

# Antibody Decoration of Neurofilaments

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**ABSTRACT** We have decorated neurofilaments with antibodies against three polypeptides (designated here as H [mol wt = 195,000], 45 [mol wt = 145,000], and 46 [mol wt = 73,000]) in an effort to understand the arrangement of these polypeptides within neurofilaments. The three polypeptides were purified and antibodies were raised against each. The cross-reactivity of the antibodies suggested that each polypeptide contains both shared and unique antigenic determinants. The differential reactivities of each antibody preparation were enhanced by adsorption with the two heterologous polypeptides, and the resulting preparations were used to decorate purified neurofilaments, which were then negatively stained and examined in an electron microscope. The appearance of the antibody-decorated structures led to the following conclusions: All three polypeptides are physically associated with the same neurofilament. However, the disposition of H and 46 within a filament is different; 46 antigens appear to be associated with a "central core" of the filament, whereas H antigens compose a structure more loosely and peripherally attached to the central core and periodically arranged along its axis. The antibody-decorated H-containing structure assumes variable configurations; in some cases it appears as a bridge connecting two filaments; in other cases it appears as a helix wrapping the central core with a period of  $\sim 1,000$  Å and an apparent unit length of  $\sim 1.5$  periods.

These configurations suggest several functional implications, including the possibility that H is a component of the cross-bridges observed between filaments *in situ*. We also note that the central core-helix relationship could be used in the design of an intracellular transport motor.

Filaments with diameters of  $\sim 100$  Å (intermediate filaments) and lengths of many microns are ubiquitous organelles in the axoplasm of neurons, where they run parallel to the long axis of the axon. The function of these neurofilaments has not been established; however, it has been suggested that they serve a structural role in the axon, or that they provide a track along which materials move down the axon in the process of axonal transport (19). Three polypeptides (with molecular weights of 195,000, 145,000, and 73,000, designated here as H, 45, and 46, respectively), which are themselves transported down the axons at a slow velocity (1 mm/d in rabbit optic neurons [25]), appear to be the major components of neurofilaments (5, 8, 14, 17, 19, 20, 26), although the subunit composition has been a subject of considerable controversy. Nothing is known about the disposition of these presumptive neurofilament polypeptides within the neurofilament structure.

In the following report we have investigated the organization of the three components within the filament by decorating neurofilaments with antibodies against each of the polypeptide components, and observing the negatively stained structures by electron microscopy. The results provide additional support for

the view that all three polypeptides (which appear to be antigenically related) are physically associated with each neurofilament. In addition, the appearance of the decorated neurofilaments raises certain possibilities concerning their structure and function.

## MATERIALS AND METHODS

### *Purification of H, 45, and 46, and Preparation of Antisera*

The following operations were performed at 4°C. Approximately 5 g of rabbit spinal cord (fresh or frozen) was homogenized in 30 ml of buffer A (5 mM EDTA, 2 mM dithiothreitol [DTT], 1 mM phenylmethylsulfonyl fluoride [PMSF], 10 mM Tris, pH 8.0) and then diluted to 360 ml in the same buffer. This homogenate was subjected to sonic oscillation (44 W) for 5 min, and was then stirred for 1 h, and centrifuged for 1 h at 100,000 g. H, 45, and 46 were precipitated from the supernate by the addition of NaCl to a concentration of 0.3 M, followed by stirring for 25 min and centrifuging for 1 h at 100,000 g. The pellets were resuspended (at room temperature) by homogenization in a total volume of 4 ml of buffer A containing 10 mM DTT, 3% SDS without PMSF. The suspensions were heated for 5 min at 90°C and centrifuged for 45 min at 100,000 g (20°C). Glycerol was added to the supernate to a concentration of 10% and the supernate was fractionated on a column (2.5 × 120 cm) of Sepharose-6B

equilibrated with buffer A containing 1.5 mM DTT and 1% SDS. The polypeptide compositions of the eluant fractions were determined by analytical SDS gel electrophoresis, and those containing H and 45, and those containing 46 were combined separately. Both samples were dialyzed against a solution of EDTA (5 mM, pH 8) and DTT (0.1 mM) to remove SDS, concentrated by precipitation with trichloroacetic acid (7.5%), and subjected to preparative SDS polyacrylamide gel electrophoresis (PAGE) as described previously (26), except that a gel containing 8% acrylamide (0.24% bis acrylamide) was employed for the fractionation of the 46-containing sample. The gel fractions most enriched in H, 45, and 46 (as judged by analytical SDS PAGE) were combined separately, dialyzed against a solution of EDTA (5 mM) and DTT (0.1 mM), pH 8, and lyophilized. The yield from such a preparation was 100–500  $\mu$ g of each protein.

For immunization, the lyophilized antigens (~100  $\mu$ g) were resuspended in 1.5 ml of 0.1% SDS, emulsified with an equal volume of complete Freund's adjuvant, and injected at ~15 sites on the backs of goats. Over a period of 6 mo, the goats were inoculated two more times with similar amounts of antigen emulsified with incomplete Freund's adjuvant; they were bled 10 d after the third inoculation, and 10 d after additional sporadic inoculation over a period of 2 yr.

