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Binding of Adenovirus to Microtubules

II. Depletion of High-Molecular-Weight Microtubule-Associated Protein Content Reduces Specificity of In Vitro Binding

JAMES A. WEATHERBEE,* RONALD B. LUFTIG, AND ROBERT R. WEIHING

Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts 01545

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A specific in vitro association between adenovirus and purified rat brain microtubules has been previously demonstrated (R. B. Luftig and R. R. Weihing, 1975). When examined by negative-staining electron microscopy, approximately 90% of the virus associated with microtubules was edge bound, i.e., associated within ±4 nm of the microtubule edge. Similar results are now found for the association of adenovirus with purified chick brain microtubules. When the content of the high-molecular-weight proteins (MAPs) normally present as projections on the surface of microtubules is depleted by fractionation of cold-depolymerized microtubules on agarose A-15M columns or by brief treatment of polymerized microtubules with trypsin, the percentage of edge-bound microtubule-associated viruses is reduced to a level close to that found for particles such as reovirus, coliphage f2, or polystyrene latex spheres, which randomly associate with microtubules (54 to 64% for column-fractionated microtubules; 45 to 68% for trypsin-treated microtubules). Counts of adenovirus particles specifically bound to microtubules, corrected for variations in microtubule and virus concentrations, gave values 2.5 to 3.5 times higher for unfractionated microtubules than for microtubule-associated protein-depleted microtubules. These results are consistent with the suggestion that the specific association between adenovirus and microtubules is mediated by microtubule-associated proteins.

Many observations have suggested that microtubules are involved in the transport of material within cells. Treatments that depolymerize microtubules also interrupt axoplasmic transport (3, 11, 21), long saltatory movements of particles in HeLa cells (8), movements of nuclei in virus-induced syncytia (10), movements of melanin granules in melanocytes (17), movements of endosomes in macrophages (1), and movements of ribosomes in telotrophic ovaries (24). In addition, various intracellular particles have been observed in close association with microtubules. These include synaptic vesicles (9, 22), mitochondria from crane fly spermatocytes (13), bean pod mottle or cowpea mosaic virus (12), and barley stripe mosaic virions (18). There have also been reports of an association between reovirus and spindle microtubules in L and FL cells (4, 6, 23). In these observations, however, the contact between virus particles and microtubules was not direct. The spindle microtubules in such infected cells were covered over several regions with a coating of dense material to which the virus particles were attached. Studies with ferritin-labeled antibodies suggested that the coating material might be some reovirus protein(s). Finally, Dales and Chardonnet (5) found an association between microtubules or vinblastine-induced paracrystals and virus particles in HeLa cells infected with adenovirus. They also found that treatment with vinblastine lengthened the time required for viral DNA to move from the cell surface to the nucleus. Based on these observations, and in light of the proposed role of microtubules in the intracellular transport of molecules and particles, it was suggested that microtubules functioned in the intracellular transport of adenovirus (and possibly of other viruses such as simian virus 40 that replicate in the nucleus) from the cell surface to the nucleus. In support of this model, Luftig and Weihing (16) reported that adenovirus particles associate in vitro with microtubules isolated from rat brain. The present work was undertaken to study this in vitro association in more detail. It has recently been reported that a high-molecular-weight protein associated with microtubules, designated as MAP (microtubule-associated protein) (7) or HMW (high-molecular-weight component) (19), is present as a structural component projecting from the surface

of microtubules. It was of interest to determine the effect of the presence or absence of this high-molecular-weight component on the binding of adenovirus particles to microtubules in vitro.

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MATERIALS AND METHODS

Preparation of microtubule protein. Microtubules were purified from the brains of rats or 1- to 3-day-old chicks by alternate cycles of assembly and disassembly in vitro. An approximately 100-g portion of fresh brains was homogenized at 0°C in a Sorvall omnimixer with a volume of polymerization mix (PM) buffer [1 mM $MgSO_4$, 2 mM ethylene glycol-bis (β-aminoethyl ether)-*N,N'*-tetraacetic acid, 0.1 mM GTP, 100 mM piperazine-*N,N'*-bis(2-ethane sulfonic acid), pH 6.9] equal to 1.5 times the wet weight of the brains. The homogenate was centrifuged for 1 h at 0°C at 17,000 rpm in the SS34 rotor of a Sorvall RC-2B centrifuge. Sufficient GTP was added to the supernatant to make the concentration 1 mM, and the solution was then incubated for 1 h at 37°C to polymerize microtubules. The microtubules were collected by centrifugation in the SS34 rotor at 18,000 rpm for 30 min at 35°C. The pellets from this centrifugation were resuspended in PM buffer (containing 0.1 mM GTP) to a volume equal to about one-third of the original extraction buffer volume. This suspension was treated briefly with a Dounce homogenizer and incubated for 1 h at 0°C to depolymerize microtubules. The suspension was then centrifuged at 40,000 rpm in a Spinco SW50.1 rotor for 1 h at 0°C to remove tubulin aggregates. The GTP concentration of the supernatant solution was again adjusted to 1 mM, and the solution was again incubated at 37°C for 1 h, and polymerized microtubules were collected as before.

