Heat Capacity Microcalorimetry of the in Vitro Reconstitution of Calf Brain Microtubules†

Hans-Jurgen Hinz, Marina J. Gorbunoff, Barbara Price, and Serge N. Timasheff*

ABSTRACT: The self-assembly of calf brain tubulin, purified by the modified Weisenberg procedure, was examined in an adiabatic differential heat capacity microcalorimeter. Tubulin solutions at concentrations between 6 and 17 mg/mL were heated from 8 to 40°C at heating rates between 0.1 and 1.0 deg/min in a pH 7.0 phosphate buffer containing 1×10^{-3} M GTP, 1.6×10^{-2} M $MgCl_2$, and 3.4 M glycerol. The heat capacity change, ΔC_p, of the microtubule growth reaction was found to be -1600 ± 500 cal/(deg mol) per 110000 molecular weight tubulin dimer incorporated into microtubules, in agreement with the reported van't Hoff ΔC_p value of -1500 cal/(deg mol) [Lee, J. C., & Timasheff, S. N. (1977) *Biochemistry 16*, 1754–1764]. The assembly reaction is characterized by a complex heat uptake pattern comprising both endothermic and exothermic processes.

The in vitro reconstitution of microtubules was described first by Weisenberg (1972), who found that this reaction was favored by raising the temperature to 37 °C. This suggested that, just as in vivo (Stephens, 1973), the in vitro reconstitution proceeds with a positive enthalpy change. In detailed studies aimed at an understanding of the mechanism of microtubule self-assembly, Gaskin et al. (1974) and Lee & Timasheff (1977) examined this process as a function of temperature, working respectively on tubulin prepared by the Shelanski et al. (1973) cycle procedure and by the modified Weisenberg method (Weisenberg et al., 1968; Weisenberg & Timasheff, 1970; Lee et al., 1973). Analyzing their data in terms of the Oosawa & Kasai (1971) theory of helical polymerization, both groups reported curved van't Hoff plots, the enthalpy change of polymerization becoming increasingly positive with a decrease in the temperature. Lee & Timasheff (1977), furthermore, calculated that the addition of each tubulin subunit to a growing microtuble was accompanied by a change in heat capacity, ΔC_p, of -1500 cal/(deg mol). In view of these reports of the van't Hoff equilibrium analysis of this process, it seemed of interest to examine this question by direct calorimetric measurements. A heat capacity microcalorimetric study was undertaken, therefore, of the self-assembly of highly purified tubulin, and the results are reported in this paper.

Materials and Methods

Calf brain tubulin was prepared by the modified Weisenberg procedure (Weisenberg et al., 1968; Weisenberg & Timasheff, 1970; Lee et al., 1973; Frigon & Timasheff, 1975). It was stored in liquid nitrogen until use. The concentration of protein was determined by diluting the sample with 6 M guanidine hydrochloride and measuring the absorbance at 274 nm by using an absorptivity of 1.15 mL/(mg cm) (Lee et al., 1973).

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. In all experiments the assembly buffer consisted of 10^{-2} M potassium phosphate, 10^{-3} M GTP, 1.6×10^{-2} M $MgCl_2$, and 3.4 M glycerol at pH 7.0.

Heat capacity measurements were carried out in an adiabatic differential heat capacity DACM-1M microcalorimeter, described by Privalov et al. (1975). Tubulin solutions in the assembly buffer at total protein concentrations between 6.4 and 16.8 mg/mL were gradually heated from 8 to 45 °C at heating rates of 0.1, 0.25, 0.5, and 1.0 deg/min. The reference cell contained the assembly buffer.

The self-assembly of tubulin was monitored by measuring the change in turbidity at 350 nm according to the method of Gaskin et al. (1974), as described by Lee & Timasheff (1975, 1977). Furthermore, most of the scanning calorimetry experiments were accompanied by a parallel turbidity experiment in which the protein solution was heated at a rate identical with that in the microcalorimeter, using a Gilford Model 240 spectrophotometer, equipped with a Gilford Thermo-Programmer, Model 2527.