### Partial Purification of IgG

Antiserum was diluted with 3 volumes of H<sub>2</sub>O and IgG was precipitated by the addition of saturated (4°C) ammonium sulfate to a final concentration of 45% of saturation at 4°C. The resulting precipitate was resuspended and equilibrated by dialysis with PBS (0.15 M NaCl, 1 mM Na<sub>2</sub>HPO<sub>4</sub>, pH 7.0).

### Adsorption of Antibodies by Affinity Chromatography

Affinity columns were prepared by resuspending the lyophilized antigens (~100  $\mu$ g, prepared as described above) in 0.65 ml of a solution of 0.2 M NaHCO<sub>3</sub>, 0.2% SDS, pH 9.5, and coupling them to 4 ml of wet packed Sepharose-4B by the method of Parikh et al. (12). The Sepharose was activated with 0.8 g of cyanogen bromide.

Serum (or partially purified IgG fractions) was applied to an affinity column (~4 ml of Sepharose) twice, and the column was washed with 20 ml of PBS. The bound antibody was eluted with a solution of 0.15 M NaCl, 0.2 M glycine, pH 2.4; fractions (1 ml) were collected and neutralized by the addition of 0.11 ml of 2 M Tris, pH 8.5, and the fractions containing protein (as judged by the optical density at 280 nm) were combined and dialyzed against PBS. The columns were prepared for further protein applications by washing them with 3 ml of a solution containing 4 M guanidine-HCl, 20% glycerol, 0.5 M sodium acetate, pH 6.5, followed by 25 ml of PBS.

In the case of the adsorption of the IgG fractions, the IgG from 10 ml of antiserum was applied to a column containing an heterologous antigen. When the column had been subjected to the cycle described above, the material that did not bind to the column (the flow through) was reapplied to the same column, and the cycle was repeated. The flow-through was then subjected to two cycles of adsorption on the other heterologous column, and finally on the homologous column; the glycine-eluate of the homologous column served as the source of IgG enriched for the specific antibodies. The glycine wash of the second cycle of each column contained between 3- and 10-fold less protein than the glycine wash of the first cycle, indicating that the columns were not saturated with antibodies on the second passage of the IgG. (The capacity of the columns was not appreciably reduced by each cycle.) The glycine wash of the first cycle for each column always contained more protein than the glycine wash of the second cycle of the previous column, consistent with the interpretation that the different columns were binding different populations of antibodies. The adsorption of unfractionated serum was performed in the same way, except that 2 ml of serum was subjected to one cycle of adsorption on each column; the amount of protein in the glycine eluates was always substantially less than the capacity of the column.

### Micro ELISA Analysis of the Adsorbed Antibodies

The relative reactivities of the three antibodies towards H, 45, and 46 were estimated by means of an enzyme-linked immunosorbent assay (ELISA). The wells of polystyrene microtiter plates (Microbiological Associates, Walkersville, Md.) were coated with antigen by incubating them with H, 45, or 46 (0.5  $\mu$ g in 0.1 ml) purified as described. The antigen-coated wells were incubated with various dilutions of anti-H, anti-45, or anti-46, in a volume of 0.1 ml. The amount of antibody retained by each well was determined by incubating each well with

0.5  $\mu$ g (in 0.1 ml) of an affinity-purified rabbit anti-goat-IgG antibody (obtained from Gateway Immunologicals, St. Louis, Mo.) that had been conjugated to alkaline phosphatase (Sigma Chemical Co., St. Louis, Mo., type VII) by the procedure of Voller et al. (22). The alkaline phosphatase retained by the wells was determined by incubating the wells with 0.2 ml of *p*-nitrophenyl phosphate (Sigma Chemical Co., 1 mg/ml) and, after stopping the reaction with 0.05 ml of 3 M NaOH, determining the optical density at 400 nm. All incubations were for 2 h at room temperature, and the buffers and washing procedure were those described by Voller et al. (22), except that Tris (0.2 M, pH 8.5) replaced diethanolamine in the buffer for the phosphatase assay, which was carried out for 30 min at room temperature.

### Purification of Filaments

The filaments used in this report were prepared by one of two procedures. In procedure I, ~1 g of fresh spinal cord from a rabbit was soaked in a 100-fold excess of a solution containing EDTA (2.5 mM), EGTA (2.5 mM), and Na<sub>2</sub>HPO<sub>4</sub> (1 mM), pH 7, for 2 h at room temperature as described by Schlaepfer (17). The swollen tissue was removed from the solution and homogenized with a loose-fitting pestle of a Dounce homogenizer (Kontes Co., Vineland, N. J.). NaCl (1 M) was added to the disrupted tissue to a final concentration of 0.15 M, and the homogenate was centrifuged at 12,000 rpm for 30 min in the SS-34 rotor of a Sorvall RC2B centrifuge (DuPont Instruments-Sorvall, DuPont Co., Newtown, Conn.) (17). The supernate was applied to a discontinuous sucrose gradient containing layers (3.3 ml each) of 2, 1.5, and 1.0 M sucrose in solution B, containing NaCl (0.15 M), EDTA (2.5 mM), EGTA (2.5 mM), and Na<sub>2</sub>HPO<sub>4</sub> (1 mM). The gradient was centrifuged for 3 h at 35,000 rpm in an SW 40.1 rotor (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.). The material at the 1- to 1.5-M interface served as the source of filaments.

