THEORY FOR MODELING THE COPOLYMERIZATION OF TUBULIN AND TUBULIN-COLCHICINE COMPLEX

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ABSTRACT Substoichiometric concentrations of tubulin-colchicine complex (TC) inhibits microtubule assembly through a copolymerization reaction between tubulin and TC. We have determined the rates and extent of TC incorporation into bovine brain microtubules and developed a theory that models copolymerization. Our analysis suggests that while the apparent association rate constants for tubulin and TC are similar, the apparent dissociation rate constants for TC are a factor of five or more larger than those of tubulin. Copolymer composition showed only slight changes during assembly despite changes in the solution phase and showed little dependence at high TC upon the initial tubulin concentration. The theory was based on coupled Oosawa-Kasai equations that allow for the co-assembly of two components, tubulin and TC. An expression was derived that relates copolymer composition to reaction mixture composition and to the affinity of microtubule ends for tubulin and TC. This expression predicts copolymer composition at TC concentrations <10 uM and correlates composition with assembly inhibition. We perceive copolymerization as a facilitated incorporation of TC requiring the presence of tubulin. TC incorporation was dependent on the ratio of total tubulin to the dissociation constant for TC bound to microtubule ends. The copolymerization reaction is thus characterized by an interplay of two effects (*a*) where tubulin facilitates the incorporation of TC into the microtubule, and (*b*) where TC inhibits the assembly of tubulin into microtubules.

INTRODUCTION

Colchicine disrupts a variety of cellular activities that are linked to microtubule function (1–4). Its inhibitory effect on microtubule assembly is presumed to occur via the tubulin-colchicine complex (TC)[1] (a 1:1 complex of tubulin and colchicine) and is evident at substoichiometric concentrations (<1:100) of TC relative to tubulin (5, 6). An elucidation of the inhibition mechanism may be relevant to understanding the in vivo control mechanisms of microtubule assembly (7, 8).

Although the molecular basis for TC inhibition of microtubule assembly is unknown, several models have been proposed (9–16). Sternlicht et al. (9, 10) suggested that substoichiometric concentrations of TC randomly copolymerized with tubulin at microtubule ends forming microtubule copolymers. Although microtubule ends were assembly competent, they had reduced tubulin affinities and altered assembly rate constants that correlate with the TC mole fraction in the microtubule phase. Inhibition at low to moderate TC concentrations did not directly involve microtubule associated proteins (MAPs), a group of non-tubulin proteins that copolymerize with tubulin and facilitate microtubule assembly (10).

Farrell and Wilson (12) attempted to reconcile copolymerization with the capping model of inhibition (13) that hypothesized assembly inhibition through TC blockage of microtubule ends. They proposed a dynamic model of inhibition involving repetitive cycles of transient TC blockage of assembly and repair by tubulin. In this model TC binds reversibly, although a single bound TC can cause a marked but transitory decrease in the rate of free-tubulin addition. At sufficiently high tubulin/TC ratios free tubulin adds over the TC block (recovery) with copolymer formation occurring through blockage-recovery cycles. Averaging this cycle over a period of time represents assembly as proposed by Sternlicht and Ringel (9), i.e., rates of copolymer formation correlate with the microtubule TC mole fraction. Margolis et al. (14) suggested that Farrell and Wilson's (12) results may be species dependent as their own data for colchicine addition to reassembled bovine brain microtubules was indicative of a cap. Lambier and Engelborghs also found TC binding was reversible and inhibitory, but did not propose a recovery phase or the conservation of assembly-competent ends (16). Copolymerization occurred through addition of tubulin subunits to TC-free protofilament ends that assembled over and trapped TC present on neighboring protofilaments. They

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[1] *Abbreviations used in this paper:* TC, tubulin dimer containing a tightly bound colchicine; MAP, microtubule associated proteins; PB-2.5 M, a microtubule stabilizing buffer (pH 6.7) consisting of 0.1 M 2(*N*-morpholino) ethane sulfonic acid (MES), 2-mM ethylenebis (oxyethylenenitrilo) tetraacetate (EGTA), 0.1 mM ethylenediamine tetraacetic acid (EDTA), 2 mM 2-mercaptoethanol, 0.5 mM $MgCl_2$, and 2.5 M glycerol; PB-OM, a similar buffer without glycerol; T_{total}, total active tubulin; D_c, the critical, i.e., minimum tubulin concentration required for assembly; X and Y, the TC mole fraction in the solution, and copolymer phases, respectively.

