Purification of Individual Components of the Neurofilament Triplet: Filament Assembly from the 70 000-Dalton Subunit†

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ABSTRACT: Mammalian neurofilaments are composed of three subunit polypeptides with approximate molecular weights of 200 000, 150 000, and 70 000 (P200, P150, and P70). These subunits were separated by ion-exchange chromatography in the presence of 8 M urea. The P200 polypeptide was differentially eluted on a diethylaminoethyl (DEAE) column. The P70 and P150 polypeptides obtained after the DEAE column were separable on a hydroxylapatite column. Under neurofilament assembly conditions, only the P70 polypeptide was able to reassemble into an intermediate filament in the absence of the other two polypeptides. The P150 and P70 polypeptides copolymerized into an intermediate filament, only if P70 was present. These results suggest that the P70 polypeptide forms the core of the filament and the other two polypeptides are tightly associated accessory proteins.

Neurofilaments are intermediate (10-nm) filaments found in the axoplasm of neurons. They are composed of three polypeptides with approximate molecular weights of 200 000, 150 000, and 70 000 (Liem et al., 1978; Schlaepfer & Freeman, 1978; Anderton et al., 1978), which copurify when the filaments are separated in situ (Liem et al., 1978; Chiu et al., 1980; Runge et al., 1981a; Shecket & Lasek, 1980) and are the slowest transported proteins in axonal transport studies (Hoffman & Lasek, 1975; Willard & Hulebak, 1977).

Efforts to separate the three subunits of the mammalian neurofilament have been hindered primarily by the difficulty of obtaining pure neurofilament proteins in quantities necessary to carry out biochemical purification. Neurofilament preparations are commonly contaminated with glial filament protein (Liem et al., 1978) or tubulin (Runge et al., 1981a). They can be obtained from peripheral nerve in relatively high purity, but the yields in these preparations are very low (Liem et al., 1978; Schlaepfer & Freeman, 1978).

In a recent communication, we have reported the successful separation of neurofilament triplet proteins from the glial filament protein as well as tubulin by hydroxylapatite chromatography (Liem, 1982). The filaments were dissolved in 8 M urea prior to chromatography and reassembled by dialysis to remove the urea. The reassembled filaments contained the neurofilament triplet and were found to be antigenically distinct from the reassembled glial filaments obtained from the same preparation.

Further separation of the subunits by gel chromatography or molecular exclusion methods even in the presence of denaturants such as guanidine hydrochloride or urea, in the absence of detergents, has so far proven to be unsuccessful. Willard and his co-workers (Willard et al., 1980; Willard & Simon, 1981) were able to purify the neurofilament subunits by fractionation on Sepharose 6B in the presence of 1% sodium dodecyl sulfate ($NaDodSO_4$). These proteins were subsequently used as antigens for antibody production and purification. The purified antibodies were then used for immunoelectron microscopic antibody decoration studies which showed that the 70-kilodalton protein appears to be associated with a central core of the filament, whereas the 200- and 150-kilodalton proteins appear to comprise a structure more loosely and peripherally associated with the same neurofilament. No assembly studies were reported on these purified subunits, and to date, information on the ability of each of the subunits to form an intermediate filament has not been published.

In this paper, we report the total separation of the neurofilament subunits by ion-exchange chromatography and the results of the reassembly studies of these subunits. These results support the suggestion that the 70-kilodalton protein is the central core of the filament and that the other two proteins are probably neurofilament-associated proteins.

