Chinese Chemical Letters Vol. 11, No. 3, pp. 243–246, 2000

DSC Study on Brain Tubulin and the Effect of Cisplatin

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Abstract: The thermal property of the polymerization of brain tubulin was studied by a high-sensitivity differential scanning calorimeter. The phenomenon that heat flows increased and decreased consistently and obviously was observed. This phenomenon was called heat flow oscillation. It was probably correlated to the dynamic instability of microtubules. The effect of cisplatin on it was reported, too.

Keywords: Tubulin, cisplatin, DSC, heat flow oscillation.

Microtubules, a class of cytoskeletal elements, appear to be present in all eukaryotic cells. The temporal and spatial control of assembly and disassembly of microtubules is believed to be a key factor in cellular function. Microtubules and their subunits (α-β tubulin heterodimers, MW 110,000) maintain a dynamic equilibrium in the cell cytoplasm. In addition, it is postulated that the lability of cytoplasmic microtubules caused by drug is related to their equilibrium. Calorimetry method has been used to observe the polymerization of brain tubulin *in vitro*, while there are varieties of results recorded by different work groups[1-6]. Cisplatin, an anti-tumor drug, could interact not only with DNA but also with microtubule[7,8].

This paper reports a phenomenon different from the others, heat flow oscillation, in the polymerization of tubulin recorded by a high-sensitivity differential scanning calorimeter (DSC). The effects of cisplatin on tubulin polymerization are also reported.

Experimental

Proteins of porcine brain microtubule were isolated and purified by two cycles of polymerization and depolymerization using a modified method of Williams[9]. This procedure of tubulin preparation can not eliminate MAPs (microtubule-associated proteins). Further purification was as follows. The sample of tubulin (10-20mg) was resuspended in 3 ml 0.1M PEM buffer (0.1M PIPES, 1 mM EGTA, 0.5 mM $MgCl_2$, pH 6.9). The equal volume of 1M PEMD buffer (1M PIPES, 1.5 mM $MgCl_2$, 2 mM GTP, 20% DMSO) was added. So the final solution included 0.5M PIPES, 1 mM $MgCl_2$, 1 mM GTP, 10% DMSO. Tubulin began to polymerize after it was kept at

37℃ for 15 min as the turbidity of solution increased. It was centrifuged for 20 min at 35℃ (25,000g). The supernatant (including MAPs) was abnegated. The volume of pellet was estimated, then the pellet of tubulin was resuspended in 4 volumes of ice-cold 0.1 M PEM buffer. After it was kept at 0℃ for 20 min, the solution was centrifuged for 20 min at 4℃ (25,000g). The supernatant contained the pure tubulin protein free of MAPs. The concentration of tubulin was determined by the method of Bradford[10] with BSA as a standard. The pure tubulin was stored at -40℃. It was diluted to 2 mg/mL by 0.1 M PEM buffer. In order to avoid the thermal process induced by dilution, the diluted solution was kept for two hours at 4℃ before DSC measurement. In the experiments containing cisplatin, cisplatin reacted with tubulin for 4 h at 4℃ before the addition of GTP so that cisplatin could react with tubulin sufficiently before it reacted with GTP. All the experiments were finished within five days.

GTP used in our experiments came from Sigma (Type Ⅲ), and cisplatin was produced by Qilu Pharmaceutical Factory.

The course of the temperature-induced polymerization of tubulin was followed by a Micro DSCⅢ differential scanning calorimeter (Setaram, France), with a high sensitivity of 0.2 μW. The heating rate can be controlled to 0.001K/min. 0.6 mL solution of tubulin (2 mg/mL) was added into the sample cell, while the same volume of 0.1 M PEM buffer was added into the reference cell. Then the solution of GTP (50 mM) was added to 2 mM in both cells at 4℃ before the cells were put into the calorimeter. After a 10-min waiting for thermal balance in the calorimeter at 4℃, the temperature was ramped up at a rate of 1 K/min. The effects of cisplatin were observed with the same procedure.