Table I: Heat Capacity Change in the Tubulin → Microtubule Reaction[a]

protein concn (mg/mL)	heating rate (deg/min)	$\Delta C_p \times 10^{-3}$ [cal/(deg mol)]
6.4	1.0	−1.8
16.8	1.0	−1.4
12.7	0.5	−1.8
10.2	0.5	−1.3
9.5	0.5	−1.8
9.1	0.5	−1.6
12.5	0.25	−1.5
		av −1.6[b]

[a] The buffer consisted of 0.01 M phosphate, pH 7.0, 0.016 M $MgCl_2$, 0.001 M GTP, and 3.4 M glycerol. [b] van't Hoff ΔC_p (Lee & Timasheff, 1977): -1.5×10^3 cal/(deg mol).

Results

Microtubule assembly from purified tubulin, free from MAPs and other complexing boosters of this reaction (Lee et al., 1978), was examined by differential scanning calorimetry. The results are presented in Figure 1 and Table I. It is seen that at all heating rates the heat uptake pattern is complex. As the temperature is raised from 8 °C, there is first a linear increase in the heat capacity of the protein solution up to about 23–24 °C. Above this temperature, the pattern is characterized first by a positive and then a negative peak, indicating a sequence of heat absorption and heat release. Finally, after an apparent small heat absorption region, a second region of monotone change in heat capacity sets in. This second region is displaced toward a lower heat capacity relative to that of

† From the University of Regensburg, Biochemie II, Fachbereich Biologie, Regensburg, West Germany (H.-J.H.), and the Graduate Department of Biochemistry, Brandeis University, Waltham, Massachusetts 02254 (M.J.G., B.P., and S.N.T.). *Received January 10, 1979.* This work is Publication No. 1254 of the Graduate Department of Biochemistry, Brandeis University. Supported by National Institutes of Health Grants GM-14603 and CA-16707 (S.N.T.), a grant from the Deutsche Forschungsgemeinschaft (H.-J.H.), and a grant from the Deutscher Akademischer Austauschdienst (S.N.T.).

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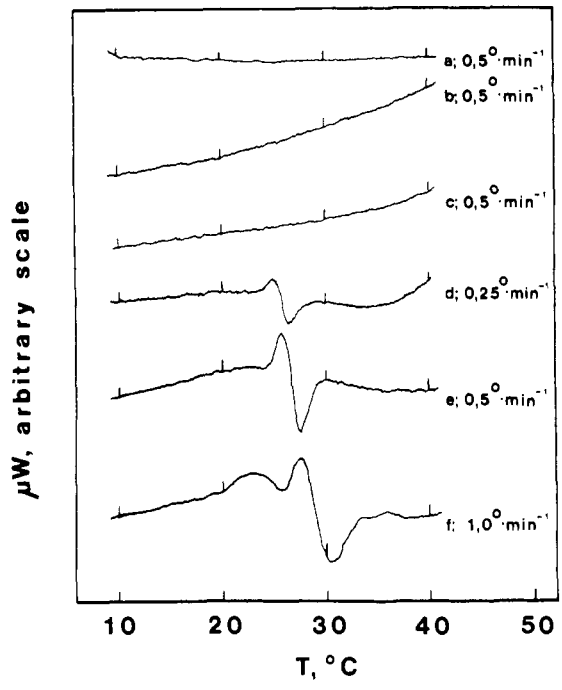

FIGURE 1: Heat uptake patterns during microtubule assembly of calf brain tubulin in an assembly buffer consisting of 10^{-2} M potassium phosphate, 10^{-3} M GTP, 1.6×10^{-2} M $MgCl_2$, and 3.4 M glycerol at pH 7.0. The heating rate is indicated next to each scan. (a) Assembly buffer alone, with assembly buffer devoid of GTP in reference cell; (b) 13.6 mg/mL tubulin in assembly buffer devoid of GTP and with the $MgCl_2$ concentration reduced to 2×10^{-4} M; (c) 10.4 mg/mL tubulin in assembly buffer containing 10^{-3} M GTP and 2×10^{-4} M $MgCl_2$; (d, e, and f) 12.5, 9.5, and 16.8 mg/mL tubulin in complete assembly buffer.

