Enthalpy Changes in Microtubule Assembly from Pure Tubulin†

H.-J. Hinz‡ and Serge N. Timasheff*

Institut für Biophysik und Physikal Biochemie, University of Regensburg, D 8400 Regensburg, Federal Republic of Germany, and Graduate Department of Biochemistry, Brandeis University, Waltham, Massachusetts 02254

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ABSTRACT: The enthalpy changes that occur in the self-assembly of tubulin into microtubules were examined by adiabatic differential heat capacity microcalorimetry and by isothermal batch microcalorimetry. Tubulin solutions at concentrations between 7 and 17 mg/mL were heated from 0 to 40 °C at heating rates of 1 or 2 deg/min in pH 6.8 or 7.0 assembly buffers containing 20 mM MES, 100 mM glutamic acid, 5 mM $MgCl_2$, 3.4 M glycerol, and either 0.5 mM GMP-PCP or 1 mM GTP. The assembly reaction in the presence of GTP was characterized by a complex heat-uptake pattern consisting of a broad endotherm with a sharper exotherm superimposed on it, similar to assembly in a GTP phosphate buffer [Hinz, H.-J., Gorbunoff, M. J., Price, B., & Timasheff, S. N. (1979) *Biochemistry 18*, 3084]. Replacement of GTP by the nonhydrolyzable analogue resulted in a pattern typical for an endothermic reaction only. These results have permitted the assignment of the endothermic process to microtubule assembly and of the exothermic process to the resultant GTP hydrolysis. In these studies equilibration was found to be slow, several hours of cooling being required for the system to return to its original state. Turbidity scans also revealed hysteresis between consecutive scans and a displacement of the depolymerization transition midpoint to a lower temperature than that of assembly. The disassembly of microtubules was examined in batch calorimetry experiments in pH 7.0 phosphate, 1 mM GTP, 16 mM $MgCl_2$, and 3.4 M glycerol, in which tubulin assembled into microtubules was diluted to below the critical concentration. The disassembly process was characterized by a heat capacity change, ΔC_p, of +2650 ± 1500 cal/(deg·mol). The absolute values of the heat release were greater than the van't Hoff enthalpy change during microtubule assembly. This difference was assigned to the nonidentity of the assembly and disassembly processes.

In a previous microcalorimetric investigation of tubulin self-association into microtubules (Hinz et al., 1979) it has been shown that this process is characterized by a complex heat absorption behavior: heat is first absorbed and then emitted before the system reaches a new state of equilibrium. A sharp increase in turbidity accompanies the second, i.e., exothermic, process. Following the suggestion by Weisenberg et al. (1976) that GTP[1] hydrolysis either accompanies or follows polymerization, it was proposed (Hinz et al., 1979) that the exothermic step may be hydrolysis of GTP to GDP during microtubule formation. In order to check this hypothesis, similar experiments have been carried out now with a nonhydrolyzable analogue of GTP, namely GMP-PCP, in place of GTP. The results are reported in this paper.

Materials and Methods

All chemicals were reagent grade, unless specified otherwise. Analytical-grade glycerol was obtained from Merck, Darmstadt. The GTP was a Sigma product, sodium salt, Type II-S. GMP-PCP was from Boeringer, Mannheim Biochemicals. Alkaline phosphatase from bovine intestine (Type VII-S), MES[1], and L-glutamic acid were purchased from Sigma. Ultrapure guanidine hydrochloride from Heico was filtered through a glass filter prior to use.

Sample Preparation. Tubulin was prepared from calf brains according to the modified Weisenberg procedure (Weisenberg et al., 1968; Lee & Timasheff, 1977; Na & Timasheff, 1981). The protein, stored in 1 M sucrose at −70 °C, was thawed and equilibrated with the proper buffer on a dry (6 mL) and then a wet (5 mL) column. In the case of GTP experiments, GTP was present in the buffer. Protein concentration was measured spectrophotometrically at 274 nm, in 6 M GuHCl, using an absorptivity value of 1.03 mL/(g·cm). All the scanning microcalorimetry experiments, as well as the Gilford ones, were made in a glycerol-containing MES assembly buffer. The buffer composition was 100 mM glutamic acid, 20 mM MES, 5 mM $MgCl_2$, 3.4 M glycerol, pH 6.8 or 7.0. The batch dilution experiments were performed in the phosphate assembly buffer, which consisted of 1 mM sodium PO_4, 16 mM $MgCl_2$, 1 mM GTP, 3.4 M glycerol, pH 7.0. The analogue was put on the tubulin following the procedure of Purich and MacNeal (1978) as modified by Wu and Timasheff (unpublished results) to ensure total replacement of GTP by the analogue. The tubulin was equilibrated with the MES assembly buffer on the dry and wet columns. It was then incubated at 37 °C for 50 min, during which time assembly took place and the exchangeable-site GTP was hydrolyzed to GDP. After 20 min of cooling in ice, $ZnCl_2$ was added to a final concentration of 0.03 mM and alkaline phosphatase to a level of 4 units/mg tubulin. This mixture was then incubated for 20 min in an ice bath to hydrolyze the remaining GTP. EDTA was then added to sequester the Zn^{2+} ions and prevent assembly to sheets (Gaskin et al., 1980; G.-M. Wu and S. N. Timasheff, unpublished results). This was followed by addition

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* Address correspondence to this author at Brandeis University.