In procedure II, 0.6 g of freshly dissected spinal cord was homogenized in solution B containing 1% Triton X-100, and the homogenate was applied directly to the sucrose gradient. The two procedures resulted in similar filament preparations as judged by the filament morphology and the polypeptide composition.

### Anti-H Fab Fragment Preparation

Fab fragments were prepared by digesting anti-H IgG with mercuripain, and fractionating the resulting Fab fragments by methods similar to those previously described (13, 28). The resulting Fab fraction was adsorbed with 46 and 45 by affinity chromatography as described above, and finally purified by affinity chromatography on H-Sepharose. The purified material had an electrophoretic mobility corresponding to a molecular weight of ~25,000, as would be expected for reduced Fab fragments (9).

### Decoration of Filaments with Antibodies

Filaments were adsorbed to Formvar-coated copper electron microscope grids by floating the grids in 25- $\mu$ l drops of the filaments (diluted to an appropriate concentration in PBS) on sheets of dental wax for 10 min at room temperature. The grids were washed by flotation on one drop of PBS, then floated on drops (25  $\mu$ l) of a solution containing the indicated concentrations of antibodies or nonimmune IgG, for 30 min at 0°C. The grids were then washed in nine drops of PBS, one drop of H<sub>2</sub>O, at 0°C, and negatively stained with an aqueous solution of uranyl acetate (1%). In the case of formalin-fixed filaments, the grids with adsorbed filaments were floated for 1 h at room temperature on 3 ml of formaldehyde (a 5% solution in PBS), and then for 10 min at 0°C in PBS before they were exposed to the antibody. In the case of the glutaraldehyde-fixed filaments, the grids with adsorbed filaments were floated in drops (25  $\mu$ l) of glutaraldehyde (1.5% in PBS) for 10 min at room temperature, and then in 30 ml of PBS (two changes) for 1 h at room temperature, before they were incubated with the antibody as described above.

## RESULTS

### Cross-reactivity of Anti-H, -45, and -46

Fig. 1 shows an example of H, 45, and 46 preparations, similar to those used to immunize goats and to prepare antigen-Sepharose affinity columns. Each preparation contains little if any of the other two polypeptides, yet a substantial fraction of the antibodies against each polypeptide bound to affinity columns containing either of the other two, as shown in Table I. This observation suggests that each of the three polypeptides shares antigenic determinants with the other two. However,

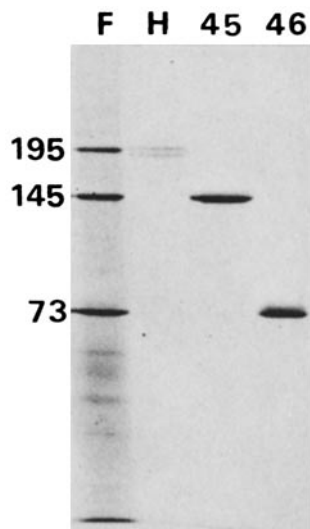


FIGURE 1 Purified protein preparations electrophoresed on SDS polyacrylamide gels. F indicates a neurofilament fraction prepared by method I (Materials and Methods). H, 45, 46 indicate the purified fractions of these proteins, similar to those used as antigens and affinity reagents. For a more heavily loaded example of H (which in this figure is from a rabbit heterozygous for two different forms of H [27]), see reference 26. The numbers indicate molecular weights in kilodaltons.

TABLE I  
Specific and Cross-reacting Fractions of Antibody

Antiserum	Cross-reacting fraction*			Self-specific† fraction
	H	45	46	
Anti-H		0.4	0.2§	0.5, 0.6§
Anti-45	0.5§		0.4	0.4, 0.3§
Anti-46	0.6	0.6, 0.5§		0.2, 0.2, 0.3§

\* The protein (estimated by OD<sub>280</sub>) that specifically bound and was eluted (by 0.2 M glycine) when the designated antiserum or IgG fraction was applied first to the affinity column containing the indicated antigen. The results are expressed as the fraction of the total protein eluted (in the glycine wash) from all three columns when the antiserum was passed through them in sequence.

† The total protein bound to and eluted from (0.2 M glycine wash) the affinity column containing the homologous antigen, when the indicated serum or IgG fraction had been previously passed sequentially through the two heterologous columns. The results are expressed as the fraction of the total protein eluted in the glycine wash of all three columns.

§ In these experiments, a partially purified IgG fraction from 10 ml of serum was subjected to two cycles of affinity chromatography on each column as described in Materials and Methods. In the remainder of the experiments, 2 ml of unfractionated antisera was subjected to one cycle on each column.

each appears in addition to possess antigenic determinants that are unique (or much more abundant or accessible than in the other two) because a fraction of each antibody preparation bound to its homologous affinity column after it had been passed sequentially through the two heterologous columns.

The passage through the three affinity columns should substantially enrich each preparation with respect to antibodies that preferentially bind to the homologous polypeptide. We tested the ability of each sequentially adsorbed preparation (prepared from whole serum as described in Materials and Methods) to bind an excess of H, 45, and 46, which were themselves adsorbed to the wells of polystyrene microtiter plates; the relative amounts of bound antibody were determined by the extent of retention of a rabbit anti-goat antibody conjugated to alkaline phosphatase, whose reaction product

served as an indicator. The assay was linear with respect to antibody concentration. Fig. 2 shows that adsorbed anti-H reacted weakly with 45 and only slightly with 46. Anti-45 showed the lowest degree of specificity; it reacted weakly with both H and 46. Anti-46 strongly favored 46. Table II shows estimates of the relative fraction of each antibody preparation that was reactive toward each polypeptide under the conditions of the ELISA assay. These differentially reactive preparations were used to decorate neurofilaments.