Preparation of MAP-depleted microtubules. MAP-depleted microtubules were prepared by treatment of polymerized microtubules with trypsin for brief intervals or by fractionating depolymerized microtubules on agarose columns. In the latter case, a column of Bio-Gel A-15M with a bed volume of approximately 50 ml was equilibrated with PM buffer containing 0.1 mM GTP at 4°C. Microtubules were prepared as described above. The pellet from the second cycle of polymerization was resuspended in approximately 2 ml of the buffer with which the column was equilibrated. The final protein concentration was approximately 2 mg/ml. The sample was maintained at 0°C for about 0.5 h, and then 1.5 ml was applied to the agarose column and eluted with PM buffer containing 0.1 mM GTP. Two-milliliter fractions were collected, and the protein concentration was monitored by determining the absorbancy at 280 nm. Peak fractions were pooled, and protein was determined by the method of Lowry et al. (14). When necessary, samples were concentrated by pressure dialysis.

Electrophoresis. Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis was carried out according to the method of Neville (20). A 50-μg portion of protein was applied to each gel. Gels were stained overnight with 0.05% Coomassie brilliant blue in 25% isopropanol and 10% acetic acid. Gels were destained in 10% isopropanol and 10% acetic acid and stored in 10% acetic acid.

Adenovirus type 5. Adenovirus was propagated in HeLa cells grown in spinner culture and purified as described previously (16).

Reovirus type 3. Reovirus was obtained from A. Hay. It was grown and purified as described previously (15).

Electron microscopy. Samples were prepared for negative-staining electron microscopy as described previously (16), except that fixation was carried out using 5% glutaraldehyde prepared in PM buffer containing 1 mM GTP at pH 6.9 rather than the cacodylate buffer at pH 7.4 used previously. Samples were examined with a Philips EM300 electron microscope.

RESULTS

A typical fractionation of purified chick brain microtubules on an agarose A-15M column is shown in Fig. 1. Fractions from the first two peaks were pooled as indicated and concentrated. The trailing peak in the elution profile consisted mainly of GTP, as determined by a spectrum of the peak fraction, but the spectrum also showed the presence of a smaller amount of material with an absorption maximum at 280 nm. SDS-polyacrylamide gel electrophoresis of

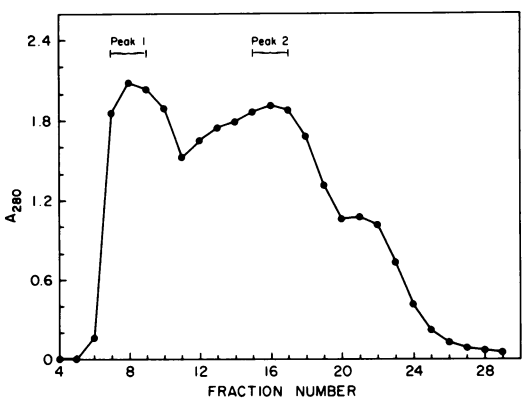

FIG. 1. *Fractionation of purified chick brain microtubules on agarose A-15M. The sample designated preparation 2 (see Fig. 2) was treated as described in Materials and Methods. Fraction volume was 2 ml. Fractions 7 to 9 were combined, concentrated by pressure dialysis from 6.0 to 1.0 ml, and designated peak 1. The final protein concentration of this fraction was 5.2 mg/ml. Fractions 15 to 17 were combined, concentrated from 6.5 to 0.9 ml, and designated peak 2. The final protein concentration of this fraction was 12.4 mg/ml.*

this fraction showed two tubulin bands and two faint bands in the MAP region as well as a number of other barely detectable bands.

SDS-polyacrylamide gel electrophoresis of the material in peak 1 showed an enrichment of the high-molecular-weight microtubule-associated proteins (MAPs) in comparison with the starting purified microtubule preparation (Fig. 2). In contrast, these MAPs were present only in barely detectable amounts in the material from peak 2. In these heavily loaded gels, a number of minor bands can be seen in addition to the major tubulin and MAP bands. It has been reported that even after six cycles of assembly-disassembly, as many as 12 protein species can be detected on gels of microtubule preparations (G. G. Borisy, K. Johnson, D. Murphy, and M. Marcum, Abstr. Cold Spring Harbor Conf. Cell Motil., p. 49, 1975). However, only the high-molecular-weight MAP proteins maintained a constant stoichiometry through this treatment. Whether the other bands represent contaminants or minor components of microtubules has yet to be determined conclusively. In Fig. 2, the gels prepared from the material from the column shown in Fig. 1 are designated as preparation 2.

In agreement with the observations of other workers (7, 19), the material from peak 1 readily polymerized at protein concentrations of 1.5 to 2.5 mg/ml in the presence of 1 mM GTP in PM buffer to produce an abundant number of microtubules (Fig. 3a, b).

At protein concentrations in this range or even higher, the material from peak 2 polymerized to give far fewer numbers of microtubules (Fig. 3c and 3d). It is now generally accepted that pure tubulin will not polymerize. Possibly our preparations contain traces of MAP sufficient to initiate the polymerization of a few microtubules. Alternatively, polymerization may be stimulated by another protein. Our peak 2 preparations show the presence of a band that migrates just behind the tubulins in the molecular weight range of 65,000 to 70,000. This band may correspond to the tau protein (25), which may also play a role in microtubule polymerization (see Discussion). In any case, sufficient microtubules are produced from peak 2 preparations to test their virus-binding properties.