the system at temperatures below the complex heat uptake zone. This indicates that the process which gives rise to the complex pattern yields a product with a heat capacity lower than that of the reactants. As seen from Figure 1 the temperature range in which the complex pattern occurs is a function of the rate of heating, being displaced to slightly higher temperatures at higher heating rates. Control experiments in which the buffer composition was varied by omitting in turn one of the components of the assembly system showed that generation of this transition pattern requires all of the components of the assembly system. Thus, as shown by curves b and c of Figure 1, omission of GTP and reduction of the $MgCl_2$ concentration from 16 to 0.2 mM resulted in patterns totally devoid of inflections, the heat capacity increasing monotonely right up to the limit of heating. In all cases, an acceleration in heat absorption was found to set in at about 40 °C, indicating most probably an incipient loosening of the protein structure. The possibility that the complex pattern is generated by nonspecific GTP hydrolysis was eliminated in experiments in which protein had been omitted, both at high and low Mg^{2+} concentrations. In such experiments heat capacity was found to be invariant with temperature, as shown in Figure 1, curve a.

Assuming that the complex transitions in heat capacity result from the polymerization of tubulin into microtubules, we calculated ΔC_p values for this reaction. In this calculation the amount of material polymerized was taken as equal to the total protein concentration less the critical concentration at the terminal temperature, taken as 1.0 mg/mL for the temperature range between 30 and 37 °C (Lee & Timasheff, 1977). The results, summarized in Table I, show that the investigated process is accompanied by a change in heat capacity per mole of tubulin dimer (M_r 110 000), ΔC_p, of

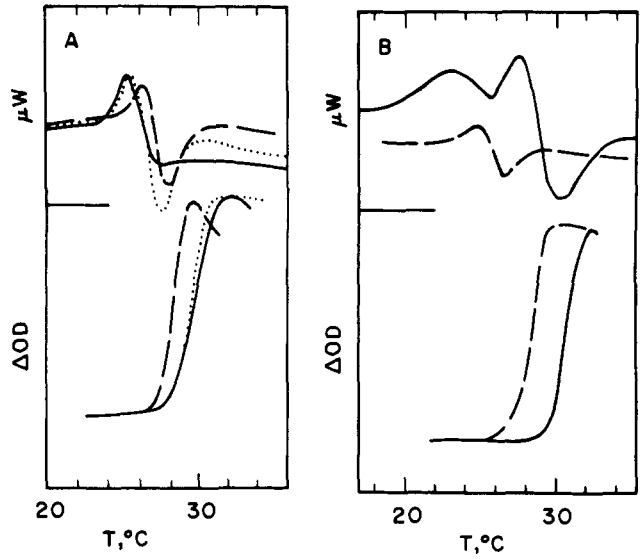

FIGURE 2: Heat uptake (upper patterns) and turbidity changes (lower patterns) as a function of heating. (A) Heating rate 0.5 deg/min, (—) 12.7 mg/mL tubulin, (---) 9.1 mg/mL tubulin, and (···) 9.5 mg/mL tubulin; (B) (—) 16.8 mg/mL tubulin heated at 1.0 deg/min and (---) 12.5 mg/mL tubulin heated at 0.25 deg/min.

-1600 ± 500 cal/(deg mol),[1] independent of the rate of heating. This value agrees well with the measured van't Hoff heat capacity change of -1500 cal/(deg mol) for the addition of each tubulin dimeric subunit to a growing microtubule (Lee & Timasheff, 1977).

That the transition seen in the heat capacity calorimeter is indeed the tubulin polymerization process was verified by turbidity experiments in which aliquots of the tubulin solutions were heated at rates identical with those of the calorimetry experiments. The results are presented in Figure 2. In all cases, an increase in turbidity sets in at a temperature within the zone of the calorimetric transition close to the beginning of the heat release stage. This is followed by a very sharp increase in turbidity which reaches a maximum after the termination of heat release. It is noteworthy that essentially no change in turbidity occurred within the first zone of heat uptake. These turbidity patterns cannot be used to calculate the degree of completion of the reaction, since, after reaching a maximum, the turbidity gradually decreased with further heating, the generated patterns being reminiscent of overshoot kinetics. Furthermore, at the high protein concentrations used, turbidity can no longer be used as a quantitative measure of the degree of assembly, as the assumptions on which the Berne (1974) theory of light scattering is based are no longer valid: the Rayleigh-Gans approximation may no longer be applicable (Heller, 1963; Kerker, 1969), secondary scattering becomes significant (Kraut & Dandliker, 1955), and a strong concentration dependence of turbidity sets in, reflecting external interference due to lack of freedom of motion of individual rods (Fournet, 1951). Nevertheless, these changes in turbidity can be used as a qualitative test of tubulin polymerization. Control experiments in which components of the assembly were omitted in turn resulted in no increase in turbidity on heating, which is a strong indication that the process being dealt with is indeed microtubule assembly.