### Decoration of Filaments with Anti-H, -45, or -46 Antibodies

We incubated filaments on Formvar-coated electron microscope grids with different concentrations of each adsorbed antibody preparation (Fig. 3). It is apparent from the increased diameters and densities of the filamentous structures (as compared to nonimmune IgG controls) in Fig. 3 that all three antibody preparations reacted with the filaments. Unreactive filaments were not observed at antibody concentrations in excess of 5  $\mu\text{g}/\text{ml}$ , supporting the idea that all three polypeptides are components of the same filament.

The filaments decorated with anti-H had the following forms: at the highest concentrations (20  $\mu\text{g}/\text{ml}$ ) of adsorbed

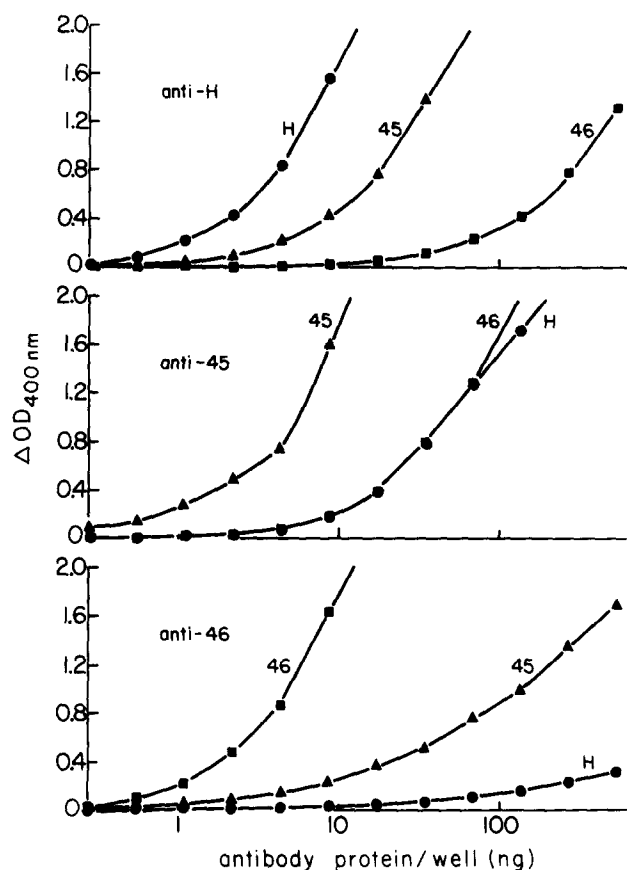


FIGURE 2 Micro-ELISA assay of the adsorbed antisera, performed as described in Materials and Methods. The indicated concentrations of anti-H, anti-45, and anti-46 (prepared by sequential adsorption of unfractionated serum as described in Materials and Methods) were incubated with constant amounts (0.5  $\mu\text{g}$ ) of H, 45, and 46 bound to microtiter wells. The amount of antibody retained was determined by incubating the wells with a secondary (rabbit anti-goat) antibody conjugated to alkaline phosphatase, and assaying the retained alkaline phosphatase.

anti-H, the decorated structures appeared as somewhat irregular solid cylinders, with diameters (often  $\sim 500$  Å) that were larger than might be anticipated for a 100-Å filament coated on either side with a layer ( $\sim 100$  Å [21]) of IgG. At lower

TABLE II  
Relative ELISA Reactivity of Adsorbed Antisera toward Antigens \*

Antibody	Antigen		
	H	45	46
H	1.0	0.2	0.01
45	0.1	1.0	0.1
46	<0.009	0.03	1.0

\* The concentration of each antibody required to give an OD<sub>400</sub> of 1.0 in its ELISA reaction with each antigen (Fig. 2) was divided by the concentration required to give an OD<sub>400</sub> of 1.0 with the homologous antigen.

antibody concentrations, the decoration had a distinct periodicity that could be best appreciated at low magnification (Fig. 4). At higher magnification (Fig. 5), the reactive antigens often appeared to compose a structure that is helically wound around an unreactive central core. The period of the helix ranged between 900 and 1,300 Å, with a mean of 1,080 Å and a standard deviation of 159 Å. The helix was often discontinuous, each segment extending for a distance between 1 and 1.5 complete turns, as in Fig. 5 *b*. In some instances, the reactive antigens appeared to be concentrated at the ends rather than dispersed uniformly along the helical structure, as in Fig. 3 *a* (2.5 μg). Infrequently, we observed two thin parallel strands running through the anti-H decorated helix, suggestive of a double helix (e.g., Fig. 5 *c*, right of center). Not all of the anti-H-decorated elements were helical, but most appeared to be peripheral to a central core; Fig. 5 *b* shows the range of appearance of the anti-H-decorated structures on a single filament. In a few instances (Fig. 6), we observed two filaments,