The binding of adenovirus particles to unfractionated microtubules, to microtubules prepared from the MAP-enriched material from peak 1, and to microtubules prepared from the MAP-depleted material from peak 2 was tested by the two general methods described previously (16). Briefly, in method A microtubules

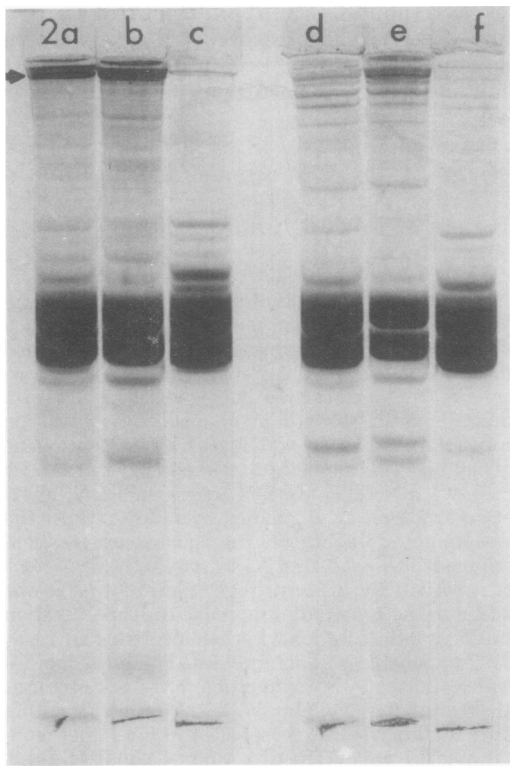

FIG. 2. *SDS-polyacrylamide gel electrophoresis of purified microtubules, MAP-enriched microtubules from peak 1, and MAP-depleted microtubules from peak 2. Gels were run using the Neville system. (a) Purified, unfractionated microtubules (preparation 1). (b) MAP-enriched microtubules (preparation 1, peak 1). (c) MAP-depleted microtubules (preparation 1, peak 2). (d) Purified, unfractionated microtubules (preparation 2). (e) MAP-enriched microtubules (preparation 2, peak 1). (f) MAP-depleted microtubules (preparation 2, peak 2). Arrow indicates the position of the MAP bands on the gels. Note that the purified microtubules from preparation 1 contain a higher proportion of MAPs in relation to tubulin than those from preparation 2.*

were adsorbed to carbon-coated grids, after which the grids were floated on drops containing virus particles. In method B, virus particles were first adsorbed to grids, after which the grids were floated on drops containing microtubules.

The results of a series of experiments using these methods with adenovirus and unfractionated and fractionated microtubules are shown in Table 1. Using method B, it was found that for unfractionated microtubules and for the MAP-enriched microtubules of peak 1, 84 to 90% of the virus particles that were associated with microtubules were bound to the edge of the

