Temperature effects on solutions of vinblastine-induced polymers assembled from brine shrimp (*Artemia*) tubulin

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Received 12 May 1986

Purified *Artemia* tubulin in the presence of neural microtubule-associated proteins and vinblastine, or with vinblastine alone, forms extensive coils. Reduction in temperature of a coil-containing solution to 4°C causes an increase in turbidity, which returns to previous levels once the solution is warmed. Examination of negatively stained samples indicates that the turbidity fluctuations are not accompanied by a pronounced change in coil structure nor by increased polymer formation. Bovine neural tubulin responds in the same way as *Artemia* tubulin to vinblastine and temperature. An interesting novel response to vinblastine, shared by tubulins from phylogenetically distinct organisms, is illustrated by our results.

Vinblastine *Tubulin* *Microtubule-associated protein* *Temperature effect* *(Artemia)*

1. INTRODUCTION

Vinca alkaloids such as vincristine or vinblastine disrupt lateral tubulin-tubulin interactions causing microtubule protofilaments to peel apart, and for neural microtubules formed in the absence of MAP, their complete disassembly. Stabilization of longitudinal interactions between tubulin dimers by MAP prevents protofilament depolymerization and leads to vinblastine-induced coil formation [1–4]. Except where vinblastine has been used to induce paracrystal formation [5–9] and for a limited examination of chicken erythrocyte tubulin [10], most studies of vinblastine effects have centered on brain microtubule proteins. As occurs with colchicine [11,12], tubulin from sources other than brain may exhibit altered sensitivity to vinblastine. We have, therefore, examined the effect of vinblastine on purified tubulin from *Artemia* [13] which, as a protosome, is phylogenetically far removed from the mammals. We describe herein, as a part of this work, an unusual vinblastine-dependent response of *Artemia* tubulin to temperature fluctuations. An identical response is shown for mammalian neural tubulin.

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Abbreviations: Pipes, 1,4-piperazinediethanesulfonic acid; MAP, microtubule-associated proteins

2. MATERIALS AND METHODS

Artemia embryos were purchased from San Francisco Bay Brand, Newark, CA. Pipes as free acid was from Research Organics, Cleveland, OH. Vinblastine as sulfate salt and GTP as sodium salt were purchased from Sigma, St. Louis, MO. Glutaraldehyde, as an 8% aqueous solution, was from Polysciences, Warrington, PA. All other chemicals were reagent grade.

Tubulin was prepared from bovine brain and *Artemia* (developed 15 h) as described in [14] except that the amount of protein applied to phosphocellulose P-11 was tripled. Pipes buffer (100 mM Pipes, pH 6.9/1 mM $MgCl_2$/1 mM EGTA) containing 1 M NaCl was used to wash

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/86/$3.50

bovine MAP from phosphocellulose P-11. The MAP were precipitated by addition of $(NH_4)_2SO_4$ to 80%, resuspended in Pipes buffer, dialyzed at 4°C and centrifuged at 40000 × *g* for 30 min at 4°C. Next, the MAP were placed in a boiling water bath for 5 min, cooled on ice and recentrifuged. The tubulin and MAP were stored at −70°C. Protein concentration was determined according to Lowry et al. [15].

A Perkin-Elmer Lambda 3B spectrophotometer equipped with a thermoelectric cuvette holder and an R100 recorder was used to measure turbidity of assembly mixtures. Assembly was at 37°C in Pipes buffer containing GTP at a final concentration of 1.8 mM. Tubulin and MAP were used at 1 mg/ml. By adding increasing amounts of MAP to a constant amount of tubulin, MAP were determined turbidimetrically to be at saturation. Addition of vinblastine to a final concentration of 40 μM was from a 400 μM stock solution made in Pipes buffer. Vinblastine additions and temperature modifications were as indicated in the figures.

