ELECTROPHORETIC CHARACTERIZATION OF RAPIDLY TRANSPORTED PROTEINS IN AXONS OF RETINAL GANGLION CELLS

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1. Introduction

Rapid axonal transport of protein from the perikaryon to the nerve terminal is likely to play an important role in the regulation of neural function. An important problem in neurobiology is, therefore, to identify the constituent proteins of this rapid intraneuronal transport in the mammalian central nervous system. Such investigations have been hampered by the fact that the major portion of these proteins are membrane-bound [1–3]; this severely restricts the number of available separation methods.

When membrane proteins are exposed to sodium dodecyl sulphate (SDS) and mercaptoethanol (ME), a large number of the hydrogen, hydrophobic and disulphide linkages are disrupted, resulting in solubilization of many of these proteins. Upon electrophoresis in the presence of SDS, the protein-SDS complexes migrate as anions, at a rate which is inversely proportional to the log of the M.W. of the polypeptide chain [4]. This technique has been used to separate the rapidly transported proteins in the optic system of the rabbit. Axonal transport of protein in this system occurs at at least four different rates (150, 40, 6–12 and 2 mm/day respectively) [5]. The present study was concerned only with the rapidly transported proteins. The results showed that most of these rapidly transported proteins consist of polypeptides with relatively high molecular weights. The major labelled polypeptide had a M.W. of approximately 107,000.

2. Materials and methods

Albino rabbits of both sexes, weighing about 2.5 kg, were injected with 50 μl of (^{3}H leucine (L-4,5-^{3}H leucine, specific radioactivity 17 Ci/mmole, conc. 1–10 mCi/ml) in a sterile aqueous solution into the vitreous body of one or both eyes. At specified time intervals after the injection, the animals were anaesthetized with sodium pentobarbital and killed by intra-cardiac perfusion with 0.9% (w/v) NaCl. The relevant parts of the optic system were immediately dissected out [5] and homogenized. The retina, optic nerve and optic tract were homogenized in a solution of 3% SDS, 3% ME, 10 mM phosphate buffer (pH 7.0) in a tight-fitting all glass homogenizer and kept at 37° during a specified time interval. The samples were then centrifuged at 10^5 g for 20 min and the resulting supernatant was subjected to polyacrylamide gel electrophoresis. The lateral geniculate body was homogenized in 2 ml 0.32 M sucrose, containing 10 mM Tris-HCl (pH 7.0), in a teflon to glass homogenizer (clearance 0.15 mm) with four up and down strokes, twice at 1500 rpm. The homogenate was centrifuged at 800 g for 10 min and the resulting crude nuclear fraction was washed twice with 0.32 M sucrose. The combined supernatants were centrifuged at 10^4 g for 20 min to give a crude mitochondrial fraction, and a supernatant which was centrifuged at 165,000 g for 60 min to give a soluble and a microsomal fraction. All the subcellular fractions were treated with 3% SDS, 3% ME in 10 mM phosphate buffer (pH 7.0) as described above, and prepared for electrophoresis. In some cases the SDS-solubilized microsomal fraction from the lateral geniculate body was dialyzed against 0.1% SDS, 0.1% ME in 10 mM phosphate buffer (pH 7.0) prior to electrophoresis.

Electrophoresis of the solubilized samples was carried out in the SDS electrophoresis system of Shapiro et al. [4], essentially as described by Weber and Osborn [6]. The gels were 110 mm long and had a diameter of 4 mm. Electrophoresis was performed, first at a constant current of 1 mA/gel for 30 min, and then at 6 mA/gel for approximately 3 hr. Gels were fixed with 20% sulphosalicylic acid for 20 hr and stained with 0.02% Coomassic brilliant blue (R 250, ICI, Ltd.) in 12.5% TCA for approximately 5 hr. The gels were sliced in 1 mm pieces with a McIlwain type tissue chopper, and the radioactivity in each gel slice was determined after treatment with soluene (Packard Co.) as described previously [7]. Standard proteins were obtained from Boehringer, Mannheim: cytochrome *c* (horse heart, M.W. 13,000), chymotrypsinogen (M.W. 25,000), ovalbumin (M.W. 45,000) and bovine serum albumin (M.W. 67,000).

3. Results and discussion

The electrophoretic patterns of the proteins of the retina, optic nerve and optic tract are shown in fig. 1. The optic nerve and tract had very similar electrophoretic patterns, which closely resembled that of the homogenate from the lateral geniculate body. The retina differed from the other samples mainly with respect to the presence of an additional major band in the anodal part of the gel. SDS electrophoresis of retinal proteins showed that the major portion of the labelled polypeptide had a M.W. higher than 35,000 (figs. 2 and 3). The two major radioactivity peaks in this region correspond to polypeptides with M.W. of 49,000 and 66,000 respectively.