‡ University of Regensburg.

[1] Abbreviations: GTP, guanosine 5′-triphosphate; GDP, guanosine 5′-diphosphate; GMP-PCP, guanosine 5′-(β,γ-methylenetriphosphate); MES, 2-(*N*-morpholino)ethanesulfonic acid; PIPES, 1,4-piperazinediethanesulfonic acid; EGTA, [ethylenebis(oxyethylenitrilo)]tetraacetic acid; MAPs, microtubule-associated proteins.

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Table I: Transitions in the Scanning Microcalorimetry of Microtubule Assembly

tubulin concn (mg/mL)	heating rate (°C/min)	time (h)	endotherm transition midpoint (°C)	endotherm ΔH_{app} (kcal/mol)	exotherm transition midpoint (°C)	exotherm ΔH_{app} (kcal/mol)
			In the Presence of GMP-PCP			
9.8	1	2	22.8	25.8		none
	1	5	18.3	22.4		
	1	8	17.3	24.0		
13.4	1	2	22.0	19.4		none
	1	5	17.1	29.8		
	1	8	17.7	21.6		
	1	28	21.9	26.1		
	1	30	23.0	9.8		
10.3	1	2	19.4	12.6	19.4	−5.5
	1	5	19.9	7.9		
	1	25	21.8	39.9		
	1	28	21.3	16.1		
16.6	2	2	23.8	7.6		
	2	4	18.7	19.3		
	2	6	16.9	18.8		
	2	8	16.4	23.6		
	2	54	18.9	15.3	20.9	−5.9
	2	57	19.0	13.5	23.5	−10.1
	2	60	18.9	11.3	22.3	−7.7
	2	63	18.9	10.9	22.1	−5.6
	2	66	19.8	8.7	22.0	−3.8
			In the Presence of GTP			
11.9	2	6	21.3	61.6	22.9	−9.4
	2	9	20.9	86.8	25.8	−55.5
	1	30	21.1	47.8	22.5	−5.9
7.2	2	6	21.0	81.0	25.5	−1
	2	9		not normal	26.8	−62.7
	2	12		not normal	27.5	−70.5
	2	15		not normal	28.6	−71.5
	2	18		not normal	30.3	−80.3
	2	21		not normal	37.0	−84.6

of GMP-PCP to the final desired concentration. The solution was then kept on ice for various lengths of time depending on the concentration of the analogue.

Scanning Microcalorimetric Measurements. The scanning microcalorimetric studies were performed with an electronically modified high-sensitivity Privalov Type DASM 1 microcalorimeter, which permits heating rates from 0.005 to 2 deg/min (Privalov et al., 1975). The heat capacity and temperature data were routinely registered every tenth of a degree by a computer. For each measurement, control runs with buffer-filled cells, which involved electrical energy calibration, were performed to establish the base line. The calculation of molar polymerization enthalpies was based on a molecular weight M of 110000, taken for the α–β tubulin dimer (Lee et al., 1973). For each experiment, the cold tubulin solution was loaded into the microcalorimeter and equilibrated to thermal stability and the heating was carried out at rates between 0.5 and 2 deg/min. Simultaneously the change in turbidity was followed on a Gilford instrument at the same rate of heating.

Isothermal Microcalorimetric Measurements. The isothermal batch microcalorimetry measurements were performed with an LKB 10700 microcalorimetry system. Tubulin solutions at concentrations between 3 and 15 mg/mL and 0.3–0.7 mL in volume, prepared in the phosphate assembly buffer, were loaded into the calorimeter cell and equilibrated for 1–2 h at the desired temperature. These solutions were then diluted to below the critical concentration by addition of appropriate amounts of the buffer, and the heat absorption (or evolution) was determined. Identical amounts of buffer were mixed in the reference cell. All the cell filling was done by weighing the samples, and the calculation of the actual amount of tubulin depolymerized by dilution took the densities of the solutions into account.

Results

Adiabatic Scanning Microcalorimetry. The results of the microcalorimetric scanning experiments are summarized in Table I. Typical scanning microcalorimetric tracings in the presence of the analogue are shown in Figure 1. The pattern is characterized by a broad endothermic reaction, the heat-uptake phase spanning some 10 °C. The reaction is reversible. Sequences of heating and cooling resulted in heat uptake during each consecutive heating phase. Comparison of consecutive scans, however, indicates hysteresis and a complex kinetic pattern. Thus, as seen in Figure 1A, consecutive cycles of heating led to a sharpening of the heat-uptake peak at each cycle with a shift of the transition to lower temperature. Prolonged cooling (overnight) resulted, however, in a loss of cooperativity, a return of the transition temperature to its original value, and a general restoration of a pattern similar to the one obtained with a fresh protein solution that had always been kept at ice temperature, as shown in Figure 1B. Integration under the microcalorimetry scans resulted in fairly constant values of the apparent heat uptake, shown in column 4 of Table I, indicating close to complete reversibility between scans. Turbidimetric experiments, shown in Figure 2, carried out in parallel with the calorimetric scans and at the same heating rate, indicated a transition at the same temperature as the maximum in the heat capacity tracing. The process was fully reversed by cooling, although the decrease in turbidity occurred at a temperature considerably below that of the turbidity increase. As seen in Figure 2A, the cooling pattern was less cooperative than the heating one. Again hysteresis