[1] The assembly of tubulin to microtubules is accompanied by a volume change of approximately 100 mL/mol of dimeric tubulin (Salmon, 1975). Calculation of the resulting apparent decrease in heat capacity showed this effect to make a contribution of ~ -100 cal/(deg mol) of dimeric tubulin, which is well within the experimental error of our measurements and does not affect significantly the conclusions of this paper.

Discussion

Examination of the self-assembly of pure tubulin into microtubules with the adiabatic differential heat capacity microcalorimeter has shown that, in our assembly buffer, the microtubule growth process is accompanied by a heat capacity change of -1600 ± 500 cal/(deg mol) for the addition of one dimeric tubulin subunit to a growing microtubule. This is in excellent agreement with the previously reported van't Hoff value of -1500 cal/(deg mol) (Lee & Timasheff, 1977).

The temperature dependence of the apparent excess heat capacity, shown in Figure 1, indicates that the microtubule formation process is complex as evidenced by the sequence of a net positive, then a negative, and possibly a second positive excess C_p change over the course of the polymerization. In fact, the positive change appears to be preceded by weak fluctuations in heat capacity. These, however, although seen on the patterns of Figure 1, are not significantly different from the base line. Such a pattern, which is reminiscent of a second-order transition, may have a variety of origins. Before discussing these, however, it must be stressed that the process is not at equilibrium over the temperature range of the complex changes in heat capacity. Equilibrium exists only at the initial and final states of the transition. In between, the heat evolution, or heat uptake, is a function not only of temperature but also of the kinetics of the reaction. Thus, at any temperature the apparent enthalpy change, ΔH_{app}, can be expressed as shown in eq 1, where C_p is excess heat capacity,

$$\Delta H_{app} = \int_{T_0}^{T} C_p[T,\alpha(T,t)]\, dT \qquad (1)$$

α is the extent of the reaction, T is thermodynamic (Kelvin) temperature, and t is time. The microtubule assembly process is known to be slow. In equilibrium experiments, polymerization is preceded by a lag period of the order of minutes, and the increase in turbidity to a plateau value also requires several minutes at 37 °C (Lee & Timasheff, 1975, 1977). At lower temperatures, the process is considerably slower. Therefore, at all rates of heating, the temperature increase in the calorimeter is too rapid for the reaction to reach equilibrium. As a result of the slowness of the process and of the temperature dependence of the enthalpy of the reaction, at any temperature T, the degree of completion of the reaction, α, is a function of the rate of heating, the same degree of completion being attained at different temperatures for different heating rates. Therefore, the total heat uptake at any given degree of completion of the reaction must also be different for different rates of heating. Equilibrium is finally attained at temperatures above 32 °C, when the critical concentration becomes essentially independent of temperature (Lee & Timasheff, 1977). It is this lag of the reaction behind the temperature increase which causes the patterns to be displaced to higher temperatures with an increase in the rate of heating.

In view of the complex dependence of heat uptake on both the kinetics and the equilibrium of the reaction, any interpretation of the patterns of Figure 1 must be approached with extreme caution. One fact is evident, however: the microtubule polymerization reaction is accompanied by both endothermic and exothermic processes, since heat is both absorbed and given out. What sort of processes could give rise to such a pattern? Let us consider three possibilities.