Materials and Methods

Neurofilament Protein Purification. Bovine brain white matter was used as starting material, and a fraction enriched in intermediate filaments was obtained by the method of Liem et al. (1978). White matter was homogenized in solution A [10 mM phosphate buffer, pH 6.8, 1 mM ethylenediaminetetraacetic acid (EDTA), and 0.1 M NaCl] containing 0.85 M sucrose, and myelinated axons were floated by centrifugation in an SW27 rotor at 10 000 rpm, for 15 min. The myelin was removed by treatment with 1% Triton X-100 in solution A, and the filaments were separated from the myelin by centrifugation through 0.85 M sucrose in solution A at 20 000 rpm for 30 min at 4 °C. The pellet, a mixture of neurofilaments and glial filaments, was dissolved in a solubilization buffer (10 mM phosphate, pH 7.4, containing 1% mercaptoethanol made up in freshly deionized 8 M urea). The neurofilament triplet proteins were separated from the glial filament protein and other contaminants by chromatography on a hydroxylapatite (Bio-Rad HTP) column. Crude filament protein (30–40 mg) in solubilization buffer was applied to a 1.5 × 10 cm column at a flow rate of 25 mL/h and eluted with 0.14 M phosphate buffer, pH 7.0, followed by 0.3 M phosphate buffer, pH 7.0 (Liem, 1982). For prevention of formation of cyanates, all buffers were made up in freshly deionized urea, prepared by passing the 8 M urea solution through a mixed-bed ion-exchange column (AG501-X8D). The fraction eluted from the hydroxylapatite column with 0.3 M phosphate buffer contained the neurofilament triplet, which was further purified by reassembly into filaments. This reassembly was accomplished by overnight dialysis against an assembly buffer which contained 10 mM phosphate buffer, pH 6.8, 1 mM ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), 1 mM $MgCl_2$, 1 mM ATP, and 1 mM phenylmethanesulfonyl fluoride (protease inhibitor) in the cold

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followed by a 1-h incubation at 37 °C. The reassembled neurofilaments were pelleted by centrifugation at 40 000 rpm for 1 h at room temperature, and the pellets were redissolved in the solubilization buffer for further chromatography.

Diethylaminoethyl (DEAE) Chromatography. Solubilized reassembled filaments were placed on a DEAE-cellulose column (Whatman DE52) for further separation of the triplet components. A 1.5 × 10 cm column was used, and 5–10 mg of purified neurofilament protein in solubilization buffer was applied to the column which was eluted with 10 mM phosphate buffer, pH 7.4, followed by 0.1 M phosphate buffer, pH 7.0, and 0.3 M phosphate buffer, pH 7.0. All buffers were made up in freshly deionized 8 M urea, and the column was eluted at a flow rate of 25 mL/h. The fractions were monitored by OD_{280}, and the material which contained the protein peaks was analyzed by $NaDodSO_4$ gel electrophoresis to establish where the triplet proteins were eluted. The fractions containing the purified triplet polypeptides were concentrated by vacuum dialysis, dialyzed against assembly buffer, and incubated for 1 h at 37 °C. The solutions were then examined by electron microscopy (EM) using negative stain as described below and pelleted by centrifugation at 40 000 rpm for 60 min in a Beckman Ti 70 rotor. The pellets were resuspended in solubilization buffer for further chromatography.

HTP Chromatography. Hydroxylapatite (HTP) chromatography was performed on the combined fractions eluted with 0.3 M phosphate buffer from the DE52 column after they were concentrated, reassembled, and taken up in solubilization buffer. Protein in solubilization buffer (~2–5 mg) was applied to a 0.75 × 5 cm column at room temperature at a flow rate of 25 mL/h. The column was successively eluted with 0.14 M phosphate buffer, 0.16 M phosphate buffer, 0.18 M phosphate buffer, and 0.3 M phosphate buffer, all at pH 7.0 and made up in freshly deionized 8 M urea. All the fractions were monitored by OD_{280}, and the fractions which contained the protein peaks were further analyzed by $NaDodSO_4$–polyacrylamide gel electrophoresis. The samples containing the purified triplet proteins were concentrated by vacuum dialysis and used for the reassembly studies described below.