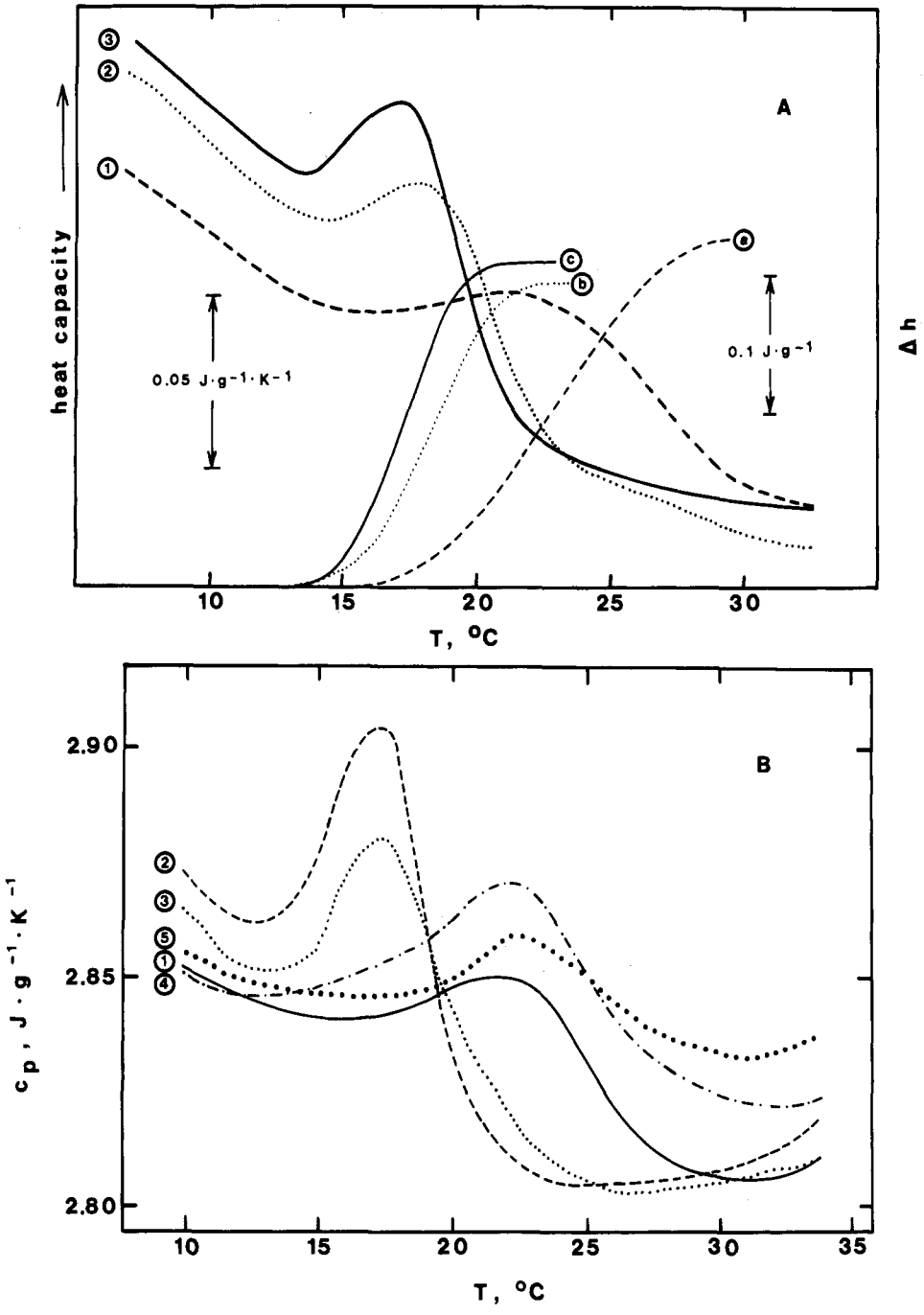

FIGURE 1: Scans of the apparent heat capacity change of tubulin. In MES assembly buffer, pH 7.0, 0.5 mM GMP-PCP; scan rate, 1 deg/min. The numbers 1, 2, and 3 indicate the first, second, and third consecutive scans of the same solution. (A) 9.8 mg/mL tubulin. The start of the consecutive heating cycles was at 2, 5, and 8 h after the preparation of the sample. The lettered curves refer to integrated heat capacity scans. These curves represent the apparent specific enthalpy change, ΔH in J/g, as a function of temperature. The letters correspond in alphabetical order to the numbers 1, 2, 3 ... (B) 13.4 mg/mL tubulin. Measurement 4 was performed after standing overnight at ca. 1 °C. The start of the consecutive heatings was at 2, 5, 8, 28, and 30 h following sample preparation for scans 1, 2, 3, 4, and 5, respectively. The values of the heat capacity and enthalpy changes, marked on this and some subsequent figures, indicate the general magnitude of the measured effect but should not be interpreted as absolute intrinsic values of these parameters for the system under investigation.

was observed, since a second heating that followed the cooling resulted in a shift of the turbidity transition during assembly to a lower temperature and a sharpening of the transition, as shown in Figure 2B.