(1) The sequence of heat absorption and evolution could simply reflect overshoot kinetics frequently seen when microtubule assembly is monitored by turbidity at high tubulin concentrations. The exothermic portion of the patterns would simply reflect evolution of heat as extra long microtubules depolymerized and reequilibration took place. This would require a mechanism somewhat more complicated than that treated theoretically by Scheele & Shuster (1974), since the reversal in the enthalpy change would imply an actual decrease in total polymer formed rather than only reequilibration of the average polymer molecular weight.

(2) The polymerization involves the sequential formation and interconversion of a series of intermediates, the formation of which is characterized by both positive and negative enthalpies of reaction.

(3) The polymerization involves several simultaneous reactions, at least one of which is exothermic. The complex pattern with sequential predominance of heat absorption and heat evolution could simply be a reflection of the heat capacity changes characteristic of the individual reactions, these changes being different in magnitude and sign for the several reactions. Since the actual addition of a tubulin subunit to a growing microtubule is known to occur with a negative heat capacity change (Lee & Timasheff, 1977), the heat uptake for this reaction should be smaller at higher temperatures than at lower temperatures. In this way its algebraic summation with the heat released by an exothermic reaction in which the enthalpy change was independent of temperature could result artifactually in patterns such as those seen in Figure 1.

Examining these in turn in the light of the observed turbidity changes (see Figure 2), it seems that the first possibility is rather unlikely. The rapid increase in turbidity takes place only in the exothermic portion of the heat uptake pattern. Since an increase in turbidity can be equated with polymerization, the observed heat evolution takes place before any possible overshooting could occur.

The second possibility is much more difficult to assess, since at present there is very little knowledge of the nature of intermediates which might be formed. A number of structures seen in the electron microscope, which might be possible candidates for this role, have been described (Erickson, 1974; Kirschner et al., 1974, 1975; Johnson & Borisy, 1975). Yet, at present, it is not possible to identify any of these observed structures with intermediates in microtubule formation since all of them could reflect independent alternate pathways of tubulin self-association.

The third possibility, that of simultaneous reactions with enthalpies of opposite signs, one of them being temperature dependent, seems more appealing. Sutherland & Sturtevant (1976), reporting on flow microcalorimetric experiments on the assembly of microtubules, concluded that the net heat of the growth process is close to zero. They proposed as one possible source of this observation the cancellation of a positive enthalpy of polymerization and of a negative enthalpy of GTP hydrolysis, the two reactions being rigorously linked. The distinct possibility of such a mechanism can be inferred from the report by Weisenberg et al. (1976) that GTP hydrolysis occurs during or immediately following the tubulin addition step to a growing microtubule, as well as from those of David-Pfeuty et al. (1977, 1978) and of MacNeal & Purich (1978) that the hydrolysis of at least one GTP molecule per tubulin dimer incorporated into microtubules occurs during microtubule growth. While a direct comparison between our study and that of Sutherland & Sturtevant (1976) is not possible since they worked with partially purified tubulin in the presence of microtubule-associated proteins which copurify with tubulin in the Shelanski et al. (1973) cycle procedure (Sloboda et al., 1976), such a situation could account for the observed heat capacity change pattern. In analogy to ATP hydrolysis, GTP hydrolysis may be assumed to be an exo-

thermic reaction with a ΔH value in the vicinity of −5 kcal/mol (Sutherland & Sturtevant, 1976; Alberty, 1969; Gellert & Sturtevant, 1960; Gerlt et al., 1975). The polymerization of tubulin into microtubules is, however, endothermic and has a large negative change in heat capacity. Therefore, the algebraic sum of these heats should become less positive, or more negative, as the temperature is increased. At low temperatures, the net observed enthalpy can be expected to be positive, while, as the temperature is increased and the positive heat contribution diminished, the exothermic reaction would become predominant, leading to a net measured negative heat absorption. GTP is known to be consumed during microtubule reconstitution (Gaskin et al., 1974; Lee & Timasheff, 1977), and recent reports (MacNeal & Purich, 1978; David-Pfeuty et al., 1977, 1978) strongly support the position that its hydrolysis is linked to microtubule growth, although the opposite position is also maintained (Penningroth & Kirschner, 1977). While our results are fully consistent with GTP hydrolysis, in the absence of direct chemical evidence during the course of the heating we cannot equate unequivocally the exothermic process which accompanies tubulin polymerization with GTP hydrolysis. The observed heat evolution could also possibly reflect the binding of a ligand or a conformational change.

