ISOLATION FROM PERIPHERAL NERVE OF A PROTEIN SIMILAR TO THE GLIAL FIBRILLARY ACIDIC PROTEIN

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1. Introduction

It will be shown in this paper that peripheral nerves do contain a protein similar to the glial fibrillary acidic protein (GFA), thus supporting the hypothesis that the protein subunits of glial and nerve filaments are alike [1,2]. The protein so isolated, which we called SN_1 protein, does not react with GFA antisera, but is related to GFA as indicated by comigration experiments on SDS-acrylamide gel electrophoresis, the type of degradation resulting from in situ proteolysis, amino acid composition and cyanogen bromide peptide mapping.

GFA protein was originally identified as the constituent of astroglial fibers since it represents the major fraction in gliosed brain tissue and because fibrous astroglia are selectively stained in immunofluorescence tests performed with diluted GFA antisera (reviewed in [3]). A recent comparative study of GFA purified from aqueous extracts of brain tissue and of the major protein contained in brain filaments isolated by density gradient centrifugation has shown that the two proteins have the same mol. wt. (54 000), and similar peptide maps [2]. The brain filaments were isolated from the axonal fraction [4] and thus presumably neurofilaments. However, electron microscopic findings [4] and more recent work in this laboratory indicate that the axonal fraction also contains fibers which may be identified as glial, on the basis of their ultrastructural features [4] and of positive immunofluorescence with GFA antisera [5]. The possibility could thus be considered that the protein similar to GFA contained in filaments isolated from axonal preparations was not related to neurofilaments but rather due to glial contamination, especially in view of the fact that electrophoretograms of axonal filaments in invertebrates do not show a major band in this molecular range [6,7].

In an attempt to solve this question – that is, whether glial and axonal filaments share a similar protein subunit – we have applied to peripheral nerve extracts the procedure used in this laboratory to isolate GFA protein from aqueous extracts of normal brain [8], under the assumption that closely related proteins will follow the same purification procedure. Peripheral nerves do not contain astroglia, while the large myelinated axons are very rich in neurofilaments both in the central and peripheral nervous system. The purification of water-soluble proteins related to nerve and glial fibers may well represent a major advance in the biochemistry of a class of filaments 7–10 nm in diameter which are particularly abundant in these structures and intermediate in size between microtubules and actin microfilaments. Isolated brain filaments are not soluble in aqueous solutions [4] and thus less amenable to biochemical studies.

2. Materials and methods

Rabbit sciatic nerves were purchased from Pel-Freez, Arkansas, and human sciatic nerves were dissected at autopsy. The time interval between death and autopsy

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varied from 2 to 24 h. Human liver, kidney, testis and serum were also used to control the specificity of the purification procedure. From none of these a protein migrating in the GFA range on SDS-acrylamide gel electrophoresis was isolated. The tissue homogenates were extracted with 0.05 M sodium phosphate buffer, pH 8.0. The extracts were adsorbed to hydroxylapatite in the same buffer and protein was eluted with 0.1 M potassium phosphate buffer, pH 8.0, after all unadsorbed protein had been removed by extensive washing with 0.05 M sodium phosphate buffer, pH 8.0. The eluates were pooled and precipitated by adding ammonium sulfate to a final concentration of 30%. The ammonium sulfate precipitates were resuspended in 0.05 M sodium phosphate buffer, pH 8.0, dialyzed against water and freeze-dried. In some experiments the sediments were redissolved with 0.05 M sodium phosphate buffer, pH 8.0, and the protein was precipitated by adding sodium acetate pH 4.7 dropwise while stirring. SN_1 protein started to precipitate at pH 6.4 and, as GFA protein [9] was completely precipitated at pH 5.0. Both proteins were redissolved at lower and higher pH. In other experiments peripheral nerves were first extracted with 0.01 M sodium phosphate–$MgCl_2$ buffer pH 6.2, a buffer used to solubilize tubulin [10], and then with 0.05 M sodium phosphate buffer pH 8.0. Both extracts were adsorbed to hydroxylapatite in sodium phosphate buffer, pH 8.0, and the procedure was continued as described before. Most of the SN_1 protein was present in the pH 8.0 extract, thus showing that the differential solubility in buffer solutions [9] was another common proterty of SN_1 and GFA proteins.