The complexity of the assembly process in the presence of the GTP analogue is further evident from experiments in which heating was started immediately after addition of the analogue, without the several hours of cold incubation. Thus, in a typical experiment, shown in Figure 3, if heating was started immediately after addition of the analogue the obtained tracing showed essentially no heat-uptake or release effects (scan 1). Cooling for 3 h resulted in a heat-uptake pattern characterized by a endothermic process (scan 2), the amplitude of the heat uptake increasing if the cooling was prolonged overnight (scan 3).

When identical experiments were carried out in a buffer containing 1 mM GTP rather than the analogue, the results were totally different. In Figure 4 are shown consecutive scans in heating and cooling cycles. It is evident that the pattern is characterized by an endothermic process on which is superimposed an exothermic one, just as had been observed in the phosphate assembly buffer (Hinz et al., 1979). The degree of cooperativity is, however, much smaller than in the phosphate buffer. The patterns of consecutive scans, however, are

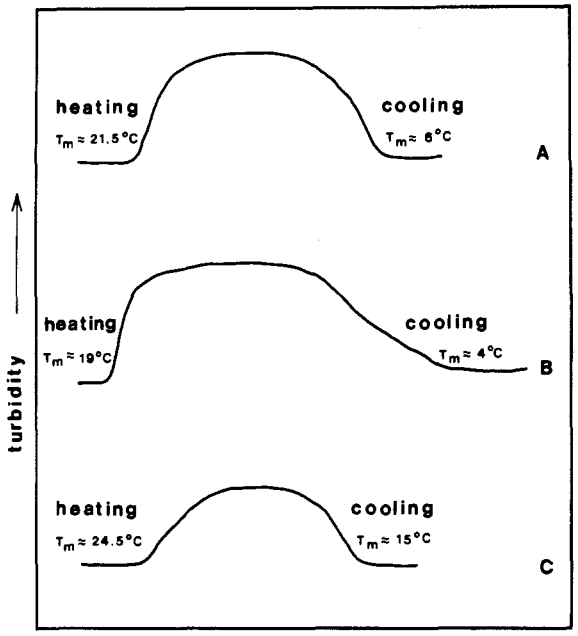

FIGURE 2: Turbidimetric scans at 400 nm of the polymerization behavior of tubulin: (A) 9.8 mg/mL tubulin in MES assembly buffer, pH 7.0, 0.5 mM GMP-PCP; heating rate, 1 deg/min; (B) repetition of A after 1 h at 2 °C; (C) analogous turbidimetric scans in the presence of 1 mM GTP. T_m is the midpoint of the apparent transition. The abscissa corresponds to temperature, first increasing and then decreasing.

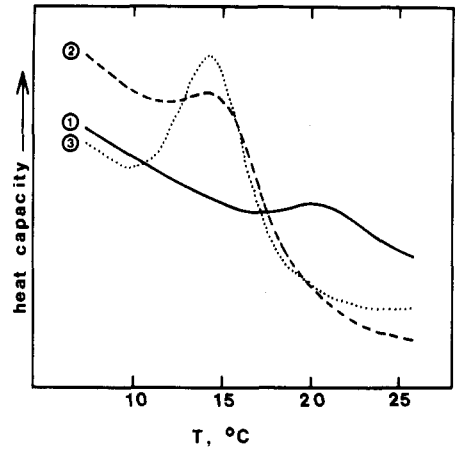

FIGURE 3: Scans of the apparent heat capacity change of tubulin (12.8 mg/mL) in MES assembly buffer pH 7.0; 0.5 mM GMP-PCP; scan rate, 0.5 deg/min. Scan 1: immediately after addition of the GTP analogue; scan 2: after 3 h, scan 3: after standing overnight at 1 °C.

not identical. Furthermore, the resolution of the exothermic contribution is a function of the rate of heating, indicating a complex kinetic pattern. In fact, consecutive heating and cooling cycles developed a series of scans, shown in Figure 5, characterized by a progressive displacement of the exotherm to higher temperatures with a small increase in the apparent heat release. Eventually this led to a total disappearance of any heat-uptake or release pattern, the scan becoming identical with the base line. Gilford experiments, carried out in parallel, showed the process to be reversible, as shown in Figure 2C. The decrease in turbidity on cooling was significantly more cooperative than its generation on heating and the transition midpoint was shifted to a lower temperature.

