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EFFECT OF HEAT ON THE MICROTUBULE DISASSEMBLY AND ITS RELATIONSHIP TO BODY TEMPERATURES

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SUMMARY

Microtubule proteins extracted from brain tissues of mouse, rat, calf and chicken have been used to study the heat induced disassembly process. The disassembly temperatures were 39.5°C, 41.5°C, 41.5°C, and 45°C respectively, to each species. These are within the range of fever temperatures of these species. Temperatures lethal to these animals cause denaturation of the microtubule proteins of the corresponding species. Purified microtubule protein is less sensitive to heat, but will reestablish its temperature sensitivity by addition of the supernatant obtained during the purification process. This study suggests that microtubule protein may have an important role in determining cellular function in the elevated temperature environment.

INTRODUCTION

The assembly and disassembly of cytoplasmic microtubules have been shown to play a vital role in expressing cellular shape, movement, secretion and cell division. Thus, the factors which influence the assembly and disassembly of microtubule (both in situ as well as in vitro) have been intensively studied. These studies demonstrate that microtubule assembly-disassembly is a temperature sensitive process. Elevated temperatures have been shown to disrupt the cytoplasmic microtubule

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organization (1-5). A similar temperature effect was also observed in isolated microtubule protein (5-7). These results are relevant to elevated temperature effects (a) on cell shape (8-10) (b) in reducing the ability of treated cells to interact with other cells, (10,11) or cultured substrates (12) and (c) in restricting the mobility of cell surface components (13).

This paper describes a comparative study of the assembly and disassembly characteristics of microtubules isolated from several homeothermy species. One of our recent studies indicates that microtubule isolated from calf (body temperature about 38.5°C) will disassemble at 41°C and will denature at 43°C(5). Since the body temperature of many homeothermy species is either higher or lower than that of bovine's body temperature, it thus would be interesting to determine whether the disassembly process of the microtubule isolated from these different species occurs at different temperatures.

MATERIALS AND METHODS

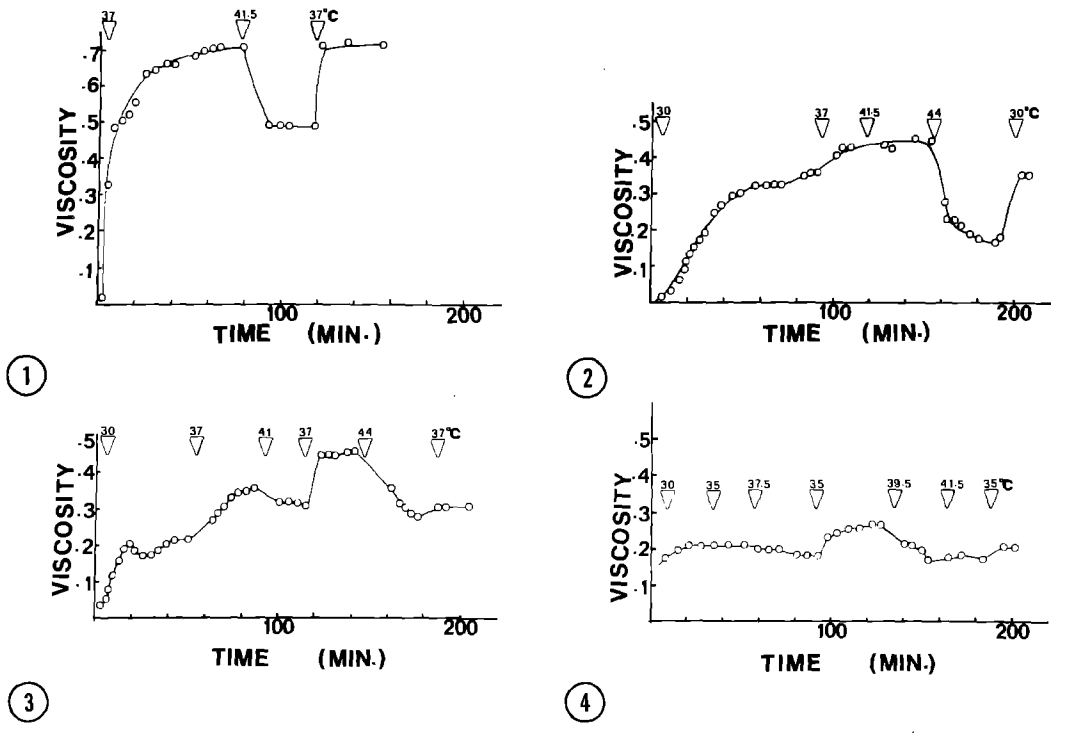
Calf brain was obtained from a local slaughter house and immediately chilled on ice. After the menings and superficial blood vessels were removed, the brain tissue was minced at 4°C in buffer (14) which contains 50 mM Mes-1mM EGTA-1mM GTP-0.5mM $MgCl_2$; pH 6.4. A motor-driven glass homogenizer with a teflon pestle was used for preparing homogenates (1 ml of buffer per gram of tissue). The homogenate was centrifuged at 100,000 x g for 1 h at 4°C. (15).