Reassembly Studies. So that the assembly of homopolymers and copolymers of the neurofilament triplet could be monitored, approximately equimolar amounts of each triplet protein dissolved in 8 M urea buffer were returned to assembly buffer by dialysis. For these experiments, polymers obtained after a 1-h incubation at 37 °C following the removal of urea with assembly buffer were monitored by EM by using negatively stained material. Formvar-coated grids were glow discharged in a Denton evaporator, and the samples were added within 30 min of discharge. The grids were washed with 2–3 drops of assembly buffer and stained with 2–3 drops of freshly filtered 1% uranyl acetate. The last drop of stain was allowed to remain for 30 s, after which it was removed with a piece of filter paper. The grids were examined in a JEOL 100S electron microscope.

The polypeptide composition of the reassembled filaments was analyzed by $NaDodSO_4$–polyacrylamide gel electrophoresis. For these experiments, the suspensions were centrifuged after reassembly in a Beckman airfuge for 1 h, and the resulting pellets were dissolved in 8 M urea to the same volume as the original reassembly mixtures. A gel buffer containing 1% $NaDodSO_4$, 0.125 M tris(hydroxymethyl)aminomethane (Tris) buffer, pH 6.8, 1% mercaptoethanol, and 2 M glycerol was added to both the pellets and supernates, and equal amounts of each were loaded on the gel. The samples were analyzed on a 7.5% polyacrylamide gel containing 0.1% $NaDodSO_4$ stained with Coomassie blue after electrophoresis.

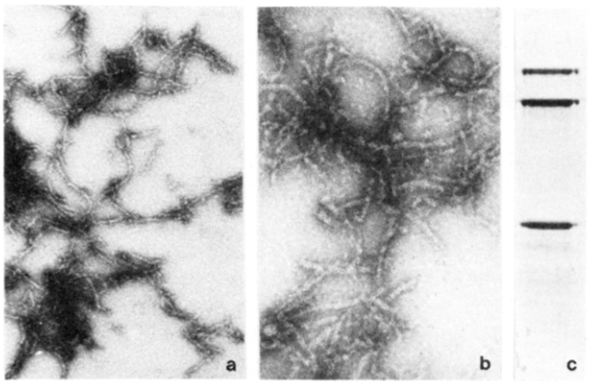

FIGURE 1: (a) Negatively stained reassembled neurofilaments purified by hydroxylapatite chromatography in 8 M urea and reassembled by replacement of the urea with an assembly buffer. Numerous long and short filaments were obtained which measured close to 10 nm in diameter (37 500×). (b) Negatively stained reassembled neurofilaments (60 000×). (c) Gel electrophoretic profile of the reassembled neurofilaments. The preparation consists almost exclusively of the neurofilament triplet polypeptides.

Results

Neurofilament Purification. Neurofilaments were separated from glial filament protein and tubulin subunits by hydroxylapatite chromatography in 8 M urea as described previously (Liem, 1982). The glial filament protein and tubulin were eluted with 0.14 M phosphate buffer, pH 7.0. We used this buffer rather than the previously reported 0.15 M phosphate buffer because the latter started to elute the 70-kilodalton neurofilament protein, which subsequently lowered the yield of the reassembled neurofilament triplet. The fractions containing the neurofilament triplet were obtained by elution with 0.3 M phosphate buffer, pH 7.0. These fractions were collected, concentrated by vacuum dialysis, and reassembled by overnight dialysis against assembly buffer, followed by a 1-h incubation at 37 °C.

In our previous study (Liem, 1982), we had observed that filament assembly is dependent on the ionic strength of the assembly solution, and we found that a buffer containing 0.1 M NaCl, 1 mM EDTA, and 1 mM ATP gave the optimum number of filaments. The nucleotide appeared to increase the yield of the reassembled filaments, even though it was not found to be an absolute requirement for assembly. We have modified the assembly conditions further by substituting KCl for NaCl and EGTA for EDTA and adding 1 mM $MgCl_2$ to the buffer. These conditions produced numerous filaments as judged by negatively stained material (Figure 1a,b) and gave better yields than the conditions we described previously; i.e., over 90% of the filament protein was assembled. The reassembled filaments were centrifuged at 40 000 rpm for 60 min in a Ti 70 rotor in a Beckman ultracentrifuge. The gel electrophoretic profile of these reassembled filaments is shown in Figure 1c. The neurofilaments, almost solely composed of the triplet polypeptides, were then used for further purification. The yield of purified neurofilament protein was approximately 10 mg per 50 g of white matter.