Comparison of the microcalorimetric scans in the presence of GTP and its nonhydrolyzable analogue again strongly suggests that the exothermic part of the scan corresponds to the hydrolysis of GTP upon assembly. This was supported

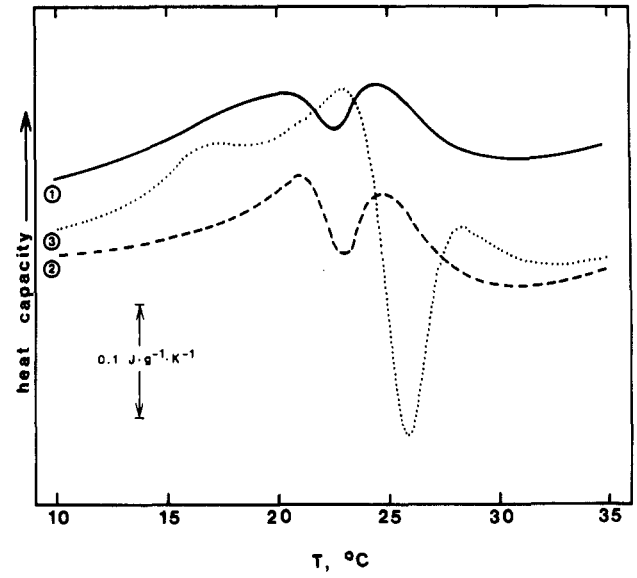

FIGURE 4: Scans of the apparent heat capacity change of tubulin (11.9 mg/mL) in MES assembly buffer pH 7.0, 1 mM GTP; scan rate, 2 deg/min for scans 1 and 2, 1 deg/min for scan 3. Tracings 1 (—) and 2 (– – –): successive scans with an interval of ca. 3 h; tracing 3 (···): scan after standing of the solution at 1 °C overnight.

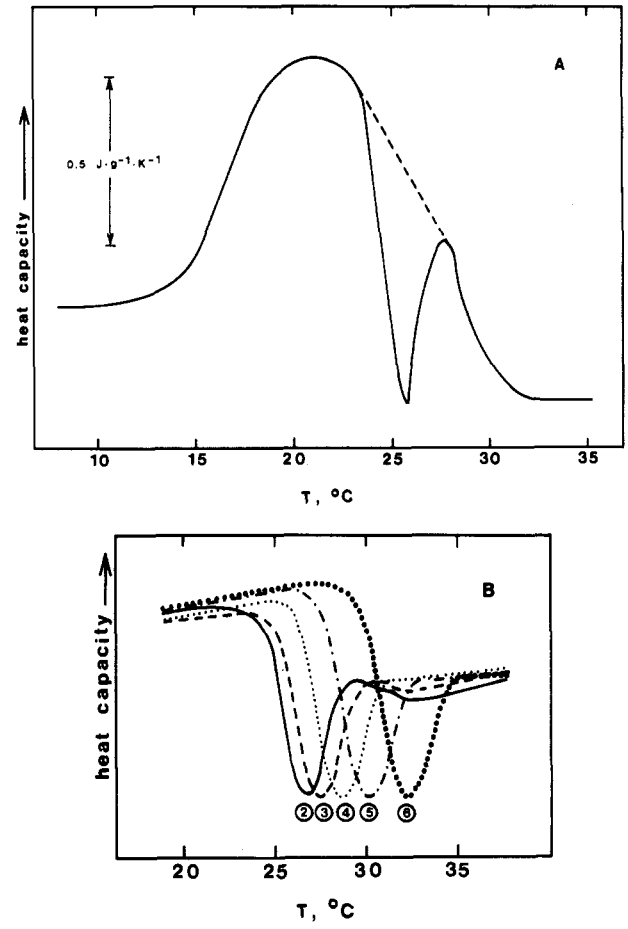

FIGURE 5: Scans of the apparent heat capacity change of tubulin (7.2 mg/mL) in MES assembly buffer, pH 7.0, 1 mM GTP; scan rate, 2 deg/min: (A) scan 6 h after thawing of frozen tubulin; (B) next 5 consecutive scans, at 3-h intervals, demonstrating the decrease of the endotherm and the shift of the exotherm to higher temperatures.

by a control experiment in which the tubulin was equilibrated with assembly buffer that contained no nucleotide. In a microcalorimetric experiment this protein showed one normal pattern during the first cycle of heating, characterized by heat

uptake and heat release. When this sample was cooled and heated for a second time, the scan obtained differed little from the base line. Addition of GTP at this point restored again the characteristic scanning pattern upon heating in the calorimeter. Thus, hydrolysis of the endogenous GTP during the first cycle of assembly exhausted the ability of this protein to assemble into microtubules and to generate either an endothermic or an exothermic effect.