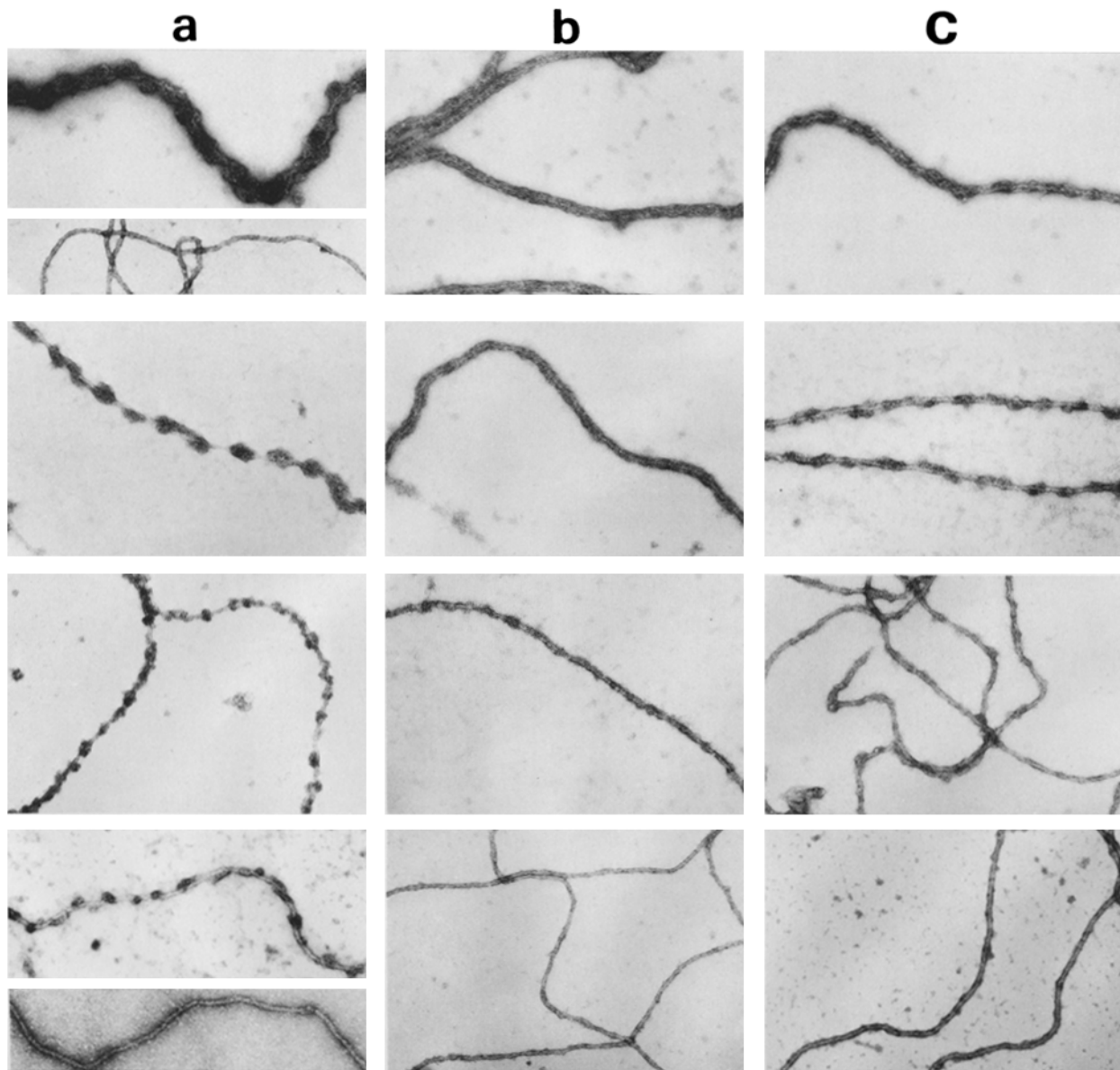


FIGURE 3 Neurofilaments decorated with adsorbed anti-H (a), anti-46 (b), anti-45 (c), or IgG from an unimmunized goat (insets to a-1 and a-4). Antibody concentrations, from top to bottom, were 20, 10, 5, and 2.5 μg/ml. a-4 was fixed with formalin; a-3, b-1, c-1, c-2, c-3, a-4-inset, were fixed with glutaraldehyde, and the remainder were unfixed.  $\times 59,000$ .

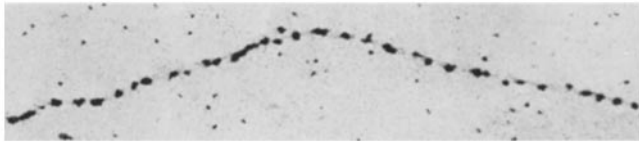


FIGURE 4 A filament (unfixed) decorated with adsorbed anti-H IgG (2.2  $\mu\text{g}/\text{ml}$ ).  $\times 24,000$ .

as far as 700 Å apart, bridged by material that was reactive with anti-H.

Fixation of the filaments with formaldehyde or glutaraldehyde before decorating them produced no systematic difference in their appearance. When filaments were decorated with adsorbed monovalent Fab fragments of anti-H IgG, helical elements were still observed (Fig. 5 *h*), indicating that the helix is not produced by the multivalency of the antibody.

