osmotic shock conducted by lowering sucrose molarity from 0.9–0.32 M for 1 h), according to [12] (overnight in 0.01 M phosphate buffer and 0.01 M mercaptoethanol, pH 6.5) or according to [10] (1 h in 0.01 M phosphate buffer). No differences were seen in the gel pattern. For the purpose of this report demyelinated axons were prepared from frozen and non-frozen bovine white matter according to [11]. A total of 25 axonal preparations were used in these experiments. The presence of EDTA (0.1 mM) throughout the procedure (including electrophoresis) did not result in changes of the gel pattern.

### 2.2. *SDS–PAGE*

Axonal preparations were resolved by phosphate buffer [13] and Tris/glycine [14] SDS–polyacryl-

amide gel electrophoresis. For the purpose of cutting the bands SDS gel electrophoresis at 7.5% acrylamide concentration was performed in 0.1 M sodium phosphate buffer. The samples containing 1% SDS, 1.5% dithiothreitol, 8% sucrose and Bromphenol blue were heated for 3 min in a boiling water bath.

### 2.3. *Elution of non-stained bands*

Elution of non-stained bands was performed as in [15]. The gels were run in 18-gel chambers, 3 gels from each run were stained with Coomassie brilliant blue and the remaining 15 gels immediately wrapped in parafilm and frozen at –20°C. For each experiment the bands from 150 gels were cut, pooled and extracted 3 times in distilled water by homogenization and centrifugation at room temperature (200 g for 10 min). The extracts were combined and centrifuged at 12 000 × *g* for 10 min at 4°C. The supernatants were dialyzed against large volumes of water for 2 h at room temperature and then for 24–36 h at 4°C with frequent changes of water. After freeze-drying, a small sample from each band was re-run on SDS gels and stained with Coomassie brilliant blue for control of purity.

Electrophoretical elution of stained bands into dialysis bags resulted in extensive degradation even if the gels were fixed in only 50% methanol and exposed to acetic acid in the staining solution for a minimum of time [10]. Stained bands were also chopped and extracted at 37°C [16]. This resulted in lower yields.

### 2.4. *Cyanogen bromide cleavage*

The eluted bands were cleaved with 1.0 M cyanogen bromide in 70% formic acid for 24 h and 48 h at 26°C, or for 20 h at 37°C. Three separate experiments were performed, each experiment representing a set of 150 gels. The cyanogen bromide peptides were separated on SDS/urea–acrylamide gel electrophoresis [17]. No differences in gel pattern were observed by increasing the period of cleavage from 24–48 h and no cleavage occurred in 70% formic acid. The cyanogen bromide-split samples were resuspended in water, and an aliquot was run on SDS gel electrophoresis in 0.1 M phosphate buffer to determine whether cleavage was complete.

### 2.5. *Preparation of tubulin and actin*

Tubulin was isolated from bovine brain by the assembly–disassembly procedure. Actin from rabbit muscle was a gift of Dr R. Niederman.

## 3. Results and discussion

Figure 1, gel 1, shows the band pattern of an axonal preparation resolved on SDS–polyacrylamide gel electrophoresis in 0.1 M phosphate buffer. The pattern is essentially identical to that in [18] using the same gel system with 0.025 M phosphate buffer. On Tris/glycine SDS gel electrophoresis the upper band of the 2 bands above 200 000 mol. wt appeared to split. Identified bands in fig.1 are those which were separately eluted from the gels.

The 2 bands >200 000 mol. wt were completely broken down when re-run on the same electrophoretic system. The pattern of the degradation products

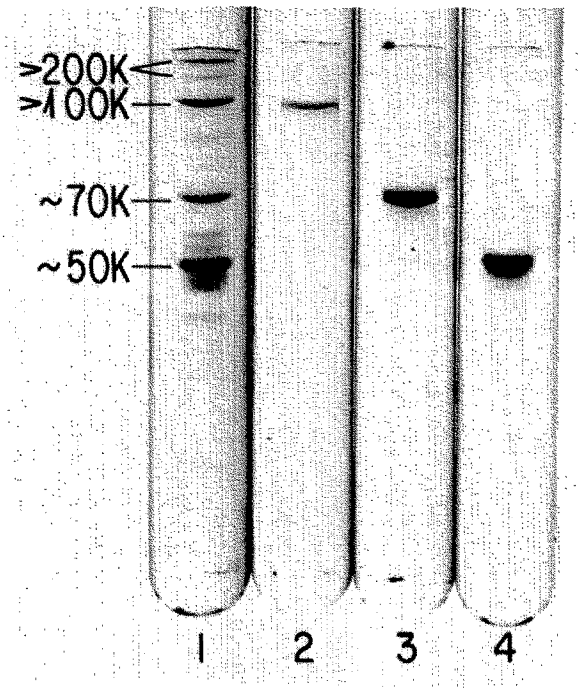


Fig.1. SDS gel electrophoresis at 7.5% acrylamide concentration of an axonal preparation (gel 1) and of proteins eluted from the identified bands which were re-run on the same gel system (gels 2–4). The bands >200 000 mol. wt were completely broken down under these conditions (results not shown). Splitting of the upper band >200 000 mol. wt occurred on Tris/glycine SDS gel electrophoresis.

varied considerably in different experiments. This, and the weak staining of the gels, prevented any definitive conclusion concerning their nature, i.e., aggregates of lower molecular weight polypeptides or authentic components of the neurofilament. The remaining bands appeared quite stable under these conditions except for minor degradation products (fig.1, gels 2–4).