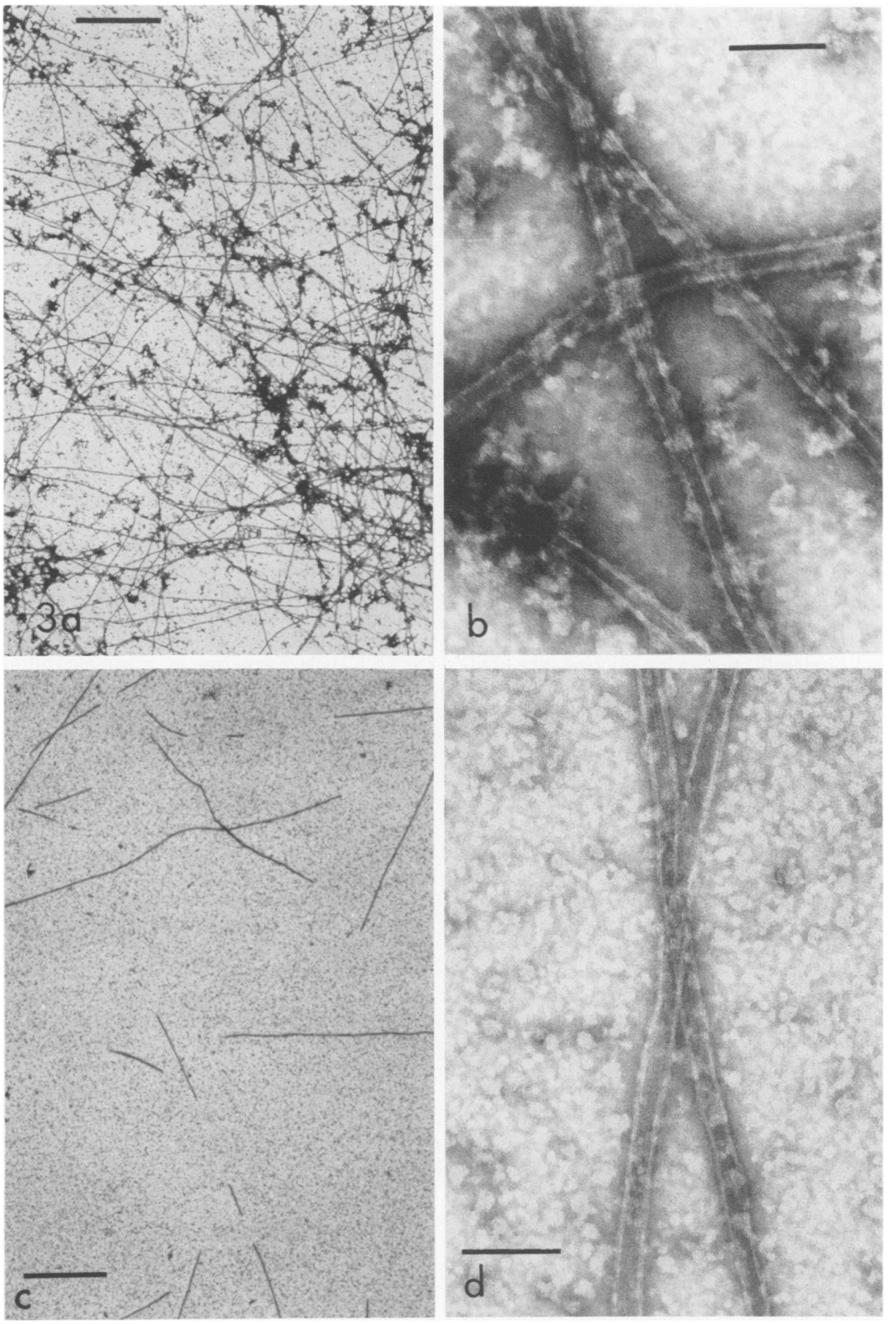

FIG. 3. *Microtubules prepared from peak 1 and peak 2 fractions. (a) Peak 1 (MAP enriched) microtubules, 6,300×. Bar = 2 μm. (b) Peak 1 microtubules, 147,000×. Bar = 0.1 μm. (c) Peak 2 (MAP depleted) microtubules, 6,300×. Bar = 2 μm. (d) Peak 2 microtubules, 147,000×. Bar = 0.1 μm. Preparation 2 microtubules were fractionated as described in the text (see Fig. 1 and 2d-f).*

TABLE 1. *Effect of MAP content on binding of adenovirus particles to chick brain microtubules*

Microtubule prepn	Method A (microtubules first)		Method B (virus first), % of bound virus on the edge
	% of virus bound to microtubules	% of bound virus on the edge	
1. Twice polymerized (usual microtubule)	Prepn 1, 77.2 (246)[a]	83.3 (120)	87.6 (226)
	Prepn 2, 61.4 (1,028)	88.1 (586)	89.8 (234)
2. MAP enriched (peak 1 from gel filtration)	Prepn 1, -	-	83.6 (278)
	Prepn 2, 73.8 (296)	89.4 (194)	87.6 (196)
3. MAP depleted (peak 2 from gel filtration)	Prepn 1, 44.7 (295)	62.5 (104)	54.2 (140)
	Prepn 2, 23.2 (786)	64.7 (128)	63.4 (360)

[a] Numbers in parentheses refer to the total number of virus particles counted.

microtubule, i.e., within ±4 nm of the edge. It has been previously shown that such a high percentage of edge binding indicates a specific interaction between microtubules and adenovirus particles (16). The MAP-depleted microtubules of peak 2, however, show a much lower percentage of associated particles bound to their edges, only 54 to 63%. These latter values are close to those found previously for particles such as reovirus, coliphage f2, and polystyrene latex spheres which do not show a specific association with microtubules. Another set of control experiments performed previously (16), in which microtubules were traced and then placed randomly on electron micrographs of virus particles, gave values of about 50% for random edge association of virus particles with microtubules.

Using method A, again a higher percentage of microtubule-associated virus particles was found to be edge associated for unfractionated microtubules (83 to 88%) and for MAP-enriched microtubules (89%) than for MAP-depleted microtubules (62 to 65%). Thus, the results from both methods suggest that the specific interaction between microtubules and adenovirus particles, which results in the phenomenon of edge binding seen in electron micrographs of fixed and negatively stained samples, is lost when MAPs are depleted. It also appears that when MAPs are depleted, fewer viruses are bound to microtubules (Table 1, column 1), again suggesting, albeit qualitatively, a loss of specific association (16). Figure 4 shows typical electron micrographs obtained with adenovirus and unfractionated or MAP-depleted microtubules using methods A and B. It can be seen that unfractionated preparations showed higher numbers of microtubules per field than peak 2 preparations when approximately equal protein concentrations were used. It can also be seen that there is a higher background level of unpolymerized protein on grids prepared by method A. Although not clearly shown in these figures, there is greater tendency for virus aggregation on these latter grids. Finally, and most importantly, it can be seen that with unfractionated microtubules, almost all of the adenovirus particles associated with these microtubules are associated with their edges. With the MAP-depleted microtubules of peak 2, however, the association with virus particles is more random, and more viruses are seen lying on top of the microtubules.

In another series of experiments, virus binding to MAP-depleted microtubules was investigated using microtubules depleted of MAP by limited tryptic digestion. Microtubules prepared from both rat and chick brains were studied. In a typical experiment, a sample containing 9.4 mg of polymerized rat brain microtubule protein per ml was treated with trypsin at a concentration of 0.025 mg/ml for 25 s at 25°C, after which the reaction was stopped by the addition of excess soybean trypsin inhibitor (2 mg of inhibitor per mg of trypsin). It had previously been determined (Weihing and Luftig, unpublished observations) that such treatment removes MAPs from microtubules without appreciably destroying tubulin or the structural integrity of polymerized microtubules. This is illustrated in Fig. 5. Measuring the association at different concentrations of trypsin, or after varying times of treatment, showed that the removal of MAPs by trypsin treatment is correlated with a loss of specific edge binding of adenovirus particles (Table 2).