The appearance of an exotherm was not limited to experiments in the presence of GTP. In some experiments with the analogue, an exotherm superimposed on the endotherm was observed on occasion and in an erratic manner. This exotherm either vanished after a new cooling and heating cycle or was displaced to a lower temperature, which is opposite to what is observed in the presence of GTP. While the reasons for the irreproducible appearance of this exotherm is not understood at present, it seems most plausible to assign it to either the process of binding of the analogue to the protein or to a conformational transition of the tubulin, if it is not an aberrant artifact of the instrument. This hypothesis is supported by the results of the experiment shown in Figure 6. Here tubulin in the presence of the analogue was subjected to an extensive sequence of heating and cooling cycles. In the first four cycles, shown in Figure 6A, the system is seen to follow the expected pattern. Heating produces an endothermic effect, the transition becoming more cooperative and its midpoint shifting to a lower temperature with each consecutive cycle of heating and cooling. After four such cycles, which took a total of 8 h, the solution was left overnight, during which time a series of repeated heating and cooling cycles was carried out automatically. The resulting pattern of tracings, shown in Figure 6B, clearly depicts a transition in the protein, the maximum in the scan shifting from a low temperature to a high temperature, with a well-defined isothermic point. Following this, the sample was cooled for 24 h and calorimetric scans were started again. These, as shown in Figure 6C, had now assumed the appearance of a broad endotherm on which was superimposed an exotherm. The endotherm was very broad, spanning over 14 °C, and the degree of cooperativity was low. Furthermore, repeated heating and cooling cycles at this point produced erratic changes both in the position and in the breadth of the pattern. However, the areas under both the heat-uptake and heat-release portions of the patterns decreased with successive scans, as shown in Table I. This strongly suggests the gradual occurrence of irreversible processes and most probably reflects progressive slow denaturation of the protein accompanied by a loss of ability to assemble into microtubules (Prakash & Timasheff, 1982) and possibly to bind the nucleotide. After 14 further cycles of heating and cooling over an additional 24 h, the calorimetric scans had lost all features and essentially resembled the base line.

The exotherms observed in the present study are not related to the strong exothermic reaction reported in the literature (Berkowitz et al., 1980). These authors had reported the presence of a strong exotherm in a GTP-containing buffer, the amount of heat released per tubulin molecule increasing greatly with a decrease in protein concentration, even below the critical concentration. To test whether this reaction is related to those reported in the present paper, control experiments were carried out with our protein in the Berkowitz et al. (1980) assembly buffer (0.1 M PIPES, 2 mM EGTA, 1 mM $MgSO_4$, 1 mM dithiothreitol, 2 mM GTP, 2 M glycerol, pH 6.9) at concentrations above and below the critical concentration for microtubule assembly. In no case was an exothermic reaction observed. Above the critical concentration the system displayed a very weak, broad endotherm, while below the critical concentration the scan deviated little from the base line, indicating the absence of any heat-releasing or heat-absorbing processes. Since our work was carried out with highly purified tubulin prepared by the modified Weisenberg procedure, while the study described in the literature (Berkowitz et al., 1980) was carried out with cycle tubulin in the presence of MAPs, we ascribe the difference in the results to the difference in protein preparations. It seems reasonable to suggest that the strong exotherm reported in the literature (Berkowitz et al., 1980) is related to a reaction involving MAPs, possibly the