Figure 2 shows the cyanogen bromide peptide maps of the bands eluted from the axonal preparation. The maps of the ~50 000 mol. wt band were markedly similar to those of GFA protein and different from those of tubulin migrating in the same molecular weight range [19]. The immunologically active peptide in the myoglobin range [20] is indicated by a bar. Since the identity with GFA of a major fraction in the 50 000 mol. wt range was demonstrated [1], this expected finding served as a control for the experimental conditions in each set of experiments and each gel run. The cyanogen bromide maps of the 70 000 mol. wt, >100 000 mol. wt and of the lower band in the 200 000 mol. wt range were different from each other and also different from those of the 50 000 mol. wt band. With respect to the upper band in the 200 000 mol. wt range, the peptide maps varied markedly in each of the 3 experiments. In fig.3 the cyanogen bromide peptide maps of actin and tubulin are compared with those of the 50 000 and 70 000 mol. wt bands from a different cleavage.

The main result of this study is that the polypeptides forming the neurofilament triplet are different from each other. With respect to the polypeptide(s) >200 000 mol. wt the results should be interpreted with caution, since the bands were completely broken down when re-run on SDS gel electrophoresis. The maps of the polypeptides forming the neurofilament triplets were also different from those of the 50 000 mol. wt band in the axonal preparation. Since the major fraction in this molecular weight range is GFA protein, our findings do not exclude the

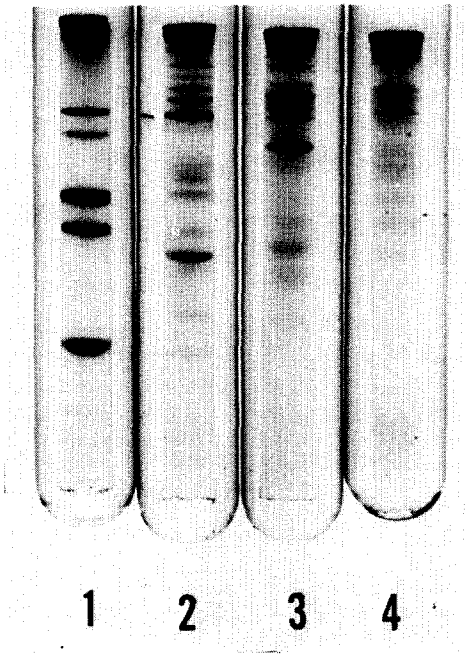


Fig.2. SDS/urea acrylamide gel electrophoresis [17] of the peptides formed by cyanogen bromide cleavage of the bands shown in fig.1. Gel 1, incomplete digest of myoglobin used as the molecular weight standard. Gel 2, ~50 000 mol. wt band. The identified band in the myoglobin range (17 200) is the immunologically active GFA peptide. Gel 3, ~70 000 mol. wt band. Gel 4, >100 000 mol. wt band.

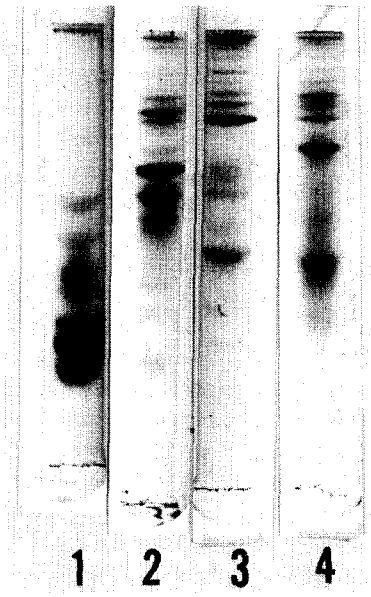


Fig.3. Comparison of the cyanogen bromide peptides of actin and tubulin with those of the ~50 000 and ~70 000 mol. wt bands in axonal preparations. The peptides are from a different cleavage compared with fig.2. Gel 1, actin; gel 2, tubulin; gel 3, ~50 000 mol. wt band; gel 3, ~70 000 mol. wt band.

possibility of a 50 000 mol. wt component related to one of the neurofilament proteins (homologies in the peptide maps could be easily missed due to the predominance of GFA).

Our findings are at variance with those in [10]. It was found that antisera raised against protein eluted from the ~70 000 and >100 000 mol. wt bands in axonal preparations precipitated the same peptides resulting from the digestion of these iodinated proteins by *Staphylococcus aureus* protease. On the other hand, similar conclusions concerning the lack of homologies between the peptide maps of neurofilament proteins in axonal preparations have been reported [21]. The method used for this paper was one-dimensional peptide mapping by partial proteolysis in SDS–polyacrylamide gels.

In conclusion, our findings suggest that the bovine neurofilament is constituted of different proteins and that, as originally proposed [7], the heterogeneity in polypeptide composition of the mammalian neurofilament is not due to degradation [22]. In this respect it is interesting to note that compared to the smooth glial filament formed by a single polypeptide chain, as indicated by assembly–disassembly experiments with GFA protein isolated by immunoaffinity chromatography [23,24], the neurofilament presents distinctive morphological features such as prominent sidearms and the ~150 Å wide lumps of irregular shape [25].

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