3. RESULTS AND DISCUSSION

Addition of vinblastine to *Artemia* tubulin in the presence of bovine neural MAP yielded coiled structures upon incubation at 37°C (fig.1). Formation of coils was accompanied by an increase in the turbidity of the tubulin-containing solutions (fig.2A). Lowering the temperature to 4°C to test the cold sensitivity of the coils caused an increase in solution turbidity (fig.2A) rather than the drop which occurs upon microtubule disassembly in the absence of vinblastine (not shown). The turbidity decreased to near previous levels when the temperature was returned to 37°C. Incubation of *Artemia* tubulin with or without neural MAP in the absence of vinblastine yielded morphologically normal microtubules (fig.3). When MAP were present the decrease in the turbidity of microtubule-containing solutions observed upon vinblastine addition was followed by a partial recovery to pre-vinblastine levels (fig.2B). The turbidity change was accompanied by transformation of

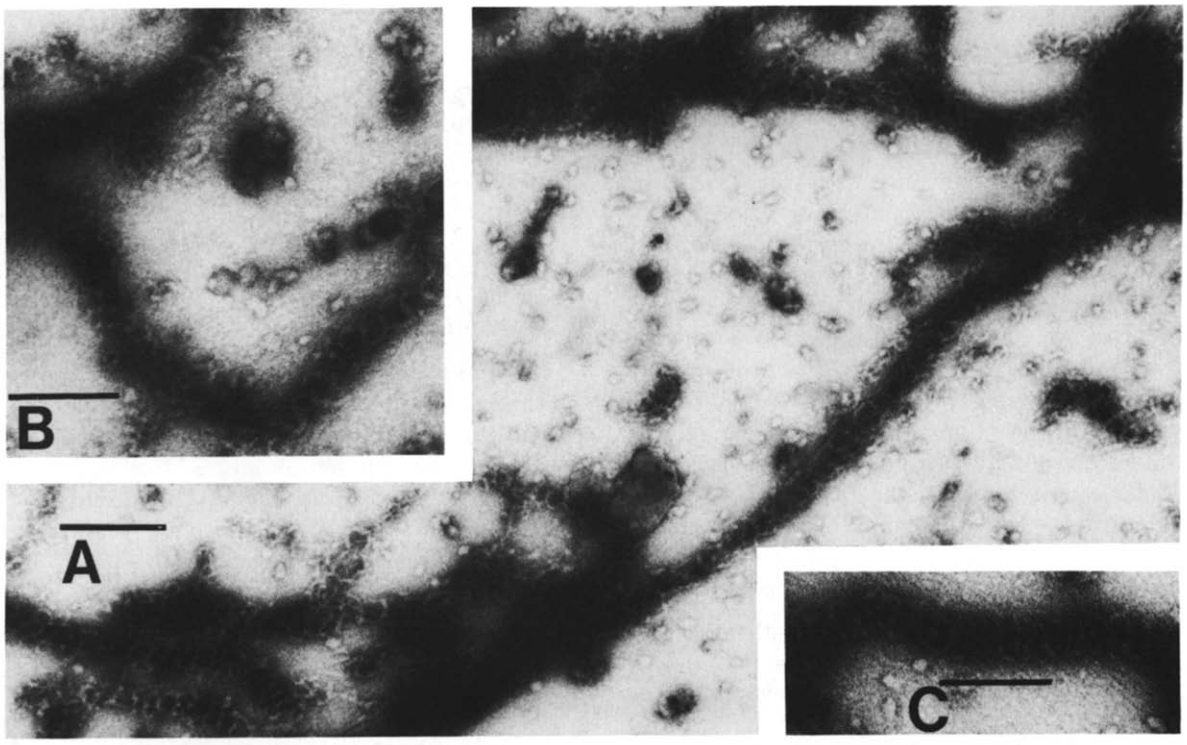

Fig.1. Electron micrographs of negatively stained coils formed upon incubation of *Artemia* tubulin and neural MAP in the presence of 40 μM vinblastine. Bar: 0.3 μm (A), 0.2 μm (B,C).