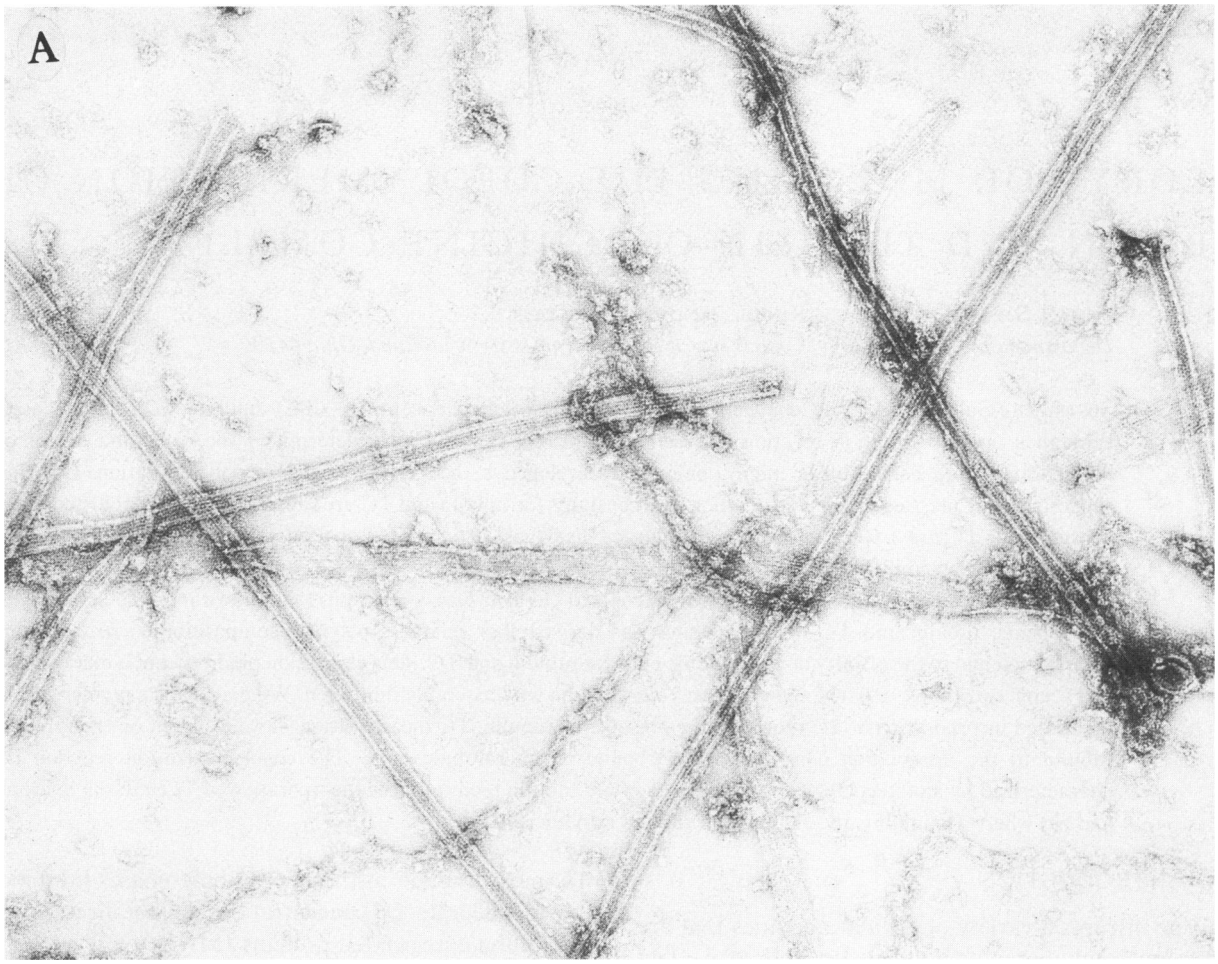

FIGURE 1 Electron micrograph comparison of microtubule structures (magnification of 325,000). 25-μM microtubule protein preparations in PB-0M buffer (10) were assembled at 37°C in (*A*) the absence and (*B*) presence of 2-μM [^{3}H] TC. Composition analysis of pellets from (*B*) indicated a $Y = 0.04 \pm 0.01$. Negative staining was done using the carbon-coated mica method as modified by Langford (36). Both (*A*) and (*B*) contain microtubules as the principal species but also contain sheet-like structures, suggestive of flattened or incompletely closed microtubules, as well as small amounts of contaminating filamentous proteins (intermediate filaments). Distinct protofilaments are evident in the sheet-like regions. A comparison of these regions in (*A*) and (*B*) indicate identical numbers of protofilaments.