While the invocation of GTP hydrolysis as part of a coupled reaction in which all of the intersubunit bonds are formed simultaneously as the source of the heat evolution observed in Figure 1 is very attractive, a close comparison between the patterns of changes in the heat capacity and in the turbidity, shown in Figure 2, suggests that the process under examination is more complicated. The strong increase in turbidity occurs only during the heat release portion of the calorimetric scan, after completion of the heat absorption stage. The same general correlation between heat absorption and turbidity is found at all rates of heating. Let us consider the implications which this pattern may have for possible pathways of microtubule assembly. Microtubules are three-start helixes with 13 subunits to a turn (Grimstone & Klug, 1966; Erickson, 1975; Cohen et al., 1975). The initiation of such a complicated structure might require several steps to construct an initial short microtubule on which growth could occur in a strictly helical polymerization manner, similar to that described by Oosawa & Kasai (1971) for actin. The assembly reaction involves the formation of two types of intertubulin bonds, one along the axis of the helix, i.e., along protofilaments, and the other along the helix, i.e., between protofilaments. Only one of these might be associated with the exothermic reaction, possibly GTP hydrolysis. Erickson (1978) has proposed that protofilament formation is, in general, the favored primary reaction. Furthermore, in an elegant statistical mechanical analysis of the microtubule growth process, Carlier & Pantaloni (1978) have deduced a model in which the initiation of a microtubule proceeds through several stages of polymerization, with the formation of fairly large structures before the onset of true helical growth. The formation of the large premicrotubule structures, which should be a cooperative process, requires the prior formation of an energetically less favorable one-dimensional nucleus as the first step in polymerization. This model in no way obviates the applicability of the nucleated cooperative polymerization theory of Oosawa & Kasai (1971) to the equilibrium analysis of microtubule growth, since the Carlier & Pantaloni (1978) analysis of the process is kinetic in nature.

The heat absorption experiments described here are nonequilibrium and, therefore, contain kinetic information.

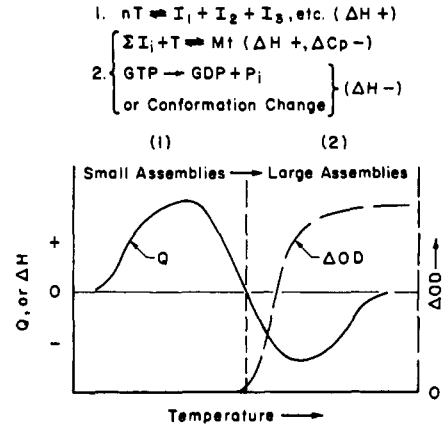

FIGURE 3: Hypothetical reaction model. In stage 1, self-assembly of tubulin takes place with the formation of various low polymer intermediates, with a positive ΔH and no turbidity detectable by the transmission technique; in stage 2, growth of long microtubules occurs with a temperature-dependent positive enthalpy and a strong turbidity characteristic of large assemblies. Definition of symbols: T, tubulin; I_i, intermediates; Mt, microtubule; Q, heat uptake.

Although the present calorimetric data are not amenable to exact kinetic analysis, they may be used to gain a qualitative insight into the pathway of the assembly process. Two conclusions are evident: the assembly process is complex and part of the heat uptake process is finished before the onset of strong turbidity. A key question seems to be: what are the processes seen calorimetrically prior to the onset of turbidity? According to the Berne (1974) theory, turbidity may be used as a quantitative measure of assembly only when the length of the rod is large relative to the wavelength of the light. These structures should give rise to a strong turbidity. The turbidity generated by short rods or other smaller assemblies, being proportional to the square of the molecular weight, will fall off rapidly as the degree of polymerization decreases and thus would not be detectable by the rather crude measuring technique which consists in the determination of the attenuation of transmitted light. Such assemblies should be readily detectable, however, in classical light-scattering experiments.