Tryptic-digestion experiments carried out with chick brain microtubules gave similar results. A sample containing 9.5 mg of polymerized chick brain microtubule protein per ml was treated with trypsin at a concentration of 0.025 mg/ml for 30 s at room temperature, after which the reaction was stopped by the addition of excess soybean trypsin inhibitor. Figure 6 shows gels of two preparations of chick brain microtubules treated as described above and control samples to which trypsin and trypsin inhibitor were added simultaneously. Again, the MAPs are virtually eliminated from the

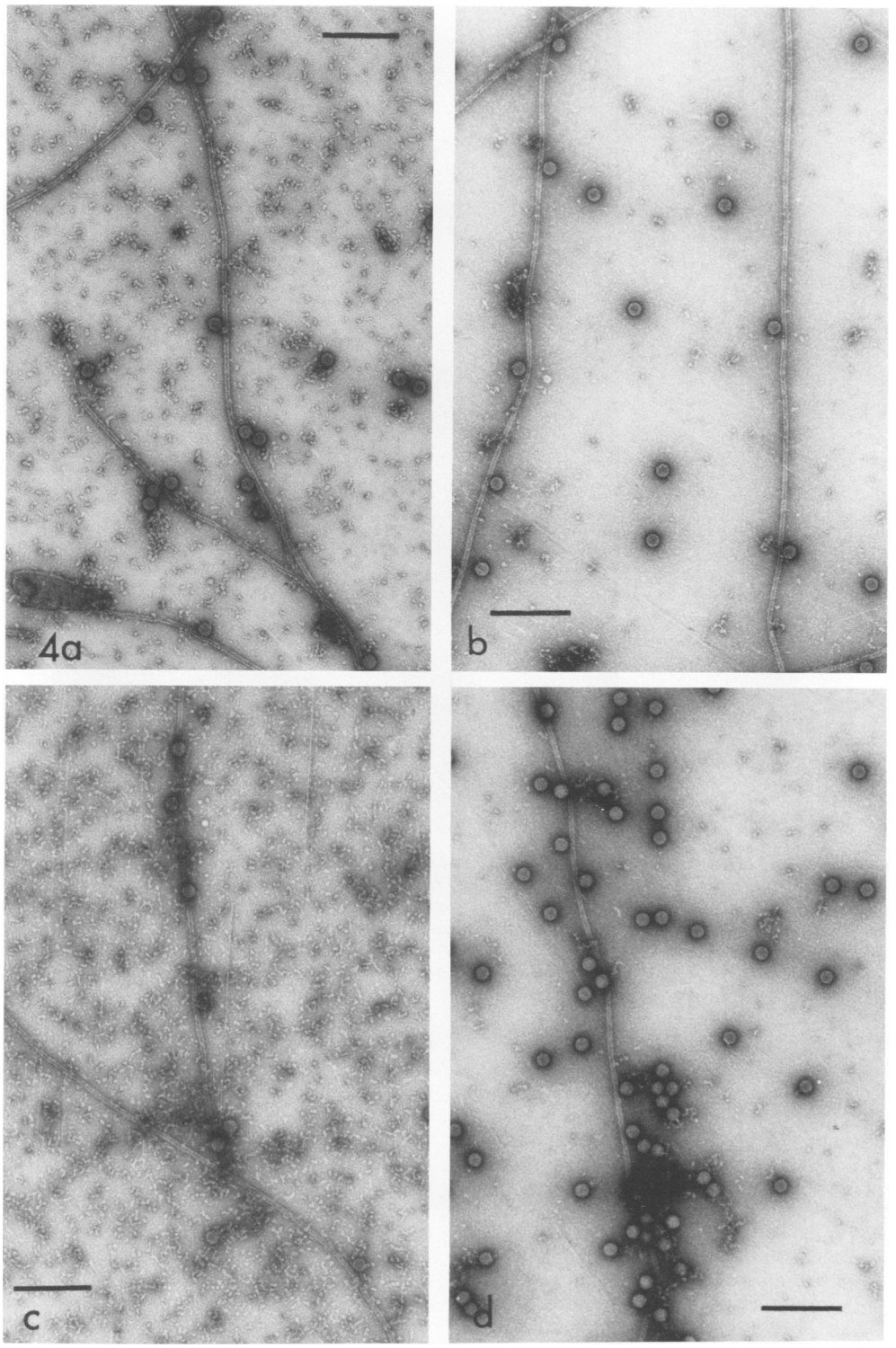

FIG. 4. *Binding of adenovirus to unfractionated and MAP-depleted microtubules. (a) Unfractionated microtubules, method A. (b) Unfractionated microtubules, method B. (c) Peak 2 microtubules, method A. (d) Peak 2 microtubules, method B. In all cases, bar = 0.5 μm and the magnification is 23,100×. All samples were from preparation 2.*