suggested that copolymerization occurs only at nonequilibrium conditions far from steady state, contrary to Farrell and Wilson (12).

Deery and Weisenberg analyzed microtubule assembly at saturating colchicine concentrations (15). They proposed that assembly proceeds through rounds of elongation and suggested capping kinetically blocks the rate-limiting step of the elongation round, i.e., the initial addition of MAP-tubulin oligomers to flat-ended microtubules. Their alternate mechanism for substoichiometric inhibition by TC was compatible with Sternlicht and Ringel's study at low to moderate TC concentrations (9).

Without considering specific inhibitory mechanisms, we have developed a quantitative theory for copolymerization based on coupled Oosawa-Kasai equations that considers the co-assembly of two monomer components (tubulin and TC) in accord with the known aspects of copolymerization. Oosawa-Kasai equations (17) have been previously used to analyze the kinetics of microtubule (18) and actin (19) assembly from single monomer components. An expression was derived that predicted copolymer compositions and permitted us to quantitatively correlate composition with assembly inhibition.

MATERIALS AND METHODS

Microtubule Protein Preparation

Microtubule protein was isolated as previously described by three assembly-disassembly cycles (9) following a procedure modified from Gaskin et al. (20). Total active tubulin concentrations ($[T_{total}]$) were derived from the total microtubule protein concentrations as determined by the Lowry method (21) and were corrected for the presence of 20% inactive tubulin and 15% nontubulin protein (9, 10).

TC Preparation

TC was prepared by incubating microtubule protein at 37°C with [^{3}H] colchicine (~50 μM, 50 Ci/mol) from New England Nuclear (Boston, MA) (9). The TC stocks contained ~25 to 35-μM total protein and ~15 to 22-μM TC. At least 90 to 95% of the radioactive colchicine in the stocks