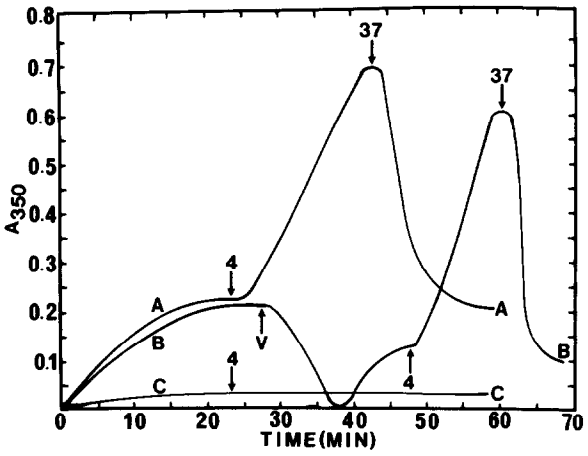

Fig.2. The effect of vinblastine and incubation temperature on the turbidity of solutions containing *Artemia* tubulin and neural MAP. (A) Tubulin polymerization occurred in the presence of vinblastine; (B) tubulin was assembled and vinblastine was added at the time indicated by the arrow labelled V; (C) neural MAP incubated with vinblastine. The arrows labelled 4 and 37 in this and subsequent figures indicate the time at which the incubation temperature was changed to these values.

microtubules to coils structurally similar to those shown in fig.1. When the temperature was decreased, there was a rise in turbidity which could be reversed by increasing the temperature (fig.2B).

Incubation of *Artemia* tubulin in the absence of MAP, but in the presence of vinblastine, caused an increase in solution turbidity which was somewhat less than if MAP were present (cf. figs 4A and 2A). Addition of vinblastine to solutions of *Artemia* microtubules assembled without MAP, in contrast to the situation with MAP (fig.2B), did not cause the turbidity to drop (fig.4C). However, in spite of these differences, and the structurally different coils which resulted upon addition of vinblastine to microtubules or tubulin in the absence of MAP (submitted), the temperature-induced turbidity fluctuations were the same as when MAP were present (cf. figs 2A,B and 4A,C).

During the turbidity fluctuations there was, as determined by examination of negatively stained preparations at various time intervals, no readily apparent change in the amount or structure of coils which would account for the observed results. The coils occasionally appeared more compact at lower temperatures but this could be due to variations in-

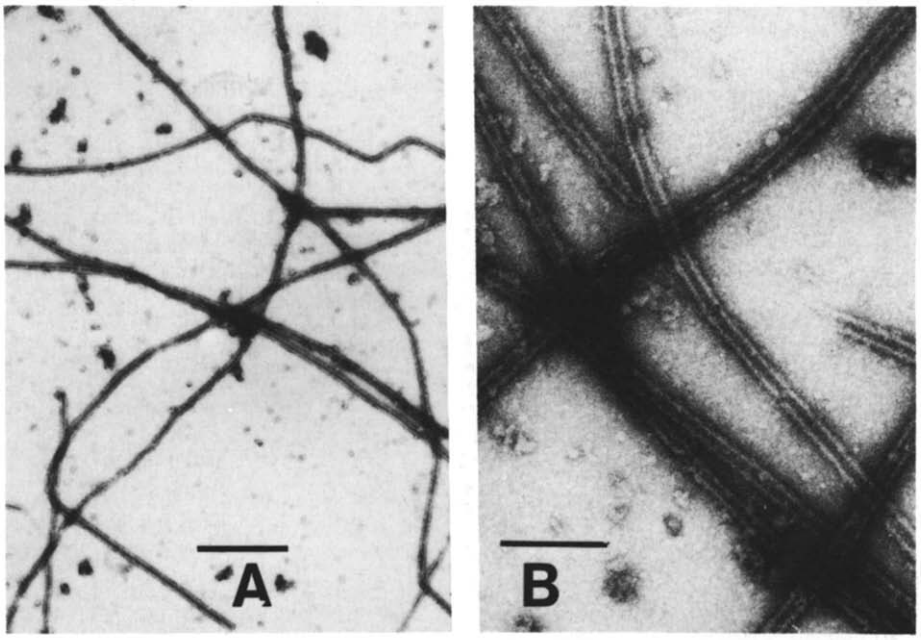

Fig.3. Electron micrographs of negatively stained microtubules formed from *Artemia* tubulin and neural MAP. Microtubules formed in the absence of MAP were structurally identical and are not shown. Bar: 0.5 μm (A), 0.2 μm (B).