SDS electrophoresis of optic nerve proteins resulted in a polydisperse labelling pattern, with the major part of the rapidly transported polypeptides having molecular weights higher than 19,000 (figs. 2 and 4). Two major radioactivity peaks were seen. These corresponded to polypeptides having M.W. of 107,000 and 21,000. The optic tract proteins showed essentially the same radioactivity pattern as the optic nerve proteins. The two major radioactivity peaks corresponded to polypeptides with molecular weights of 102,000 and 20,000 (figs. 2 and 5). The microsomal fraction from the lateral geniculate body showed a polydisperse labelling pattern, with most of the labelled polypeptides

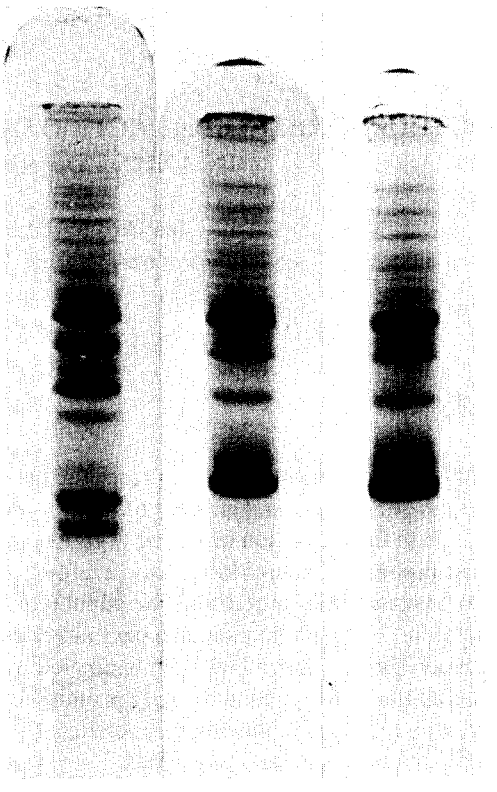

Fig. 1. Electrophoretic patterns of retinal proteins (left), optic nerve proteins (middle) and optic tract proteins (right). Electrophoresis as described in Materials and methods.

having a molecular weight higher than 34,000 (figs. 2 and 6). One major radioactivity peak was seen. The mobility of this peak corresponded to a protein of M.W. about 105,000–110,000. The radioactivity peak in this region was repeatedly found and was the largest one observed in all experiments performed. This was true for all the different procedures used to solubilize the microsomal fraction (3% or 5% SDS at 45° for 4 hr, 3% SDS for 2–19 hr at 37°, or 3% SDS at 4° for 40 hr. The radioactivity pattern was also the same when the samples were dialyzed into 0.1% SDS prior to electrophoresis. In five separate experiments the M.W. for this peak was found to be 107,000 ± 3000.

In conclusion, the constituent proteins of rapid axonal transport consist of polypeptides with relatively high M.W. The major labelled component has a M.W. of approximately 100,000–110,000 in the optic

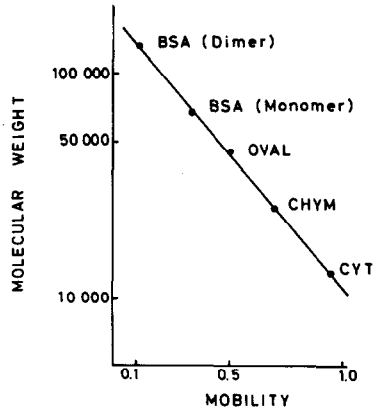

Fig. 2. Comparison of the molecular weights of some standard proteins with their electrophoretic mobilities (1.0 = mobility of tracer dye, bromphenol blue). BSA: bovine serum albumin, OVAL: ovalbumin, CHYM: chymotrypsinogen, CYT: cytochrome *c*.

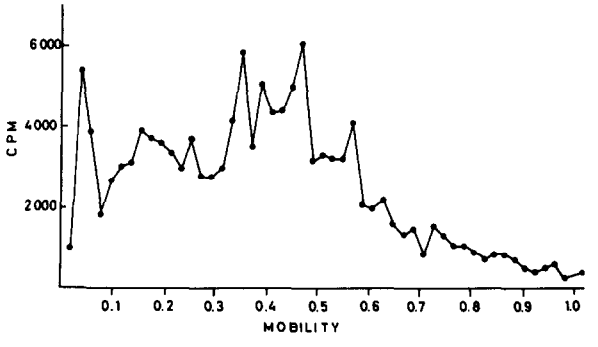

Fig. 3. SDS electrophoresis of retinal proteins. Animals were injected with 500 μCi (^{3}H) leucine into each eye, and were sacrificed 7 hr after the injection, i.e. at a time when the radioactivity in the retina has reached its maximal value [5]. The SDS treatment used solubilized more than 70% of total protein and radioactivity in the retina. All of the protein-bound radioactivity that was applied to the gel could, after electrophoresis, fixation, staining, destaining and slicing of the gel, be recovered in the individual gel slices. Most of the labelled polypeptides of the retina had electrophoretic mobilities less than 0.6, and the two major radioactivity peaks were found to have mobilities of 0.47 and 0.36.