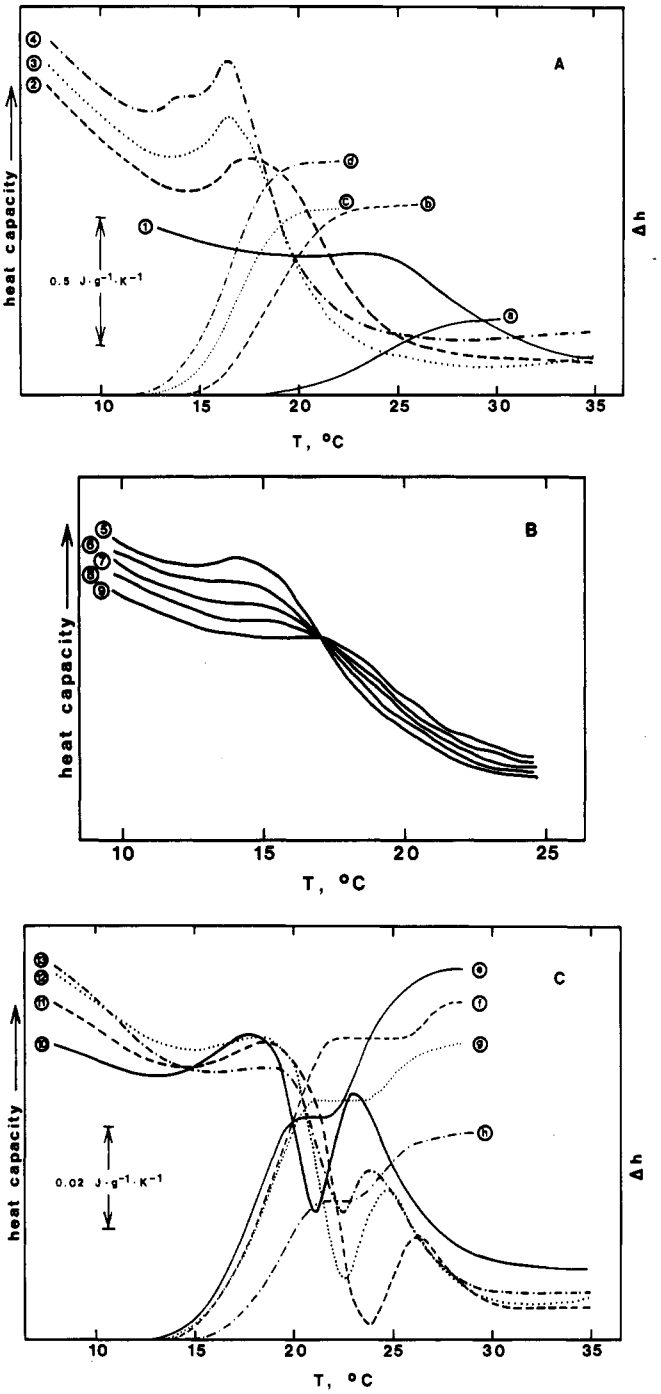

FIGURE 6: Scans of the apparent heat capacity change of tubulin (16.6 mg/mL) in MES assembly buffer, pH 7.0, 0.5 mM GMP-PCP; scan rate, 2 deg/min; (A) first four heating and cooling cycles, at 2-h intervals; (B) next 5 heating and cooling cycles, at 2-h intervals; (C) scans after cooling for 24 h at ca. 1 °C. The time intervals between scans for scans 10–13 are ca. 3 h each. The letters refer to the integrated $\Delta H = f(T)$ curves ((e) corresponds to scan 10, (f) to scan 11, etc.).

perturbation of a tubulin–MAP interaction.

In order to ascertain that the various effects seen in the scanning microcalorimetry experiments are related to tubulin self-assembly, a number of control experiments were carried out. These comprised (i) a solution of alkaline phosphatase and analogue in the assembly buffer, but no tubulin, to see whether any of the signals are due to possible contamination of the analogue with GTP; (ii) no addition either of analogue or of GTP to the tubulin solution, following the alkaline phosphatase treatment, to see whether any of the signals were due to either a tubulin–phosphatase interaction or an extraneous protein reaction, such as a temperature-dependent conformational change; (iii) a complete assembly system following the full preparatory procedure, but the tubulin being diluted to below the critical concentration just prior to calorimetry, to see whether any of the observed effects were due simply to a tubulin conformational change in the absence of assembly. In none of these experiments was there any deviation of the heating pattern from that of the base line between 0 and 40 °C, indicating that the observed effects are, in fact, related to the microtubule assembly process.

Scanning microcalorimetric experiments were also carried out in the presence of the analogue in the MES assembly buffer, but with the zinc ion sequestering step omitted from the sample preparation procedure. These again gave endothermic patterns that were slow to reverse on cooling. In fact, in the Gilford experiments, there was little disappearance of turbidity even when the temperature was decreased to 0 °C. Wu and Timasheff (unpublished results) have shown that failure to sequester the zinc ions results in the formation of sheets that may coexist with microtubules. These sheets are known to be difficult to disassemble (Gaskin & Kress, 1977). The present results demonstrate, however, that the intertubulin contacts formed during sheet formation, as well as during assembly into microtubules, are characterized by a positive change in enthalpy.

Isothermal Batch Dilution Microcalorimetry. The complexity of the kinetic processes involved during assembly makes it very difficult to interpret the values of the apparent enthalpy changes that accompany microtubule assembly, listed in Table I. The positive values obtained are of the same order of magnitude as those calculated from the van't Hoff analysis of assembly in the phosphate buffer (Lee & Timasheff, 1977). These values, however, cannot be regarded as true measurements of the enthalpy of microtubule assembly, since during the heating process the system is never at equilibrium, the heating rate being always faster than the assembly (Hinz et al., 1979). Attempts were made, therefore, to measure directly the enthalpy of assembly by dilution experiments in a batch microcalorimeter. To this end, tubulin solutions that had been assembled into microtubules in the phosphate assembly buffer were diluted with the same buffer to below the critical concentration and the heat emission was determined. Two series of experiments were carried out with two different preparations of tubulin. In each, the heat release on dilution was measured at two temperatures to see any possible change in apparent heat capacity during the disassembly process. The results are summarized in Table II. Even though the points are greatly scattered, the general pattern is one of heat release during the disassembly, i.e., an enthalpy change in the opposite direction from that obtained on assembly (Lee & Timasheff, 1977). Also, the heat change is greater at the lower temperature, just as in the assembly process. The difference between the apparent ΔH values at the two temperatures corresponds to an apparent change in heat capacity, ΔC_p, of +2500 ± 1500 cal/(mol·deg) that is the reverse of what had been measured for the assembly process both by van't Hoff analysis (Lee & Timasheff, 1977) and by microcalorimetry (Hinz et al., 1979). The absolute values of the apparent enthalpy changes, however, are considerably greater than the van't Hoff values measured for the assembly process. This, however, is not surprising, since the assembly and disassembly processes are not simple reversals of an equilibrium but differ by at least one irreversible step, namely, the hydrolysis of GTP to GDP. This difference may reflect not only the enthalpy change due to the GTP hydrolysis but also that resulting from conformational changes in the protein that must follow both the assembly, inducing GTPase activity, and the GTP hydrolysis, since the nucleotide in the GDP state when incorporated into microtubules becomes unavailable to contact with solvent, as it can neither be exchanged with solvent GTP nor phosphorylated to the GTP state (Carlier & Pantaloni, 1981; David-Pfeuty & Huitorel, 1980). While the current results are fully consistent with the above qualitative interpretation, a quantitative analysis of the heat-release pattern is precluded by the great scatter of the points. This is due most probably to a partial inactivation of the protein during the several hours of standing in the calorimeter while the system is brought to thermal equilibrium. Tubulin is known to undergo a slow transition on standing in solution (Prakash & Timasheff, 1982), one of its consequences being loss of ability to assemble into microtubules.