Anti-46-decorated filaments were different from those decorated with anti-H; their diameters ( $\sim 300$  Å) were smaller at high antibody concentrations (20  $\mu\text{g}/\text{ml}$ ), and at lower concentrations the decoration was more uniform (Fig. 3 *b*). We did not observe undecorated segments of the filament. In some instances, there was a hint of periodicity (e.g., Fig. 3 *b*, 5  $\mu\text{g}$ ) but of much smaller amplitude than with anti-H.

Filaments decorated with adsorbed anti-45 usually appeared less dense and, at the highest concentrations of antibody, tended to have smaller diameters (300–400 Å) than those decorated with anti-H (Fig. 3 *c*). At lower antibody concentrations, the anti-45 decoration also appeared periodic and in some cases helical (Fig. 3 *c*).

## DISCUSSION

The electron microscope observation of organelles decorated with specific antibodies has been a useful approach for determining the disposition of different proteins within complex organelles, such as phage (29) and ribosomes (7). Here we have examined the possibility (initially suggested by Schlaepfer's report that the interaction between antibodies and intermediate filaments could be readily detected by electron microscopy [15]) that such an approach could be used to analyze the molecular architecture of neurofilaments. The following considerations are relevant to the interpretation of the results. First, we assume that our filament preparation contains predominantly filaments of neuronal origin because the anti-H antibody, which reacts with the vast majority of the filaments in the preparation, appears to react specifically with neurons when it is used to immunofluorescently stain sections of nervous tissue (manuscript in preparation). Consistent with this assumption, polypeptides of 50,000–60,000 mol wt (the proposed molecular weight of the subunits of intermediate filaments from several non-neuronal sources including glia) were not major components of our filament preparation (Fig. 1). Second, the assumption that the three antibody preparations are differentially reactive towards H, 45, and 46 is based both upon the method of their preparation (i.e., the elution profiles of the affinity columns indicated that they were effective in adsorbing cross-reacting antibodies) and upon their differential reactivities with H, 45, and 46 in the ELISA assay. The distinctive decoration patterns produced by the preparations provide an additional argument for their differential reactivity. Third, the disposition of the antibody in a decorated filament will accurately reflect the disposition of a polypeptide only if the reactive antigenic determinants are uniformly distributed

in the polypeptide and are uniformly accessible to the antibody. Finally, in spite of our efforts (fixation and the use of Fab fragments) to reduce its likelihood, the possibility that aspects of the appearance of antibody-decorated structures are generated by the antibody-antigen interaction cannot be excluded; nor can we rule out the possibility that some of the features were generated during the disruption of the axoplasm and the preparation of the filaments. These considerations should be kept in mind when evaluating the following interpretations.

## Polypeptide Composition of Neurofilaments

Hoffman and Lasek (5) originally proposed that three polypeptides similar in size to H, 45, and 46 were components of neurofilaments, and considerable evidence has supported this view (e.g., references 8, 14, 17, and 26). The results reported here, that antibodies with differential specificities for H, 45, and 46 can, with similar concentration dependencies, decorate filaments, provide an additional argument that all three polypeptides are physically associated with neurofilaments. Furthermore, the observation that most of the filaments were decorated by each antibody indicates that each filament contains all three polypeptides.

The three neurofilament polypeptides appear to be structurally related, as indicated by the cross-reactivity of their antibodies (8, 26, 30). Assuming that common antigenic determinants are equally accessible in the three Sepharose-coupled polypeptides, and that the polypeptides have indeed been separated as indicated (Fig. 1), the elution profiles of the affinity columns suggest that each polypeptide has both unique and shared antigens and, further, that the domain shared by any two polypeptides overlaps with, but is not identical to, the domain shared by either of these with the third polypeptide. Direct evidence that the three polypeptides are encoded by different genes has recently been reported (3); the antigenic relationship of the three polypeptides suggests that their genes evolved from a common ancestral gene.

## Filament Structure

The difference in morphology between H- and 46-decorated filaments shows that the two polypeptides are not equivalently disposed. Most probably, H is located more peripherally and is more loosely attached along its length than 46. Such a configuration is consistent with: (a) the large diameters of some anti-H decorated filaments; (b) the peripheral appearance of anti-H decoration in some filaments; and (c) the occasional observation of anti-H-decorated material spanning the distance (often several filament diameters) between two filaments. Furthermore, the presence of bare, undecorated segments between the anti-H-decorated segments of the filament suggests that H may not be uniformly required for the filamentous morphology. These considerations lead us to hypothesize that neurofilaments comprise a central core, composed of at least in part of 46, and a more peripheral structure, composed in part of H.

The most regular configuration displayed by antibody-decorated H *in vitro* is a filamentous helix wound around the unreactive central core. The H helix has a period of  $\sim 1,000$  Å, and in many cases appears to be divided into segments, each making  $\sim 1.5$  complete turns about the central core and covering a distance of  $\sim 1,400$  Å along the filament. The length of one segment of the H helix itself (as measured from Fig. 5 *b*) is  $\sim 2,000$  Å; it is interesting to note that this distance could be spanned by a single H polypeptide in an  $\alpha$  helical configuration.