DEAE Chromatography. When the purified neurofilaments were applied to a DEAE column, the 200-kilodalton neurofilament polypeptide was eluted with 0.1 M phosphate buffer, while the other two polypeptides did not appear until 0.3 M phosphate buffer was used to elute the column (Figure 2). As can be seen from the gel profile, purification of the 200-kilodalton protein was complete, with none of this protein remaining in the second peak. The 10 mM phosphate wash

FIGURE 2: Gel electrophoretic profile of the pooled fractions obtained after DEAE-cellulose chromatography. (a) Material eluted with 0.1 M phosphate buffer, pH 7.0, which consists almost exclusively of the P200 polypeptide. (b) Material eluted with 0.3 M phosphate buffer, pH 7.0, which consists of the P70 and P150 polypeptides as well as several other polypeptides with molecular weights intermediate between these two proteins.

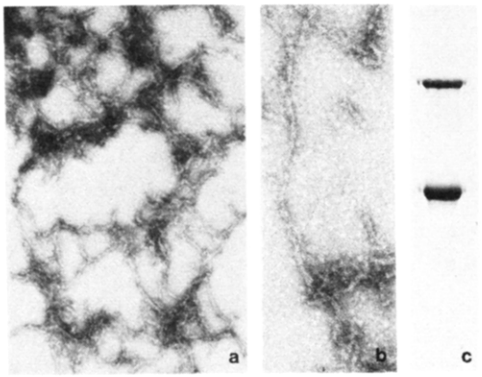

FIGURE 3: (a) Electron micrograph of the negatively stained reassembled P70 and P150 mixture obtained after DEAE-cellulose chromatography (37 500×). (b) 60 000×. (c) Gel electrophoretogram of the pelleted filaments, which shows that only the P70 and P150 polypeptides are reassembled into filaments. The apparent degradation products of intermediate molecular weight remain in the unassembled supernate.

eluted some of the nonneurofilament contaminants which had remained in the starting material. The fractions containing the neurofilament polypeptides were concentrated by vacuum dialysis, and the peak containing the 150- and 70-kilodalton proteins was reassembled as described above and examined by negative staining. Filaments are still abundant in this preparation (Figure 3a,b) and do not differ significantly in appearance from those obtained by reassembly of the whole neurofilament triplet, indicating that the two polypeptides are capable of reassembling into a neurofilament without the presence of the 200-kilodalton component. The gel electrophoretic profile of this material shown in Figure 3c clearly shows that these two proteins are the only proteins present in this supernate. When the gel electrophoretic pattern of the reassembled P150 and P70 proteins obtained after pelleting at 40 000 rpm for 60 min is compared with the material which was obtained from the DEAE column, it is apparent that some additional proteins with molecular weights between 70 000 and 150 000 which were present in the starting material (Figure 2b) are no longer present in the assembled material. These polypeptides are likely to be degradation products of the P150 protein, because a specific antibody against the P150 protein reacted with these polypeptides in immunoblotting experiments (J. S. Pachter & R. K. H. Liem, unpublished results). Yields of the P200 protein and the reassembled P70 + P150 proteins were 1 and 5 mg per 10 mg of neurofilament triplet protein, respectively.