Table II: Apparent Enthalpy Changes during Microtubule Disassembly

temp (°C)	tubulin concn (mg/mL) starting	final	ΔH_{app} (kcal/mol)
	Series I		
25	6.2	0.56	−50.4
	7.0	0.67	−62.5
	9.5	0.80	−52.8
	10.2	0.98	−41.7
			av −51.9
37	6.2	0.61	−20.4
	10.2	0.97	−22.0
	12.5	1.25	−34.9
			av −25.8
	$\Delta C_{p,app}$ = +2200 cal/(deg·mol)		
	Series II		
25	5.4	0.51	−86.0
	6.8	0.65	−75.5
			av −80.8
35	5.6	0.56	−44.7
	7.2	0.74	−55.1
	8.0	0.82	−49.7
			av −49.8
	$\Delta C_{p,app}$ = +3100 cal/(deg·mol)		
	$\Delta C_{p,app,av}$ = +2650 ± 1500 cal/(deg·mol)		

Discussion

The results reported in this paper are fully consistent with the conclusion that, in the presence of GMP-PCP, microtubule assembly is a fully reversible (G.-M. Wu & S. N. Timasheff, unpublished results; Carlier & Pantaloni, 1986) endothermic process. The long times required to reach equilibrium point to a complex kinetic mechanism that may involve either the binding process of the analogue to the protein or a conformational transition in the tubulin. Thus, while the calorimetric analysis clearly shows the difference between the assembly process in the presence of GTP and GMP-PCP, the exact contributions to the heat changes of the various reactions going on during assembly cannot be assessed. It is known (G.-M. Wu & S. N. Timasheff, unpublished results) that the initial

and final states of the process of assembly in GMP-PCP are tubulin monomer and microtubules, but it is not clear for this or any other microtubule assembly system how much oligomerization takes place prior to microtubule formation. As a result, the heat changes along the polymerization route cannot be interpreted in more than a qualitative way. The slowness of the reversibility is fully consistent with the observations of Wu and Timasheff (unpublished results) that full assembly into microtubules requires several hours of incubation with the analogue prior to heating. Furthermore, the degree of cooperativity is different during assembly and disassembly, the first process being more cooperative, which is exactly the opposite of what is true when the nucleotide in the system is GTP. In that case, however, assembly takes place in the presence of GTP, while disassembly occurs with the protein in the GDP state, and the extent of cooperativity reflects possibly the relative stability of microtubules in these two states. Another factor pointing to the complexity of the assembly process in the presence of the nonhydrolyzable analogue is the shift in the midpoint of the transition, the increase in cooperativity with consecutive heating and cooling cycles, and the reversibility to the initial state after prolonged cooling. Therefore, assembly and disassembly in the presence of the analogue cannot be regarded as a simple easily reversible equilibrium. In fact, both reactions must involve a complexity of processes, the nature of which is not known at present.

The experiments in the presence of GTP are fully consistent with the above observations. Again assembly is characterized by an endothermic process. In this case, however, an exothermic process is always superimposed on the heat uptake. Since the exothermic reaction requires the presence of GTP, it may be equated with the hydrolysis of GTP, which is known to accompany normal microtubule assembly. The displacement of the exothermic step along the endothermic peak during consecutive scans shows that the coupling between the assembly reaction and GTP hydrolysis is loose. This is consistent with the proposal, first made by Weisenberg et al. (1976) and clearly demonstrated by Carlier and Pantaloni (1981) (see also: Carlier, 1982) that GTP hydrolysis follows self-assembly and is, therefore, a consequence of assembly and not its source of energy. It is also a direct demonstration that GTP hydrolysis is not closely linked kinetically to assembly but is a reaction that follows loosely the assembly step, a conclusion that has been reached by Carlier and Pantaloni from kinetic experiments (1981).

The present results may serve to clarify the observations reported earlier on the assembly of tubulin into microtubules in phosphate buffer (Hinz et al., 1979). From the patterns of overlap between the endothermic and exothermic processes in the MES–glutamic acid assembly buffer, described here, it is evident that the two thermal processes are not consecutive as might be inferred from the calorimetric scans in the phosphate buffer but overlap on each other, the endothermic process being broad. The great increase in the breadth of the transition in the MES buffer over the PO_4 buffer is consistent with the known fact that, in phosphate buffer, microtubule assembly has a much more cooperative character and occurs more rapidly than in the sulfonic acid buffers. This is supported by the fact that in the Gilford turbidity scanning experiments cooperativity did not seem to differ in the MES assembly buffer whether the solvent contained GTP or GMP-PCP.

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