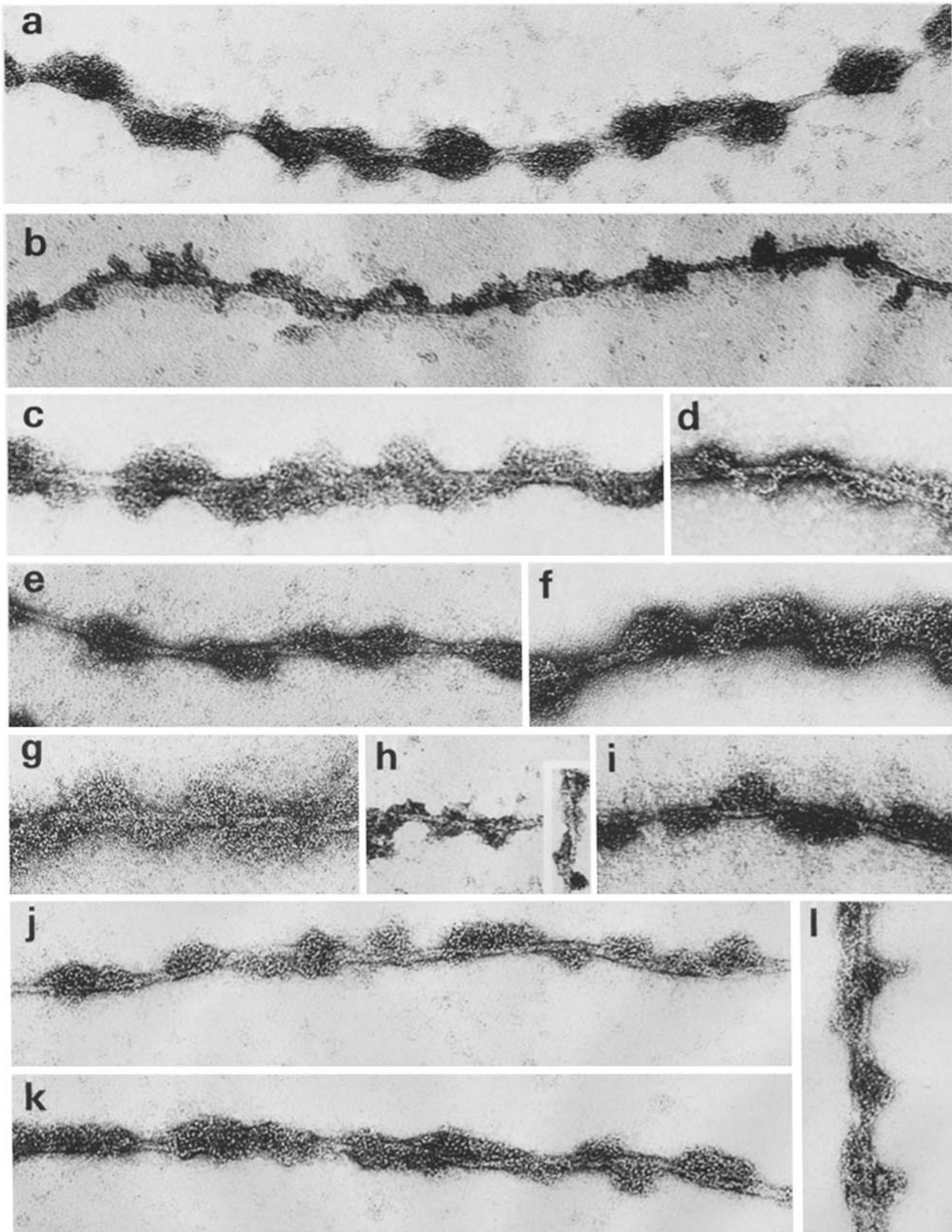


FIGURE 5 Filaments decorated with anti-H. (a) 10  $\mu\text{g}/\text{ml}$  adsorbed serum; (b) 2.5  $\mu\text{g}/\text{ml}$  adsorbed IgG; (c and d) 20  $\mu\text{g}/\text{ml}$  whole (unadsorbed, but affinity purified) IgG; (e) 10  $\mu\text{g}/\text{ml}$  adsorbed serum; (f) 20  $\mu\text{g}/\text{ml}$  adsorbed serum; (g) 20  $\mu\text{g}/\text{ml}$  whole IgG; (h) 13  $\mu\text{g}/\text{ml}$  adsorbed Fab fragments; (i) 5  $\mu\text{g}/\text{ml}$  adsorbed IgG; (j and k) 10  $\mu\text{g}/\text{ml}$  adsorbed IgG; (l) 20  $\mu\text{g}/\text{ml}$  whole IgG. Unfixed filaments were used except that in d and h the filaments were fixed with formalin.  $\times 200,000$ .

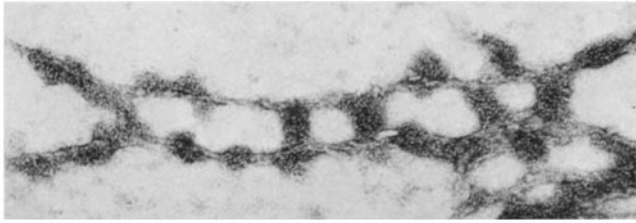


FIGURE 6 Filaments cross-linked by anti-H- (10 µg/ml adsorbed serum) decorated material.  $\times 115,000$ .