3. Results and discussion

SN_1 protein isolated from rabbit sciatic nerves yielded a single band on SDS gel electrophoresis at 5%, 7.5% and 12.5% acrylamide concentration (fig.1, gel 1), co-migrating with GFA protein, 54 000 daltons [8], in mixing experiments. SN_1 protein isolated from human sciatic nerves showed a multiple band pattern suggestive of degradation [8,11] (fig.1, gel 3). As previously described for GFA protein from human, bovine and rat brain [8,11], a marked enrichment in the lower mol. wt. species was obtained by incubating sciatic nerves at 24°C for 24–48 h. After 72 h only polypeptide co-migrating with highly degraded GFA protein, 45 000 – 40 500 mol. wt. [11] were present. The preparations used for amino acid analysis (table 1) were isolated from sciatic nerves dissected shortly after death and showing little degradation.

Reduced and non-reduced preparations of SN_1 protein had markedly different electrophoretic mobility on SDS gels. As observed with GFA protein (Dahl and Bignami, in preparation), without dithiothreitol in the sample nondegraded protein stayed at the origin of the gel (fig.1, gel 2). With degraded protein, the lower mol. wt. components were selectively included into the gel (fig.1, gel 4). The

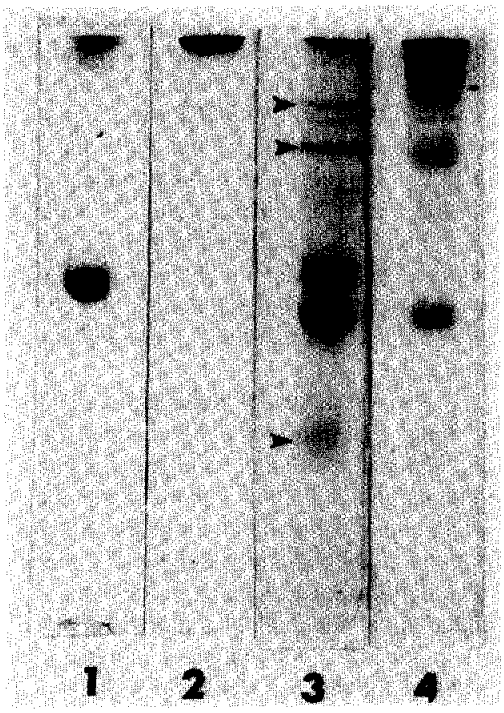

Fig.1. SDS gel electrophoresis at 7.5% acrylamide concentration [18] of reduced and non-reduced SN_1 protein. Gel 1, reduced rabbit SN_1 protein migrates as a single band at 54 000 daltons; gel 2, the same protein as in gel 1 without dithiothreitol in the electrophoretic sample. Most of the protein stays on the top of the gel. Comparable results were obtained with non-reduced GFA protein; gel 3, reduced human SN_1 protein isolated from sciatic nerves dissected 24 h after death. Arrowheads indicate contaminants (purification was less complete with autolyzed tissue); gel 4, the same protein as in gel 3 without dithiothreitol in the electrophoretic sample. Degraded protein is selectively included into the gel.

Table 1
Amino acid analysis of SN_1 and GFA proteins

Amino acid	SN_1 protein Human[a] (4)	Rabbit (4)	GFA Human[a] (2)	Bovine (2)
	moles %			
Lysine	5.7	5.3	6.1	4.4
Histidine	2.1	1.9	2.2	2.3
Arginine	5.9	5.4	4.8	5.7
Cysteine[b]	1.4	nd[c]	nd[c]	1.5
Aspartic acid	10.9	10.5	10.7	10.3
Threonine	5.1	5.6	6.0	5.8
Serine	6.4	7.0	6.3	6.2
Glutamic acid	16.3	15.2	16.5	15.0
Proline	4.4	5.2	4.1	4.9
Glycine	8.4	11.4	9.4	7.5
Alanine	7.7	8.4	8.6	8.5
Valine	5.1	5.1	5.9	5.7
Methionine	2.0	2.3	2.5	2.6
Isoleucine	3.8	3.4	3.7	3.8
Leucine	9.4	7.9	8.4	8.6
Tyrosine	2.7	2.8	2.1	3.2
Phenylalanine	3.1	3.0	2.7	3.9

Average of 24 and 72 h hydrolysates. Number of determinations in parentheses.