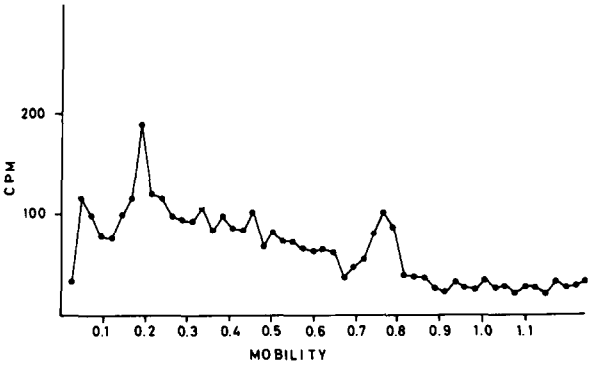

Fig. 4. SDS electrophoresis of optic nerve proteins. Animals were injected with 500 μCi (^{3}H) leucine into each eye, and were sacrificed 7 hr after the injection, i.e. at a time when radioactive proteins of the two rapid phases of axonal transport are present in the optic nerve [5]. The SDS treatment used solubilized 95% of total protein and radioactivity in the optic nerve. 98% of applied radioactivity entered the gel. The major portion of the labelled polypeptides in the optic nerve had an electrophoretic mobility less than 0.8. Two major radioactivity peaks were seen and their mobilities were 0.19 and 0.77.

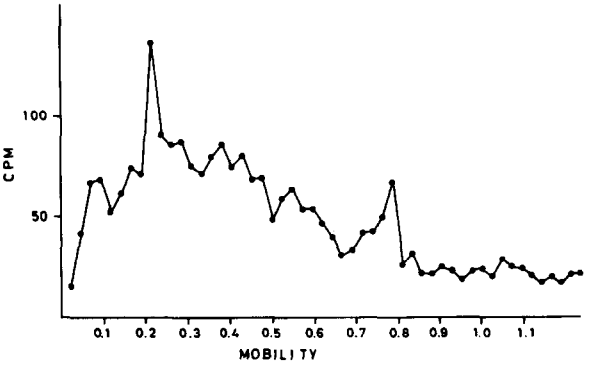

Fig. 5. SDS electrophoresis of optic tract proteins. Animals were injected with 500 μCi (^{3}H) leucine into each eye and were sacrificed 7 hr after the injection, i.e. at a time when radioactive proteins of the two rapid phases of axonal transport are present in the optic tract [5]. The SDS treatment solubilized more than 99% of total protein and radioactivity in the optic tract. All of the applied radioactivity entered the gel. The major portion of the labelled polypeptides in the optic tract had an electrophoretic mobility less than 0.8. Two major peaks of radioactivity were seen and their mobilities were 0.21 and 0.78.

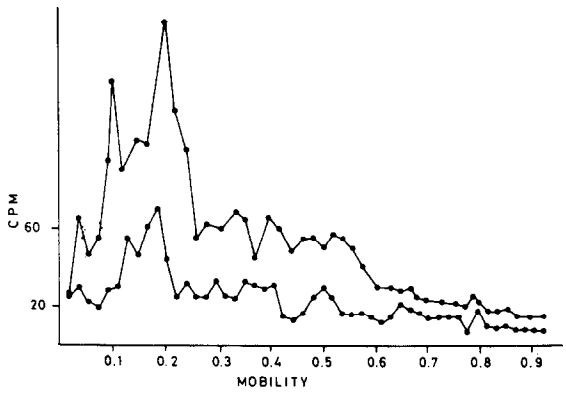

Fig. 6. SDS electrophoresis of microsomal proteins from the lateral geniculate body. Animals were injected with 500 μCi (^{3}H) leucine into each eye and were sacrificed 7 hr after the injection, i.e. at a time when radioactive proteins of the most rapid phase of axonal transport are present in the lateral geniculate body [5]. The SDS treatment solubilized more than 99% of total protein and radioactivity in the microsomal fraction of the lateral geniculate body. Lower curve shows the labelling pattern of a microsomal fraction treated with 3% SDS at 37° for 2 hr. Upper curve shows the pattern of the same microsomal fraction treated with 3% SDS for 19 hr, and then dialyzed against 0.1% SDS for 20 hr. Twice as much protein was applied to the latter gel. The major portion of the labelled polypeptides had an electrophoretic mobility less than 0.6. One major radioactivity peak was seen, and its mobility was 0.18.

nerve, optic tract and in the microsomal fraction from the lateral geniculate body. A labelled polypeptide with a M.W. of about 20,000, which was seen in the optic nerve and tract, was not prominent in the microsomal fraction from the lateral geniculate body. This labelled component could be a constituent of the second phase of rapid axonal transport, which has not yet reacted the lateral geniculate body at the time period studied. Alternatively, it could be localized in other subcellular fractions from the lateral geniculate body. In an investigation of the transport of fucose-containing glycoproteins in the rabbit optic system [8], the M.W. pattern of the transported glycopeptides was similar to that of the radioactive proteins in the present study. Thus, many of the rapidly transported leucine-labelled proteins may be glycoproteins.

Acknowledgements

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