The above considerations suggest that the most plausible explanation of the heat absorption and turbidity change patterns might be afforded by a combination of the second and third possibilities described above. According to this model developed in light of the analyses of Erickson (1974, 1978) and of Carlier & Pantaloni (1978) and depicted schematically in Figure 3, heating of the tubulin solution under assembly conditions would lead to the following sequence of events: first, formation of nuclei with one type of intertubulin contact only; second, two-dimensional addition of more tubulin to the growing structures or combination of small oligomers. This step should be more favorable than the initial linear polymerization and, therefore, should proceed more rapidly and in a cooperative manner. If both processes are endothermic, one should witness absorption of heat but little change in turbidity. Finally, once short microtubules were formed, growth could proceed by the helical addition of individual subunits in the classical Oosawa & Kasai (1971) manner with the generation of strong turbidity (Berne, 1974). At some late point in the process, GTP hydrolysis would occur. If heat evolution is indeed caused by GTP hydrolysis, it seems that this reaction should follow the addition of tubulin to the growing structure, as proposed by Weisenberg et al. (1976), since it might have to be preceded by a conformational change in the protein which accompanies the dislocation of planar intermediates into the cylindrical assembly. The requirement

of such a conformational change for induction of GTPase activity in tubulin is vividly suggested by the observation of David-Pfeuty et al. (1977) that the binding of colchicine to tubulin induces enzymic acitivity.

The above general pathway can account for both the observed sequence in heat absorption and release and the delay in the development of turbidity relative to the heat change process. The gradual increase in temperature in our experiments should help to resolve sequential slow reactions. In particular, the negative heat capacity change associated with microtubule growth should result in a decrease at higher temperatures of the positive contributions to ΔH, bringing out more vividly any exothermic reactions occurring late in the process. Furthermore, the weak fluctuations in heat capacity which precede the heat uptake step suggest the occurrence of earlier events, possibly a conformational change or early stages of nucleation. At present, however, this pathway, while consistent with observations, must be regarded as a working hypothesis and its validity must be tested by further detailed investigations. Furthermore, the intermediates are yet to be identified, a task best pursued by nonequilibrium solution experiments. It is certain, however, that the formation of a complex structure, such a microtubules, which requires assembly of a minimum of some 42 subunits to exist, must involve a sequence of intermediate assemblies culminating in its first manifestation.

A question that should be considered at this point is: why is the exothermic process seen in microcalorimetry not seen in the van't Hoff analysis, $\Delta H°$ being positive? The answer is found in the different natures of the two types of measurements. In a complex process, the heat changes measured calorimetrically and from van't Hoff equilibrium plots are effective quantities. The summations involved are a function of the manner in which the measurements are made. In calorimetric measurements, all heat processes should be manifested and the heats should be additive, the measured value being their algebraic sum. van't Hoff heats, however, are the result of more complex summations of all the individual heats through a proper combination of equilibrium constants. Indeed, they are a function not only of the way in which the equilibrium reaction is formulated but also of the experimental technique used to follow the processes and of the relative concentrations of the reactants. In the present studies, all experiments were carried out in a large excess of GTP. In calorimetry, all the heat evolved during GTP hydrolysis should be measured, independently of the excess of GTP present. In the equilibrium measurements, on the other hand, the GTP concentration remained at an essentially steady-state saturation level, and its depletion by hydrolysis during the reaction did not affect the measured equilibrium constant. As a result, the heat of this reaction could not be reflected in a van't Hoff plot and should not manifest itself in the calculated $\Delta H°$ value.

Finally, one might ask: does the initial linear nucleation process contribute to the observed heat changes? Sutherland (1977) states that the van't Hoff heats observed are actually measurements of the heat of nucleation rather than of microtubule growth. This conclusion was based on the assumption that the equilibrium constants for addition of a subunit to a growing nucleus, K_n, and for helical polymer propagation, K_g, are close to identical: $K_n/K_g = 0.8$. Such a situation, however, is inconsistent with the Oosawa & Kasai (1962, 1971) theory of nucleated polymerization which requires that the free energy of polymer growth be much larger than that of nucleation, with $K_n/K_g < 0.1$. An increase of this ratio to the value assumed by Sutherland (1977) would simply reduce the process to one of essentially isodesmic polymerization. No critical concentration could be observed, and the degree of polymerization would vary hyperbolically rather than sigmoidally with the protein concentration. Lee & Timasheff (1977) examined the meaning of the observed van't Hoff enthalpy in terms of the Oosawa & Kasai (1962, 1971) theory. They found that when the criterion for measuring equilibrium was critical concentration, C_r, the van't Hoff heat of the reaction is given by eq 2, where ΔH_g is the enthalpy of polymer