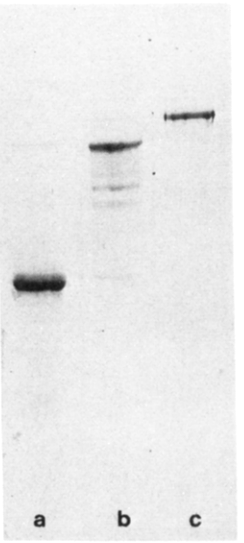

FIGURE 4: Gel electrophoretic profile of the separated neurofilament subunits. (a) Fractions eluted with 0.16 M phosphate buffer from the second hydroxylapatite column after they were pooled and concentrated. This material consists almost exclusively of the P70 neurofilament subunit. (b) Fractions eluted with 0.30 M phosphate buffer from the second hydroxylapatite column after they were pooled and concentrated. This material contains the P150 protein as well as several proteins of lower molecular weight, which appear to be degradation products of the P150 protein. (c) P200 protein obtained from the DEAE column.

HTP Chromatography. The reassembled P70 and P150 proteins obtained after DEAE-cellulose chromatography were dissolved in solubilization buffer and applied to a second hydroxylapatite column. The column was first eluted with 0.14 M phosphate buffer to remove any nonneurofilament contaminants, which may still be present in the preparation. When the column was eluted with 0.16 M phosphate, the P70 protein was eluted in a broad peak. With 0.18 M phosphate buffer, the remainder of the P70 protein was eluted as well as some of the P150 polypeptide. We found it necessary to elute with this buffer although it reduced the yield of the P150 protein, because it removed almost all of the remaining P70. The purified P150 protein was obtained from the elution with 0.3 M phosphate buffer. The proteins containing the purified P70 and P150 polypeptides were concentrated by vacuum dialysis, and their gel electrophoretic profiles are shown in Figure 4. The P70 protein was greater than 90% pure; however, the material obtained from the 0.3 M phosphate buffer elution still contained several contaminants in the molecular weight range of 70–150 kilodaltons in addition to the P150 protein, resembling those obtained in the supernate when the P150 and P70 proteins were reassembled after the DEAE column. These polypeptides were not present in any quantity in the material which was placed on the column (Figure 3c), which is a further indication that they are degradation products of the P150 polypeptide as discussed earlier. Yields of the P70 and P150 proteins were 2 and 0.5 mg per 5 mg of material placed on the column, respectively. The yield of the P70 protein could be increased significantly by eluting the initial HTP column with 0.16 M phosphate buffer before the 0.3 M phosphate buffer elution. This step would result

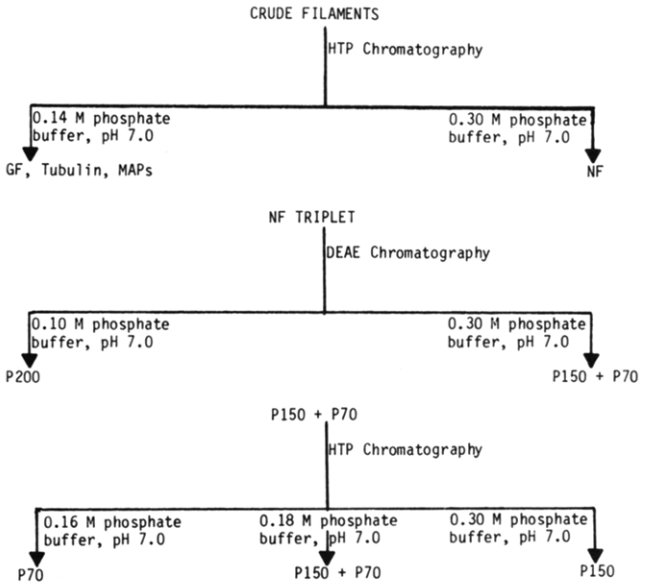

FIGURE 5: Flow charts of the chromatographic steps described in the text to obtain purified triplet polypeptides from crude intermediate filaments from brain.

in 5 mg of P70/10 mg of neurofilament triplet protein; however, the P150 and P200 proteins, which were obtained in the elution with 0.3 M phosphate buffer, were contaminated with degradation products, which were not easily removable by subsequent chromatography.