Results and Discussion

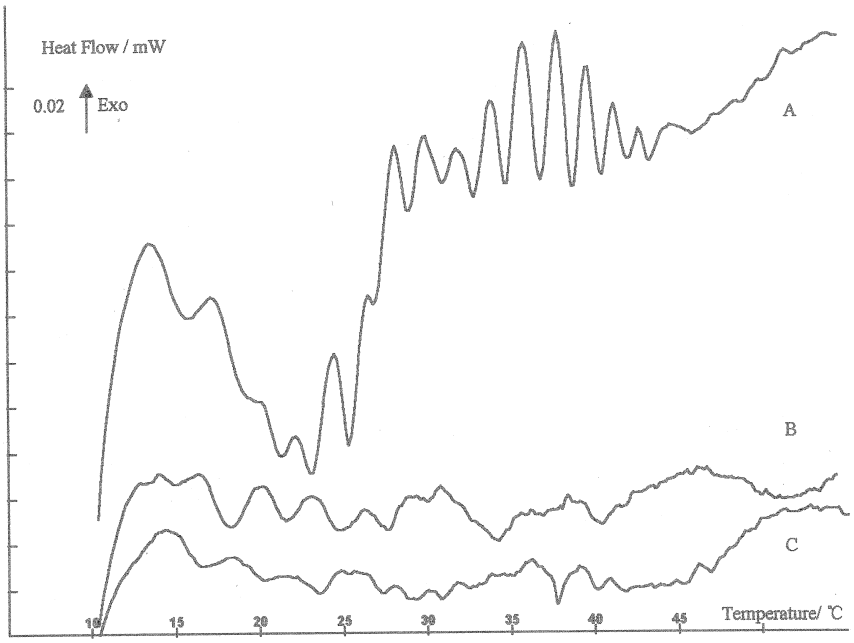
The DSC curve of tubulin polymerization is shown in **Figure 1 A**. Different from the other experiments[2-4], it was not showed as one peak or two peaks. Instead, the phenomena that heat flows increased and decreased consistently and obviously were observed. This phenomenon is called heat flow oscillation. The shapes of the curves in several repeated experiments were not identical completely, but the phenomena of oscillation repeated well. It is seen from **Figure 1** that the swing is up to 60 μW. The interval between peak and peak is about 2.4 ~ 4℃. It continues until nearly 45℃.

Curve B and curve C show the effects of cisplatin at different concentrations respectively. The oscillation still exists in the low concentration of cisplatin (7 μM), while the swing decreases to less than 20 μW, and it remains to the temperature lower than 30℃. When the concentration of cisplatin increases to 95 μM, the oscillation nearly disappears.

In the studies of the polymerization of tubulin by DSC, different work groups observed the various phenomena. Berkowitz *et al.*[3] observed an exothermic process, while Klump *et al.*[4] observed an endothermic process. Hinz *et al.*[2] observed a more complex process comprising an endothermic and an exothermic peak. In our experiments, a heat flow oscillation was observed. Such a variety of phenomena show the susceptibility of tubulin to the environment. There are many factors that exert influence on the polymerization of tubulin, such as the state of protein sample,

the concentration of tubulin, the ingredient of buffer, and so on. The presence of MAPs exerts an important effect on it. In the presence of MAPs, the polymerization of tubulin is inclined to show a single peak in DSC[3,4]. However, in the absence of MAPs, the polymerization of tubulin is inclined to show a complex thermal process, such as the results of Hinz[2] and ours. These phenomena illustrate the complicacy of the actions of tubulin. The important functions of microtubule *in vivo* are related to the complicacy. The dynamic instability of single microtubule[13-15] was discovered. The oscillation we observed in heat flow might be the reflection of the dynamic instability of the population of microtubules.

Figure 1. DSC curves of tubulin polymerization and the effect of cisplatin



A) tubulin (2mg/ mL) + GTP (2mM) B) tubulin (2mg/ mL) + cisplatin (7 μ M)+ GTP (2mM)
C) tubulin (2mg/ mL)+ cisplatin (95 μ M)+ GTP (2mM)

With the increasing of cisplatin, the heat flow oscillation was obscured. It suggests that cisplatin can suppress the dynamic instability of microtubule. This is probably related to the anti-tumor activity and side effect of cisplatin.

In our experiments, the oscillation of heat flow was observed. It is not occasional. The history of oscillations in chemical systems is long, and its richness has increased greatly over the last three decades of intense activity[11]. In an oscillating chemical reaction the concentrations of reagent undergo oscillations in time. This behavior is driven by the Gibbs-free-energy decrease of an overall chemical reaction occurring far from thermodynamic equilibrium. Some phenomena of oscillation in biology were reported, such as the oscillation of the viscidity in the

process of actin polymerization[12], the dilution of tubulin[5]. The phenomenon of heat flow oscillation is significant in cell and might be a commonness of the proteins, which can assemble and be disassembled. The micromechanism of the oscillation in chemical and biological system is extremely complex. The deep research on it is highly needed.

Acknowledgment

This work was supported by the National Natural Science Foundation of China (29873005).

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Received 13 October 1999