[a]Isolated from tissue dissected shortly after death.
[b]Determined as carboxymethylcysteine.
[c]Not determined.

effect of reducing agents may be explained by recent studies indicating that the 54 000 polypeptide chains of GFA protein are covalently assembled into large polymers by interchain disulfide bridges [12]. The selective inclusion of degraded protein into the gels suggests that cleavage occurs in the region of the molecule containing the intermolecular disulfide bridges and that the 45 000 – 40 500 degraded species are not covalently linked.

The effect of reducing agents on the mol. wt. of non-degraded SN_1 and GFA proteins is of interest considering that the amino acid composition of these proteins (table 1) is very similar to tubulin [10,13]. In agreement with literature data [13], we did not observed marked differences in the electrophoretic mobility on SDS gels between reduced and non-reduced tubulin. Disulfide bonds, if present in tubulin, appear to be intramolecular rather than intermolecular [14]. Further evidence that our preparations are not contaminated by tubulin is provided by the fact that the amino terminal amino acid of non-degraded GFA protein is blocked [15], while both the alpha and beta subunits of tubulin have methionine as the amino terminal residue [13].

The cyanogen bromide peptide maps of highly degraded GFA protein from multiple sclerosis plaques [11], bovine GFA, and human SN_1 protein are shown in fig.2. A 17 000 mol. wt. peptide was common to all preparations.

The isolation of a protein similar to GFA from peripheral nerve but not reacting with GFA antisera, with the same procedure to purify GFA from the brain, raises a major question, i.e., whether both glial and nerve fibers are the source of the protein isolated from normal brain. The apparent selectivity of the antisera for the astroglial component could be explained with the fact that the original preparations used as antigen were isolated from tissue selectively enriched in glial fibers (multiple sclerosis plaques) [16,17]. However, we have recently prepared antisera

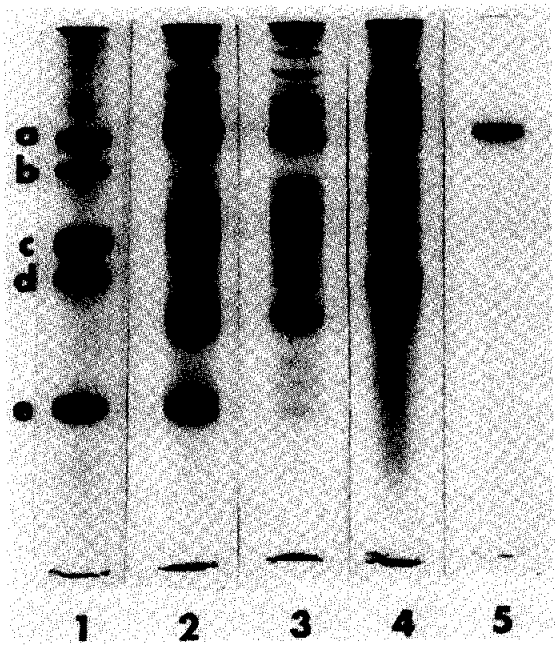

Fig.2. Cyanogen bromide peptides of SN_1 and GFA proteins on SDS/urea gel electrophoresis [19]. A cyanogen bromide peptide migrating in the myoglobin range 17 200 mol. wt. is common to all preparations. Slower migrating bands are probably the result of aggregation since they were not observed on SDS gel electrophoresis. Gel 1, incomplete digest of myoglobin used as the standard [19]: (a) myoglobin, 17 200; (b) peptides I + II, 14 900; (c) peptide I, 8270; (d) peptide II, 6420; (e) peptide III, 2550; gel 2, digest GFA protein isolated from gliosed human brain [11]; gel 3, digest of bovine GFA protein; gel 4, digest of human SN_1 protein; gel 5, cyanogen bromide peptide in the myoglobin range isolated from the digest of bovine GFA protein by hydroxylapatite chromatography.

with the same specificity using GFA protein isolated from normal spinal cord (Dahl and Bignami, in preparation), thus suggesting that if the glial and neuronal components are isolated in the same fraction, they differ markedly in immunogenicity.

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