Figure 5 shows a flow chart of the chromatographic steps described to obtain the purified polypeptides from the crude brain intermediate filament preparation.

Reassembly Studies. Figure 6 shows the result of the reassembly studies by negative stain of each of the separated proteins. As can be seen (Figure 6a), the P70 polypeptide is capable of forming intermediate filaments in the absence of the other two. A mixture of long and short filaments is evident, similar to those obtained for the neurofilament triplet. No filaments can be seen by EM when the material prior to dialysis was examined. The diameter of the filaments was not obviously different from those formed by the whole triplet (Figure 1) or by the P70 and P150 subunits together (Figure 3). The P150 and P200 polypeptides are evidently not able to form filaments in the absence of P70 (Figure 6b,c). When the subunits are mixed, the P70 polypeptide appears to be a necessary requirement for the formation of the copolymers (Table I). When the P200 and P150 proteins are mixed, no filaments are formed. Filaments are only formed by EM criteria if the P70 polypeptide is present in the reassembly mixture.

When the gel electrophoretic profile of the polypeptides obtained after reassembling different mixtures of the three polypeptides and centrifugation at 100000*g* for 60 min is examined, we find a significant amount (up to 15%) of both the P150 and P200 polypeptides in the pellet even though no filaments were seen by electron microscopic examination. This result would indicate that these proteins do form nonfilamentous aggregates under assembly conditions. For the P150 protein, these aggregates were found to consist only of the P150 protein; the lower molecular weight components seen in Figure 4b again remained in the supernate.

Table I: Ability of Different Mixtures of Neurofilament Polypeptides To Form Intermediate Filaments As Judged by EM Using Negatively Stained Material

assembly mixture	assembled filaments
P70	+
P150	–
P200	–
P70 + P150	+
P70 + P200	+
P150 + P200	–
P70 + P150 + P200	+

Because of the high degree of self-aggregation of these proteins in assembly buffer, we were not able to do quantitative experiments on the assembly of these filaments, or to determine the stoichiometry of the subunits. However, we were able to determine that the presence of the P70 protein enhances the amount of the other two polypeptides found in the pellet. When P70 is in excess (Figure 7a,b), all the P150 and P200 protein was removed from the supernate and found in the pellet. When P150 and P200 were in excess, there remained a significant amount of these two polypeptides in the supernate (Figure 7c,d). In both cases, almost all of the P70 polypeptide was found in the pellet. Similar results were obtained when the P150 or P200 proteins were mixed separately with P70.

Discussion

The results presented in this paper support the interpretation that the mammalian neurofilament is composed of a core filament made up of the P70 protein with P200 and P150 polypeptides serving as accessory proteins. This interpretation is based on the fact that purified P70 can reassemble into intermediate filaments, but the P150 and P200 proteins cannot (Figure 5). Although these experiments do not exclude the possibility that the P150 and P200 polypeptides are able to polymerize into filaments under different conditions than those employed in this study, the two proteins do appear to be competent to reassemble, since they can be incorporated into filaments when the P70 polypeptide is present (Table I). The requirement of the P70 polypeptide under our experimental conditions for the coassembly of the other two proteins into filaments may indicate that the P150 and P200 proteins are more peripherally associated with the filament. These results are consistent with the report by Willard & Simon (1981), which showed by immunoelectron microscopic decoration

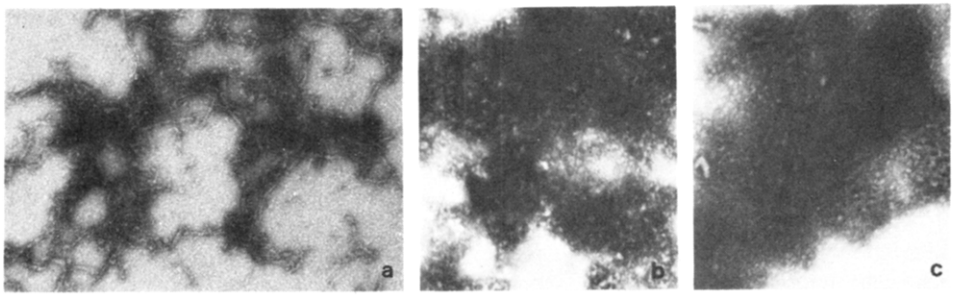

FIGURE 6: Electron micrographs of the negatively stained neurofilament subunits after they were placed under assembly conditions. (a) P70 (28 125×); (b) P150; (c) P200 (27 000×). Only P70 can form intermediate filaments in the absence of the other two subunits.