The pellet was discarded and the supernatant as crude extracts was used in experiments. Some experiments were done with purified microtubule protein isolated by two cycles of temperature dependent assembly-disassembly process (15). The same procedure as above was used to prepare crude extracts from chicken (from local poultry farms), rat and mouse (common laboratory strains). Microtubule assembly-disassembly was determined by viscometry (7) or by turbidity (16) assays.

RESULTS

Temperature sensitivity of calf microtubule extracts

Viscometers containing aliquots of an extract were incubated at 37°C until a stable viscosity was reached; the samples were then shifted to various experimental temperatures for various specific viscosity. Crude extracts of calf brain microtubule prepared both in the presence and absence of glycerol (final concentration 4M) were used in our experiments. We observed slightly different initial viscosity development kinetics between these extracts. The brain extract prepared with glycerol and incubated at 37°C showed a gradual increase in its specific viscosity obtaining the maximum level of stable specific viscosity in about 60 min. Whereas the brain extract prepared without glycerol and incubated at 37°C showed a very rapid



Figures 1-4: Temperature effect on the viscosity development (viscosity without colchicine - viscosity with 0.1mM colchicine) of microtubule extract from calf brain (Figure 1); chicken brain (Figure 2); rat brain (Figure 3) and mouse brain (Figure 4).

increase in its specific viscosity-within 10 ∿ 15 min. However, the specific viscosity value also falls rapidly and gradually levels off to a lower value of stable specific viscosity than the extract prepared with glycerol. The total time of incubation needed to reach final stable specific viscosity was again about 60 min. Despite the initial assembly kinetic differences between these two kinds of extract preparation, their temperature sensitivities were identical. When the stable specific viscosity was attained, the viscometer was quickly moved into another water bath set at the desired temperature. We consistently observed a decrease in their specific viscosity after incubation at 41 ∿ 41.5°C with all of the calf brain extracts (whether it was prepared with or without glycerol). There was no further change in viscosity if the protein was maintained at 41 ∿ 41.5°C. On the other hand, the specific viscosity value was fully regained soon after the protein was cooled back to 37°C (Fig. 1). In the control experiment, identical amounts of brain extract containing 0.1mM colchicine were incubated at various temperatures in parallel, but no changes in specific viscosity were observed. Thus, this

result suggests that the observed changes in specific viscosity were due to the assembly or disassembly of microtubules.

The purified microtubule protein is not as sensitive to 41°C as crude extracts. To determine whether the temperature (41°C) sensitivity could be reconstituted, we added back the supernatant fraction obtained during the purification process and we observed the same temperature response as before. However, the disassembled microtubules could not be promoted to reassemble by shifting back to 37°C.

Temperature sensitivity of chicken microtubule extract

Incubating chicken brain extract at 30°C gradually increases its viscosity over a period of 90 min. Contrary to the calf brain extract, the viscosity of the chicken extract could be further increased when the incubating temperature was shifted to 41.5°C. However, warming it up to 45°C effectively disassembled the microtubules, and this was reversed after the incubation temperature was returned to 30°C (Fig. 2).

Temperature sensitivity of rat microtubule extract

The effect of temperature on rat brain extract was similar to that of calf brain extract. Temperatures up to 37°C enhanced the assembly whereas 41.5°C disassembled the microtubule, and this process again was reversible. However, the disassembly which occurred at 44°C was not reversible (Fig. 3).

Temperature sensitivity of mouse microtubule extract

In comparison with the other three kinds of brain extracts that have been tested, mouse brain extract requires the lowest temperature to disassembly its microtubule. Microtubule assembly was enhanced at 35°C and the reversible disassembly occurred at 39.5°C. Incubating mouse extract at 41.5°C caused the microtubule to irreversibly disassemble (Fig. 4).

Temperature sensitivity of purified calf microtubules mixed with mouse microtubule or chicken microtubule extracts.