$$\Delta H_{app} = -R\frac{d \ln C_r}{d(1/T)} = -\Delta H_g + 2R\frac{d \ln (K_g - K_n)}{d(1/T)} \quad (2)$$

growth. Since $K_n < 10^{-1}K_g$, this equation reduces within a close approximation to $\Delta H_{app} = \Delta H_g$. Therefore, the van't Hoff enthalpy determined from critical concentration measurements is the heat of growth, with little contribution from the enthalpy of nucleation. The same holds true for the calorimetrically measured heats. Again, the ratio of the equilibrium constants shows that the concentration of the protein found in linear nuclei should be considerably smaller than that in the two- and three-dimensional polymers. The heat generated by the one-dimensional nucleation would, therefore, be undetectable by present-day instrumentation.

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Isolation and Characterization of Basement Membrane Collagen from Human Placental Tissue. Evidence for the Presence of Two Genetically Distinct Collagen Chains†

Thomas F. Kresina and Edward J. Miller*

ABSTRACT: A unique collagen fraction containing basement membrane collagen molecules has been isolated from limited pepsin digests of placental tissue utilizing selective precipitation procedures and chromatography of the collagen in native form on CM-cellulose. Characterization of the denaturation products of this collagen has revealed the presence of two classes of components. The more acidic components (C′, C, and $50K_1$) represent, respectively, a modified form of the pro-C chain, a collagen chain virtually identical with the α-chain-sized component derived from limited pepsin digests of isolated basement membranes, and a mixture of smaller molecular weight components arising from proteolysis of either the pro-C or C chain. The more basic components (80K and $50K_2$) represent, respectively, the major portion of a genetically distinct chain, designated the D chain, and a mixture of proteolytic cleavage products of the latter chain. The C chain and the 80K component are readily distinguishable on the basis of chromatographic properties, compositional features, and their respective cyanogen bromide cleavage products. In addition, both chains appear to be derived from molecules originating in basement membrane structures since both components are observed in the denaturation products of collagen solubilized from isolated basement membranes. It is concluded from these data that basement membrane collagen molecules are comprised collectively of at least two genetically distinct collagen chains.

Basement membranes may be regarded as specialized extracellular matrices which serve to form the boundaries between various tissue compartments. In this role, the membranes may function as selective permeability barriers as well as supporting elements. They are, therefore, of critical importance in the maintenance of tissue integrity.

Early studies on the chemical composition of the larger and more readily isolated basement membranes such as the lens capsule, glomerular basement membrane, Reichert's membrane, and Descemet's membrane definitely established that such structures contain a high, albeit variable, proportion of molecules with collagenous sequences (Kefalides, 1973). However, the precise nature of the collagenous constituents and their molecular organization within basement membranes have not been well-defined and are the subject of some controversy. In this regard, studies on the pepsin-solubilized collagen from several basement membranes have led to the isolation of a single a1-like chain which exhibits a number of unique compositional features relative to other known collagen chains (Kefalides, 1971; Dehm & Kefalides, 1978). In addition, recent studies on basement membrane biosynthesis employing organ cultures of parietal yolk sac endoderm (Minor et al., 1976) and lens capsules (Heathcote et al., 1978) have shown that these tissues synthesize a high molecular weight collagenous protein comprised of three apparently identical procollagen-like chains, each of which exhibits an apparent molecular weight in the range of 160000–180000. Pulse chase experiments, however, indicated that the basement membrane procollagen species did not undergo a time-dependent conversion to a smaller molecular weight molecule on incorporation into the basement membrane structure. These results have led to the view that the collagenous constituents of basement membranes may be characterized as a homogeneous population of molecules (type IV collagen), each of which contains three identical chains (α1(IV) chains) and which are present in a form resembling procollagen molecules.

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