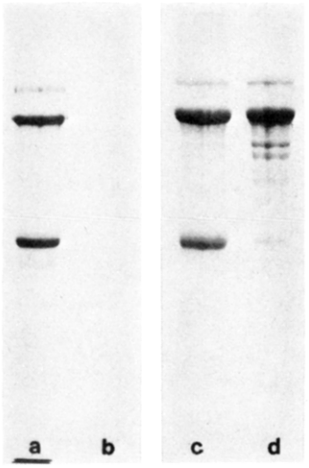

FIGURE 7: Gel electrophoretic profile of the pellets and supernates obtained when different amounts of neurofilament subunits are placed under assembly conditions. (a) Pellet and (b) supernate obtained when an excess of P70 is present; (c) pellet and (d) supernate obtained when P150 and P200 are present in excess.

studies that the neurofilament consists of a core of P70 protein with the P150 and P200 proteins winding around this central core.

The results of Steinert et al. (1976) on the assembly of the different keratin filament subunits have shown that a number of these keratin filament subunits can polymerize into filaments independently of the others. In contrast, our results indicate that for neurofilaments only a single subunit (P70) can form an intermediate filament; the other two subunits can associate with an intermediate filament only if the P70 polypeptide is present in the reassembly mixture. It is not clear whether they bind to the same site of the P70 protein; however, either protein appears to coassemble with P70 (Figure 7).

In a recent communication, Moon et al. (1981) also reported that the P150 and P70 subunits assembled together into intermediate filaments, whereas the P200 subunits appeared as globular structures. They also reported that the presence of P200 subunits is required for the formation of longer filaments. We found that a mixture of longer and shorter filaments was present in all the material in which the P70 protein was present. This inconsistency may simply be due to the difference in isolation and reassembly conditions and does not alter the conclusion that the P70 polypeptide forms the neurofilament core or backbone structure.

The purification of the neurofilament subunits in the absence of detergents has been relatively unsuccessful. Willard et al. (1980) have been able to purify a P200 protein (H) from rabbit spinal cord by extraction with low ionic strength solutions followed by gel filtration on a Sepharose 6B column in the presence of 1% $NaDodSO_4$. This protein was extracted by the low ionic strength solution, although significant amounts of the P70 and P150 proteins were still found in the extract. These polypeptides were apparently only removed by gel filtration on Sepharose 6B containing 1% $NaDodSO_4$. In concurrence with this report, results in our laboratory (R. K. H. Liem, unpublished experiments) have shown that the neurofilament triplet does not separate even in the presence of 8 M urea on a number of gel exclusion columns, such as Sepharose 4B or AcA 34, unless 1% $NaDodSO_4$ is added to the elution buffer.

Recently, Moon et al. (1981) have reported the separation of the P200 subunit by CM-cellulose chromatography. We have also separated this polypeptide from the other two by ion-exchange chromatography (on DEAE-cellulose), indicating that this polypeptide has a sufficiently different isoelectric point from the other two to be separated in this manner. The P70 and P150 polypeptides were much harder to separate and required fairly sharp gradients on hydroxylapatite. The present report is therefore the first indication that the two polypeptides are separable without the addition of $NaDodSO_4$.