As described earlier (5), if the calf brain extract was separated into microtubule protein and supernatant fractions, the microtubules become less sensitive to 41°C. However, the sensitivity could be re-established if the two fractions were combined. Thus, it would be of interest to determine the temperature sensitivity of microtubules when the microtubule protein and non microtubule supernatant fractions from different species are mixed together. In preliminary studies, we observed that the addition of mouse microtubule extract to purified calf microtubule protein would change the calf microtubule disassembly temperature from 41°C to 38°C (Fig. 5). This is close to the characteristic temperature for mouse microtubule disassembly. In a similar experiment, a supernatant portion from chicken brain was added to purified calf microtubule protein. The disassembly temperature was shifted to 44°C

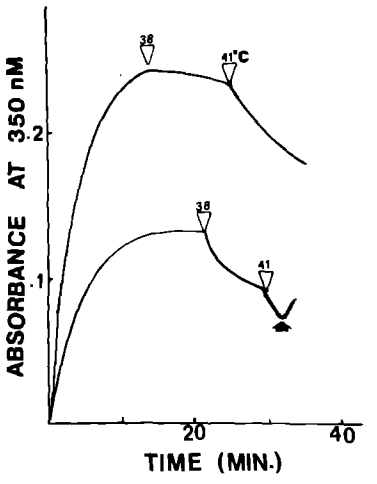


Figure 5: Temperature effect on the turbidity development of purified calf microtubule protein (top line) and purified calf microtubule protein with equal amounts of mouse microtubule extract added (bottom line) and were monitored at 350mM. After the absorbance attained at plateau at 35°C, the temperature was changed to 38°C then to 41°C. Denaturation occurred at the point indicated by .

which approximates the disassembly temperature of chicken microtubule (data not shown).

DISCUSSION

In agreement with that reported by others (1-7), elevated temperatures disassemble microtubule and can also cause the microtubule to denature. However, our results emphasize the following:

1. Crude extracts of microtubules from different species have different polymerization and depolymerization temperatures which are in the close range of physiological and high fever temperatures. The body temperatures for mouse, rat, bovine and chicken are 36.5, 38.2, 38.5 and 41.9°C respectively. Thus, the microtubule assembly temperatures are slightly less than 37°C for mouse, slightly greater than 37°C for rat and calf, and greater then 41.5°C for chicken. The microtubule disassembly temperatures are 39.5°C for mouse, 41.5°C for rat and calf, and about 45°C for chicken. Figure 6 suggests a direct correlation between body temperature and microtubule depolymerization in temperature ranges which closely approach lethal body temperatures for their respective species (17). Elevated temperatures have been shown to be able to disrupt microtubule organization in vivo and to affect cellular function and behavior which depend on the microtubule organization. In addition, elevated temperatures can

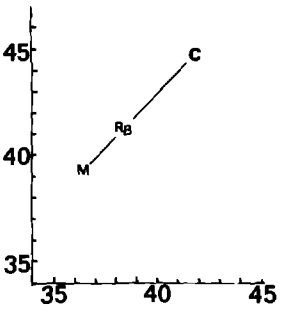


Figure 6: Correlation between normal body temperature (→) and microtubule depolymerization temperature (↑). M=mouse; R=rat; B=bovine; C=chicken.

produce lethal cytotoxic effects. Forty three degrees celsius, a temperature which can denature proteins (18), has been considered to be a critical temperature in hyperthermic cytotoxic analysis (18,19). The species of the cells frequently used for cytotoxicity studies are human and small mammals. These have either similar or lower normal body temperatures than bovine body temperatures. To further emphasize the relationship between normal body temperature and lethal temperature of cells in culture, maximum temperature of survival for chick fibroblast (normal body temperature is about 42°C) and gonadal cells from rainbow trout (normal body temperature is about 12°C) had been shown to be 46.5°C and 26°C, respectively (20,21). It is thus conceivable that microtubule organization could serve as thermomediator(s) which modulate heat responses. This notion agrees with the earlier suggestion by Atema (22) that microtubules are active, functional units in the reception and transduction of environmental stimuli via stimulus-specific conformational changes. In this regard, heat treatments have been shown to block and reverse early stages of oral morphogenesis in Tetrapymenas. This is attributed to the repression of tubulin synthesis by heat or merely the expression of a conformational change induced by heat (23).

2. It appears that microtubule from the brain crude extract and from the purified microtubular proteins respond to temperature differently, suggesting that the heat sensitivity of the microtubule might be changed by depolymerization factors (DPF) in the supernatant. This hypothesis is supported by the experiments mixing the microtubule and extract supernatant from two different species. There are several possible mechanisms which are relevant to the disruption of microtubules induced by elevated temperatures. The cold temperature used during the purification process may cause the release of the DPF. This DPF is probably different from the cold stabilizing factor (CSF) reported recently (24) due to the facts that CSF does not

dissociate from a microtubule at cold temperatures and that EGTA was used in our buffer which would minimize any dissociation of CSF from microtubule caused by Ca^{2+}. Elevated temperatures may promote the binding of DPF to microtubule. Or simply the complex of microtubule and DPF has an altered conformation which is a temperature sensitive entity.

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