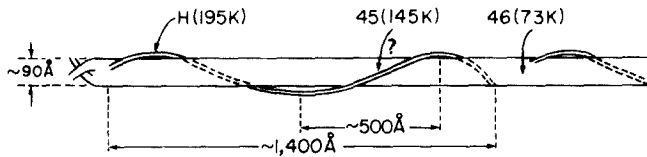


FIGURE 7 A schematic summary of certain aspects of the structure of neurofilaments suggested by the antibody decoration. Filaments comprise a 46-containing central core; H, and possibly 45, compose a structure (one of whose *in vitro* configurations is the illustrated helix) loosely and peripherally attached to the central core.

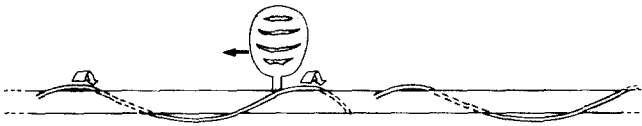


FIGURE 8 A schematic diagram illustrating how any filamentous structure comprising a central core wrapped by a helix could function as a rotary motor for intracellular transport. The helix rotates around the central core (arrows), powered by a central core-dependent hydrolase associated with the helix. The transported material (represented by the vesicle) associated with the groove of the helix is propelled down the filament by a worm-screw mechanism.

The decorated H helix appears to be displaced from the surface of the filament by  $\sim 100$  Å over most of its length; because we have never observed such a structure in an undecorated filament, it is either cryptic to negative staining or, more likely, it is pulled away from the surface of the filament by the reaction with the antibodies. Fig. 8 schematically summarizes the helix. The anti-H-decorated structure assumes other, less defined configurations, as illustrated in Fig. 5. The helix and these other configurations could reflect multiple functional configurations of H (i.e., it is a protean protein) or represent different states of collapse of its native configuration (e.g., the helix). Alternatively, H could project away from the surface of the filament *in situ* and collapse onto the surface of the central core only when the axoplasm is disrupted. *In situ* decoration of filaments might resolve these alternatives.

Because the anti-45 antibody is less specific than the others and appears to require somewhat greater concentration to decorate filaments (Fig. 3), the interpretation of the anti-45 decorated filaments is the least clear. With this reservation, the appearance of some anti-45-decorated filaments suggests that 45 may also be a component of a helical structure.

Helical relationships in neurofilament structure have been previously indicated from X-ray diffraction patterns of annelidan axoplasm (4) and from electron microscope observation of mammalian (10, 16), moluscan, and annelidan filaments (6). It has been proposed that neurofilaments comprise two protofilaments twisted helically together. On the other hand, our observations indicate that the anti-H-decorated structure is not

one of two equivalent protofilamentous partners; it is more peripheral to the axis of the filament than 46-containing structures and, in its helical configuration, appears to "wrap" the central core. Although this interpretation is different from previous proposals, it is not incompatible with them, as the central core may itself include helically intertwined protofilaments. Consistent with this possibility, we have occasionally observed (when viewing photographs such as Fig. 5 *a* at 10-fold magnification) indications of two, and sometimes three, protofilaments ( $\sim 20$  Å in diameter) running through the central core of decorated filaments. They sometimes appear to run in parallel for short distances, sometimes cross each other (as would be expected of helically twisted protofilaments), and sometimes form more complex patterns.

### Implications for Function

The interpretation that neurofilaments comprise a central core and a peripheral structure containing H places H in a position to mediate interaction between the filament and its environment. In particular, the H structure could be the cross-bridge that links filaments with other filaments and other organelles *in situ*; we have observed anti-H-decorated interfilamentous linkages spanning distances comparable to those observed *in situ* (although these *in vitro* linkages may well be mediated by the divalent antibody). As a cross-bridge, the H structure could function to maintain order and spacing among intracellular elements, or conceivably mediate mechanochemical coupling of organelles with filaments. If instead, the physiological configuration of the H structure is the helix wrapping a central core, the structure could serve to regulate interactions between the central core and other elements, such as tropomyosin (which is also helically related to the central actin filament) regulates the interaction between actin and myosin.

Finally, it is interesting to note that the principle of a central core wrapped by a helix could serve as the structural basis for a hypothetical mechanism of energy transduction that would have certain advantages for intracellular transport. If the helix rotated around the central core (driven perhaps by a helix-associated central core-dependent hydrolase), an organelle situated in the groove of the helix would be translocated down the filament by a worm-screw mechanism (Fig. 8). A rotational velocity of 35 revolutions/s could be sufficient for an H helix to translocate materials at velocities up to 300 mm/d (the range of rapid axonal transport); another rotary motor, the bacterial flagellar motor, can rotate in excess of 100 revolutions/s (1). Any filamentous organelle employing the helix-core relationship could serve as the basis for such a rotating-helix transport motor.

An advantageous characteristic of a rotating-helix motor is that it is stationary; none of the elements involved in generating force need to move in the direction of the transported material, and therefore don't have to be recycled. In contrast, models for transport (e.g., reference 11) based on analogy to the sliding of actin and myosin filaments in muscle require that one member of a sliding pair moves in the direction of transport at least as rapidly as the material being transported. However, the most obvious candidates for such sliding filaments (e.g., actin, myosin, tubulin, and neurofilament proteins) have generally been observed to move down axons much more slowly than the rapidly transported proteins (2, 5, 23, 24). Although this discrepancy does not rule out linear sliding filament mechanisms (e.g., the filaments could be locally recycled), it is alter-

natively attractive to consider stationary motors, of which the rotating helix is one example.

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