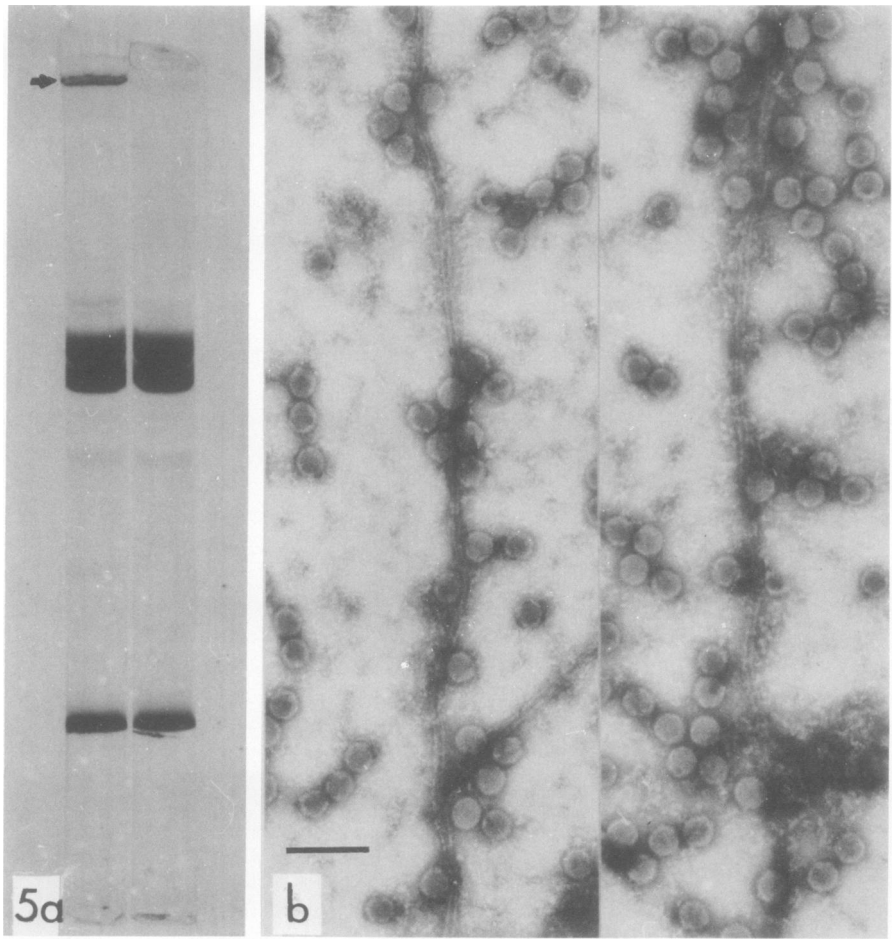

FIG. 5. *Binding of adenovirus to unfractionated and trypsin-treated rat brain microtubules. (a) Left-hand gel: SDS-polyacrylamide gel electrophoresis of purified rat brain microtubules. Arrow marks position of MAP bands. Right hand gel: SDS-polyacrylamide gel electrophoresis of rat brain microtubules treated with trypsin as described in the text. (b) Electron micrographs of trypsin-treated microtubules interacting with adenovirus. The grid was first coated with virus and then with microtubules (method B). Particles are associated with the edge, the top, and the bottom of the microtubules. Compare this figure with Fig. 4a,b showing edge association of virus with microtubules containing MAPs. 43,670×. Bar = 0.25 μm.*

trypsin-treated samples.

Table 3 shows the effect of the trypsin treatment on the binding of adenovirus to chick brain microtubules. Using method B, it was found that 60 to 62% of the adenovirus particles associated with trypsin-treated microtubules was edge bound. The value for binding to the control microtubules was 78 to 88%. Using method A, 50 to 68% of the adenovirus particles associated with trypsin-treated microtubules was edge bound as compared to 88 to 89% for control microtubules. Again, treatment that has been shown to deplete microtubules of MAPs abolishes specific virus binding.

Effect of varying virus and/or microtubule concentration on binding affinity. In previous studies (16), where it was possible to maintain a relatively constant total amount of microtubular material and a constant total number of virus particles per standard field in the electron microscope assay, the percentage of the total number of virus particles on the grid that were associated with microtubules was taken as a measure of the affinity of virus particles for microtubules relative to their affinity for the carbon film of the grid. This measure was only used with method A where microtubules were first adsorbed to the grid and virus particles were free to diffuse onto the microtubule-coated grids.

In the present study this type of analysis was not so useful, because the microtubule concentration varied widely between grids prepared from unfractionated or peak 1 (MAP enriched) microtubules and peak 2 (MAP depleted) microtubules, viz., the latter showed far less total microtubule contour length per standard field at a magnification of 7,700×.

In an attempt to obtain some quantitative measure of the relative affinity of adenovirus particles for treated and untreated microtubules using method A under the present conditions, a different type of analysis was employed. It was assumed that the amount of association of virus particles with microtubules (both specific binding and random association) would increase with increasing virus particle concentration and with increasing microtubule concentration. To correct for the variation of these factors, primarily the variation in microtubule concentration, the number of specifically bound virus particles per standard field at a magnification of 7,700× was divided by the total microtubule contour length per field and the total number of virus particles per field. The number of specifically bound virus particles was estimated by subtracting the number of virus particles associated on top of microtubules from the number of particles that were edge associated; previous work suggested that top association could be used as a measure of random association (16). Thus, each field counted was provided with an internal control. Finally, for this analysis, only single virus particles were counted to eliminate the ambiguities introduced by the presence of virus clumps in some cases.

Table 4 shows the results of this type of analysis when applied to the association of adenovirus particles with unfractionated microtubules and with microtubules depleted of MAPs by agarose column chromatography or by brief trypsin treatment. For comparison, the results obtained using this analysis on the association of reovirus particles with unfractionated and peak 2 microtubules are also shown. This virus is similar in size and density to adenovirus, but has been previously shown not to bind specifically to microtubules (16).

The results shown in Table 4 show that the corrected specific binding of adenovirus particles to unfractionated microtubules is at least two to three times as high as to microtubules that have had their MAP concentration depleted by column chromatography or trypsin treatment. The values obtained for reovirus particles, although somewhat higher than the values obtained with adenovirus and MAP-depleted microtubules, are still much lower than the values obtained with adenovirus particles and unfractionated microtubules. There is also no significant difference between the values obtained for association of reovirus particles with unfractionated microtubules or with

TABLE 2. *Effect of trypsin treatment on binding of adenovirus to rat brain microtubules*[a]

Parameter studied	Time of exposure to trypsin (s)[b]	Final trypsin concn (mg/ml)[c]	% of bound virus on the edge[d]
Kinetics of trypsinization of microtubules	0		76 (85), 88 (115)[e]
	25		50 (205)
	60		52 (99), 56 (41)[e]
	90		48 (66), 56 (71)[e]
Concn effect of trypsin treatment		0	87 (68)
		0.025	45 (77)
		0.05	57 (122)
		0.11	41 (27)[f]
		0.22	43 (14)[f]

[a] Data were obtained using method B, where particles and then microtubules, respectively, were applied to the grid. See text for details.