The suggestion that the P150 protein is an accessory protein of the neurofilament is consistent with the proposal that neurofilament–microtubule interactions may be mediated through this protein (Leterrier et al., 1981). Interaction studies showed that neurofilament proteins were able to bind to microtubule accessory proteins as well as to phosphocellulose-purified tubulin (Shelanski et al., 1981). Runge et al. (1981b) have shown ATP-induced formation of a complex between neurofilament and phosphocellulose (PC)–tubulin, and Leterrier et al. (1981) have shown the cAMP-dependent phosphorylation of the P150 component of the neurofilament by the MAP_2-stimulated protein kinase (Vallee, 1980) of the microtubule proteins. These interactions would be more easily mediated by the P150 protein if this protein is not part of the core of the filament but represents instead a structure peripheral to this core as suggested by Willard & Simon (1981). The role of the P200 component as an accessory protein has been postulated by these authors to be in the formation of cross bridges between neurofilaments.

The stoichiometry of the three polypeptides associated with the neurofilament has varied from preparation to preparation in different laboratories (Shecket & Lasek, 1980; Chiu et al., 1980; Moon et al., 1981). Our results indicate that this variation may be due to the amounts of P150 and P200 removed from the filaments during the isolation procedure, by either extractions or shearing during the homogenization, because these polypeptides are peripherally located on the filaments. The copolymerization studies presented in this paper show that the amount of coassembly is critically dependent on the presence of P70. The P150 and P200 polypeptides in the mixtures will coassemble until all the P70 is used up (Figure 7).

Five classes of intermediate filaments can be distinguished by biochemical and immunological criteria. Intermediate filaments from glial cells (Eng et al., 1971; Yen et al., 1976), mesenchymal derived cells (Starger & Goldman, 1977; Franke et al., 1978), and smooth muscle (Cooke, 1976; Lazarides & Hubbard, 1976; Small & Sobieszek, 1977) consist primarily of a single subunit, with a molecular weight of around 55 000. Epithelial cell intermediate filaments have several subunits with M_r's between 50 000 and 60 000 (Steinert et al., 1976; Sun & Green, 1978; Yen et al., 1980), which can polymerize into intermediate filaments requiring at least two different subunits (Steinert et al., 1976). In addition, Steinert et al. (1978) have shown that intermediate filaments from BHK21 cells (vimentin or decamin) and epidermal keratin filaments have similar structures on the basis of X-ray diffraction data. Similarities in amino acid composition have also been reported for the intermediate filaments from different cell types (Starger et al., 1978). These four classes of intermediate filaments appear therefore to be closely related in their subunit structure. The fifth class of intermediate filaments, the neurofilament, seemed to be somewhat of an anomaly. It appeared to consist of three subunits of widely divergent molecular weights, which were tightly associated in a filament (Liem et al., 1978; Schlaepfer & Freeman, 1978).

The results presented in this paper suggest that the core of the mammalian neurofilament is composed of a single polypeptide with a molecular weight which is higher than that, but

not widely different from that, of the other four classes of filaments. The other two polypeptides appear to be tightly associated proteins which are peripherally located. Hence, the neurofilament does not represent an anomalous intermediate filament. Zackroff & Goldman (1980) have shown that squid brain filaments also consist primarily of a single subunit with a molecular weight of 60 000 with an apparent associated protein with a molecular weight of 200 000. This latter protein is a more major component in the squid giant axon.

We have presented in this paper a method for the purification of the subunits of the neurofilament and by reassembly studies have shown that the P70 polypeptide by itself is capable of forming an intermediate filament, whereas the other two are not. The precise geometry of the subunits within the neurofilament, the ability of the two accessory proteins to stimulate assembly of the filament, the structure of these proteins in solutions, and the physiological importance of these structures remain to be worked out. The purification method presented in this paper shows that we are able to obtain assembly-competent neurofilament protein subunits in biochemical quantities, which would enable us to do these studies.

Added in Proof

Since submission of the manuscript, Geisler & Weber (1981) have also reported the separation of the components of the neurofilament proteins and the self-assembly of the 68 000-dalton subunit.

Acknowledgments

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