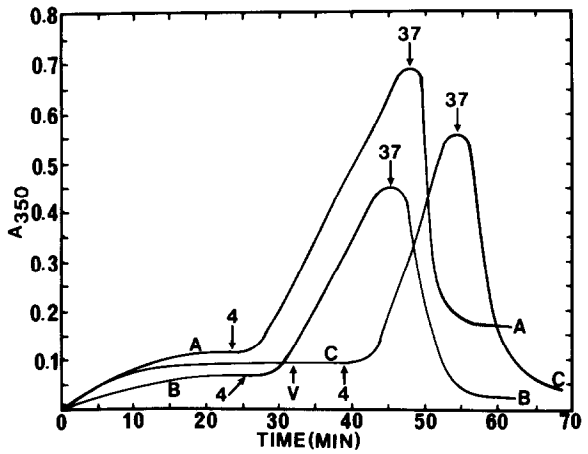

Fig.4. The effect of vinblastine and temperature on the turbidity of solutions containing tubulin in the absence of MAP. (A) *Artemia* tubulin polymerized in the presence of vinblastine; (B) neural tubulin polymerized in the presence of vinblastine; (C) *Artemia* tubulin was assembled and vinblastine was added at the time indicated by the arrow labelled V.

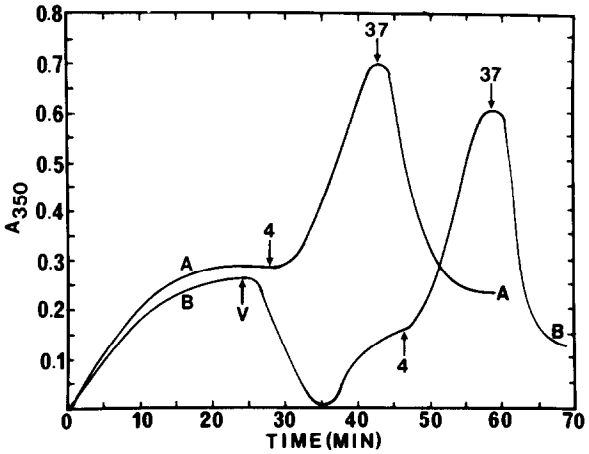

Fig.5. The effect of vinblastine and temperature on the turbidity of solutions containing bovine neural tubulin and MAP. (A) Tubulin polymerization occurred in the presence of vinblastine; (B) tubulin was assembled and vinblastine was added at the time indicated by the arrow labelled V.

troduced by fixation and negative staining. The turbidity change as shown here was dependent on the combined presence of tubulin and vinblastine. Tubulin and MAP in the absence of vinblastine (not shown) or MAP and vinblastine in the absence of tubulin (fig.2C) did not undergo an increase in turbidity upon reduction in temperature.

Exposure of bovine neural microtubule proteins to vinblastine and temperature fluctuations yielded turbidity changes identical to those obtained with *Artemia* tubulin (figs 4B, 5). It was not possible to examine the response of neural microtubules formed in the absence of MAP to vinblastine since neural tubulin did not assemble under the conditions we used unless MAP were present.

To the best of our knowledge there is only one other report where the turbidity of a coil-containing solution was monitored during temperature reduction [16]. In that study, the turbidity of a solution containing coils formed from bovine brain tubulin at 10 μM vinblastine dropped when the temperature was lowered, with little apparent loss of polymer. The effect of rewarming was not reported. Although there are variations in our tubulin purification and incubation conditions as compared to those of Himes et al. [16], it is difficult to understand why our solutions of vinblastine-induced coils should undergo a different response to temperature reduction. We have shown, however, that the *Artemia* coils, probably due to the binding of vinblastine to tubulin [2], are resistant to cold depolymerization. Further, the results demonstrate that purified tubulin from two very different sources share an essentially identical response to vinblastine, suggesting, as have our earlier results ([13]; submitted) a conservation of the vinblastine-binding site across extreme phylogenetic distances. Similar polymerization in the presence of MAP and vinblastine is particularly interesting since *Artemia* tubulin, in the absence of the drug, assembles more readily than does brain tubulin and its assembly is disrupted by Mg^{2+} and glycerol, agents which normally promote microtubule formation [14,17].

ACKNOWLEDGEMENTS

The work was supported by grants from the Natural Sciences and Engineering Research Council of Canada and the Banting Research Foundation to T.H.M. and by Robert Welch Grant AQ-726 and NIH grant GM23476 to R.F.L. The authors thank Ms Mary Primrose for assistance with the photography and Ms Christyne Mason for assistance with the electron microscopy.

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