[b] Microtubules (final concentration, 6.2 mg/ml) were digested with trypsin (final concentration, 0.1 mg/ml) at 25°C for the times indicated, and then the reaction was stopped with a twofold excess of soybean trypsin inhibitor (2 mg of inhibitor per mg of trypsin).

[c] Microtubules (final concentration, 9.4 mg/ml) were digested with trypsin at the final concentrations indicated. Digestion was carried out at 25°C for 25 s and stopped with excess inhibitor.

[d] Numbers in parentheses refer to the total number of virus particles counted.

[e] Replicate experiments were performed on two different preparations of microtubules.

[f] Fewer microtubule-associated particles could be counted at these trypsin concentrations because microtubules were less frequent on the grid. This suggests that trypsin was digesting tubulin as well as MAP at these concentrations.

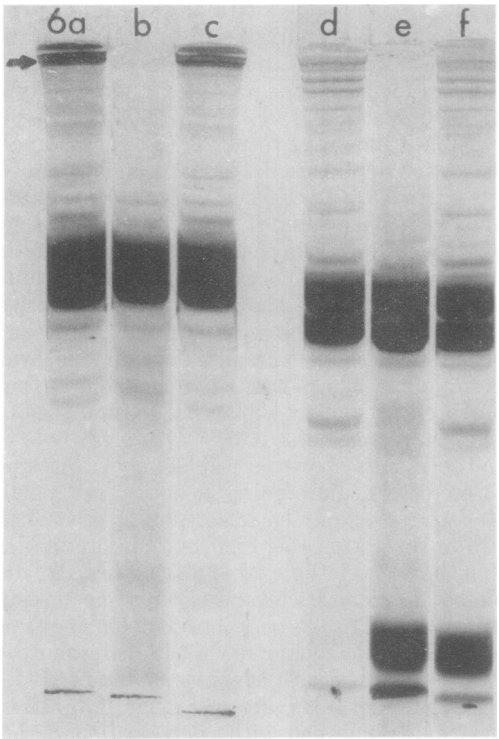

FIG. 6. *SDS-polyacrylamide gel electrophoresis of purified microtubules from chick brain and microtubules subjected to limited tryptic digestion. Gels were run using the Neville system. (a) Freshly isolated and purified microtubules (preparation 1). Arrows point to position of MAP bands. (b) Polymerized microtubules treated briefly with trypsin (preparation 1). See text for details. (c) Gels of microtubules polymerized at the time of the trypsin treatment experiment (preparation 1). This sample was used as a trypsin treatment control in that the trypsin inhibitor and trypsin were added simultaneously to these microtubules. (d) Freshly isolated and purified microtubules (preparation 2). (e) Polymerized microtubules treated briefly with trypsin (preparation 2). (f) Gels of microtubules polymerized at the time of the trypsin treatment experiment (preparation 2). The trypsin inhibitor and trypsin were added simultaneously as in (c). The trypsin-treated and control microtubules were used immediately after treatment in virus-binding experiments. The small difference in the distance of band migration of the left hand set of gels compared to the right hand set is due to a change in the pH of the electrophoresis reservoir buffer. Conditions were identical for gels within each set.*

MAP-depleted microtubules.

It should be noted that the microtubules polymerized from preparation 2 at the time of the trypsin experiment showed a somewhat decreased level of adenovirus binding than microtubules polymerized from this same preparation when it was fresh. Gels of these microtubules prepared from material stored at −80°C show a diminished MAP content compared to microtubules polymerized when preparation 2 was freshly purified (compare Fig. 6d and 6f). It has been reported that MAPs are labile when stored at 0°C (K. Sloboda and J. L. Rosenbaum, J. Cell Biol. **67**:405a, 1975). Although our preparations were routinely stored at −80°C, they were periodically thawed briefly for removal of portions for experiments. This treatment may account for a gradual decrease in the amount of MAP present in microtubules polymerized from preparations stored for some time. It should also be noted that microtubules polymerized from preparation 1 always showed more prominent MAP bands on gels than microtubules polymerized from preparation 2 (compare Fig. 2a and 2d). It can be seen from Table 4 that preparation 1 microtubules also showed a higher level of corrected specific binding than preparation 2 microtubules. Qualitatively, at least, these two sets of observations are consistent with the suggestion that a higher MAP content is correlated with a higher level of adenovirus binding for unfractionated microtubules.

DISCUSSION

The evidence presented in this paper is consistent with the suggestion that the specific in vitro association between adenovirus particles and microtubules is mediated by the high-molecular-weight proteins associated with the microtubules. Treatments that deplete the MAP content of microtubules destroy the specificity of the microtubule-virus association. Evidence that the component of the virus involved in the interaction is the hexon capsomere has previously been presented (16). Brief formamide treatment, which removes pentons and fibers (but not hexons) from the virions, produces particles that still bind specifically to microtubules.

The exact function of the high-molecular-weight MAPs has not yet been clearly defined. It has been suggested that MAPs play a role in microtubule polymerization (2, 7) because MAP-depleted tubulin polymerizes only slowly and inefficiently and readdition of MAPs restores microtubule polymerization. However, a similar stimulation of the polymerization of purified tubulin has been reported for another protein isolated from microtubule preparations, the tau protein studied by Kirschner and coworkers (25). In addition, it has been shown that a number of polycations, e.g., DEAE dextran or histone, are effective in initiating the

TABLE 3. *Binding of adenovirus to chick brain microtubules subjected to limited trypsin digestion*

Microtubule prepn	Method A (microtubules first)		Method B (virus first), % of bound virus on the edge
	% of virus bound to microtubules	% of bound virus on the edge	
Trypsin-treated microtubules (see Fig. 6)	Prepn 1, 11.0 (400)[a]	50.0 (44)	60.2 (128)
	Prepn 2, 57.6 (1,180)	68.0 (704)	62.4 (423)
Control microtubules (see Fig. 6)	Prepn 1, 41.4 (643)	88.6 (238)	87.7 (359)
	Prepn 2, 71.6 (1,362)	88.0 (926)	78.2 (182)

[a] Numbers in parentheses refer to the total number of virus particles counted.

TABLE 4. *Binding of adenovirus and reovirus particles to unfractionated and MAP-depleted microtubules corrected for nonspecific binding and for variation in microtubule and virus concentrations*

Microtubules	Corrected specific binding of virus particles to microtubules ($\times 10^5$)[a]
Unfractionated	
Prep 1 + adenovirus	555 ± 151
Prep 2 + adenovirus	381 ± 49
Prep 2 + reovirus	211 ± 43
MAP depleted	
Prep 2 peak 2 + adenovirus....	135 ± 60
Prep 2 (trypsin treated) + adenovirus[b]	189 ± 36
Prep 2 peak 2 + reovirus	229 ± 59

[a] This value represents the total number of specifically bound virus particles (the total associated particles minus the number of particles associated nonspecifically on top) divided by the total microtubule length (centimeters) per standard field (at a magnification of 7,700×) and the total number of virus particles per standard field. An average of 14 fields was counted for each value. Fields that contained greater than 450 cm of total microtubule contour length or fewer than 20 virus particles were not counted. The values reported represent average corrected specifically bound virus particles plus or minus the standard error of the mean.

[b] A control performed for this sample by simultaneously mixing trypsin and the trypsin inhibitor gave a value of 267 ± 29. This value, although higher than the values for these particular MAP-depleted microtubules, is lower than the value found for this preparation when it was first purified. See text for a more complete discussion.

polymerization of tubulin into microtubules (H. P. Erickson, J. Cell Biol. 67:110a, 1975). The tubules formed with the polycations, however, show structural abnormalities, such as double walls. In any case, although MAPs can stimulate polymerization in vitro, this effect is not unique, and it remains to be determined whether MAPs or other factors control in vivo polymerization.

Whether or not MAPs have an in vivo function in microtubule polymerization, they do seem to be incorporated into the structure of microtubules assembled in vitro. Murphy and Borisy (19) observed that they appear as projections measuring 18.9 by 5.6 nm spaced along the entire microtubule length, having a longitudinal periodicity of about 32.5 nm. In transverse sections of embedded microtubules, the projections are found at all azimuthal angles (7, 19). Furthermore, the ratio of high-molecular-weight protein to tubulin remained constant in microtubules that had been carried through six cycles of polymerization and depolymerization (2). These results seem unlikely if the MAPs represent a nonspecific contaminant.

It has been suggested that MAPs may be analogous to the dynein cross-bridges found on cilia and flagella. The analogy is not perfect, however, since the major MAPs can be electrophoretically separated from dynein and the dimensions of the MAP projections on microtubules differ from the dimensions of dynein cross-bridges (19). It has also not yet been established whether or not MAPs possess any ATPase activity.

It has been previously emphasized (16) that the physical basis for edge binding is obscure. From their dimensions in electron micrographs after negative staining, microtubules are apparently present as long, somewhat flattened cylinders. Adenovirus particles appear spherical or occasionally in hexagonal profile under these conditions. A consideration of the three-dimensional geometry and dimensions of the structure involved shows that any postulated surface-to-surface interaction between a virus particle and a microtubule would result in an overlap of the virus over the tubule when viewed two-dimensionally on an electron microscope grid after drying. However, when it is considered that the surface of the microtubules is covered with periodically spaced 18.9-nm projections and if, as the evidence presented in this paper suggests, these projections are the components of the microtubules to which the virus

binds, then the situation becomes clearer. Attachment of virus particles to MAPs would hold them away from the microtubule surface. Upon drying, virus particles on the sides of microtubules would be found beside the microtubule surface rather than overlapping it. Since MAPs are distributed over the whole surface of microtubules, presumably viruses bound to MAPs on the tops of tubules would be lost or fall down to the sides of the tubules during drying.

The in vitro association between microtubules and adenoviruses observed here strengthens the suggestion that microtubules function in the transport of virus particles from the cell surface to the nucleus and additionally suggests the intriguing possibility that this transport is mediated by MAPs. There is still, of course, no evidence whether MAPs function actively in virus transport or only serve as the site of attachment for particles that are then transported by another mechanism, such as selective polymerization and depolymerization of microtubules.

Future work that will be able tc provide quantitative data more conveniently than the electron microscopic assay which has been used so far will be directed toward developing new methods for studying virus-microtubule interaction.

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