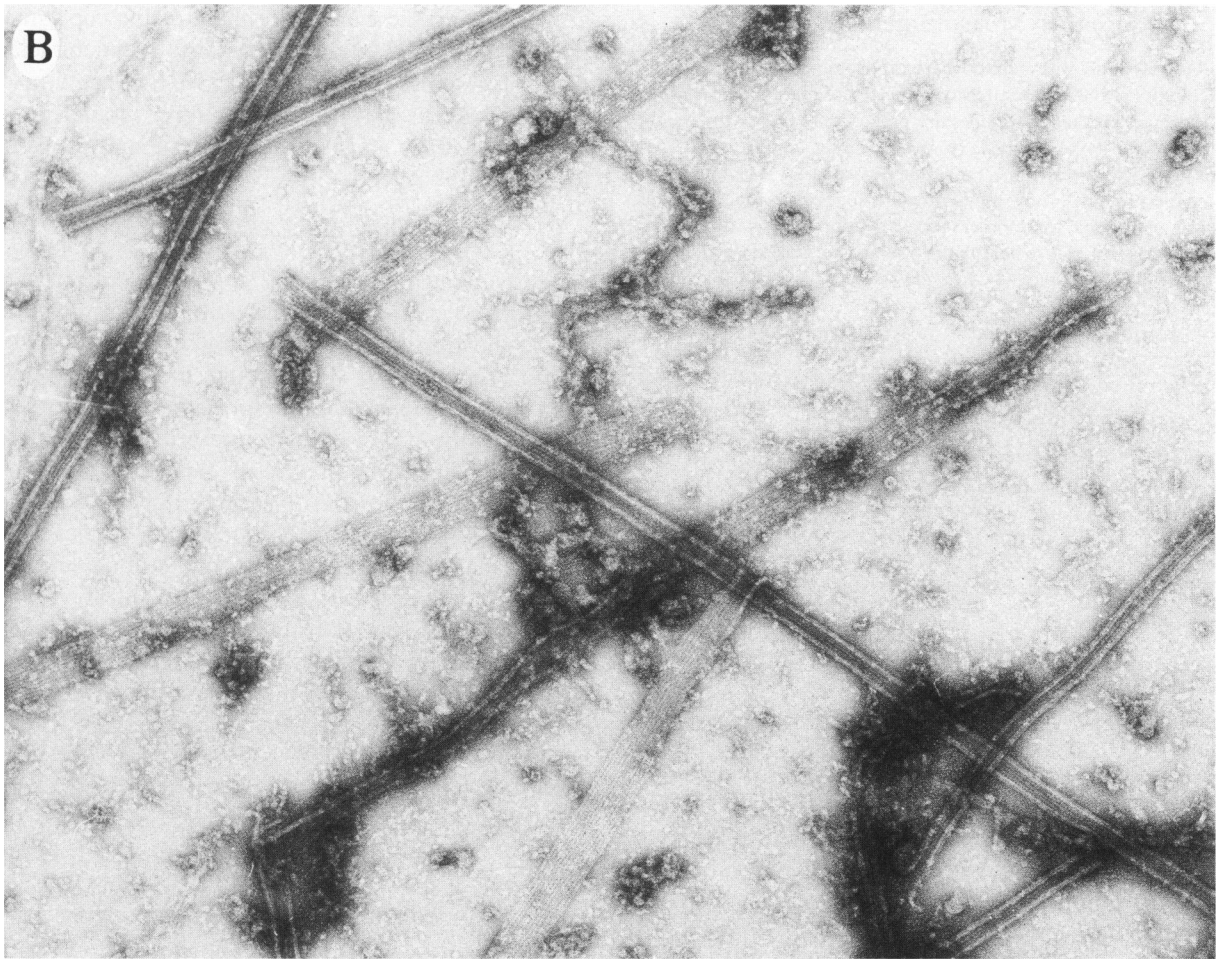

FIGURE 1 Continued

was present as TC (9). TC is relatively stable with little dissociation ocurring over several hours at 37°C (22) or several days at −20°C.

TC Mole Fractions in the Microtubule and Solution Phases

Microtubule copolymers were analyzed as previously described (9, 10). Microtubule protein solutions (~0.5 to ~14 mg/ml) containing various concentrations of [^{3}H] TC (<10 μM) were assembled at 37°C in the presence of 1 mM GTP. Assembly was measured by monitoring the absorbance at 350 nm (A_{350}) (23). Following centrifugation, the pellet TC mole fraction (Y) was estimated from the radioactivity and the protein mass (corrected for nontubulin protein) and set equal to $(TC/TC + T)_{MT}$, the TC mole fraction in the microtubule phase.

The TC mole fractions at steady state and during assembly in the solution phase (X) were set equal to the $[TC]_{sol}/([T]_{sol} + [TC]_{sol})$ ratio. $[T]_{sol}$ and $[TC]_{sol}$ were estimated from polymer yields at the time of colchicine blockage, from Y values in the pellets, and from $[T]_{total}$ and $[TC]_{total}$: $[T]_{sol} = [T]_{total} - [1 - Y)\ (T + TC)_{MT}$; $[TC]_{sol} = [TC]_{total} - Y(T + TC)_{MT}$. $(T + TC)_{MT}$, the tubulin plus TC concentration in the microtubule phase, was estimated from A_{350} (see below). Colchicine blocks were established by adding colchicine (50–150 μM) to assembly reactions at specific times during assembly.

The kinetic model for TC inhibition of assembly presented below is an extension of earlier studies of assembly inhibition (9, 10) and presumes that Y values reflect a copolymerization reaction in which TC adds to microtubule ends in a manner similar to tubulin. This perception is consistent with electron microscopy studies that suggested that in the presence of TC, microtubule protein assembled primarily into microtubules that appeared indistinguishable from the microtubules formed in the absence of TC (Fig. 1). Furthermore, all efforts to ascribe the measured Y values to processes other than copolymerization, e.g., contamination or adventitious binding of TC to microtubules were unsuccessful. In one set of experiments designed to detect contamination (9), for example, microtubules were assembled from mixtures of microtubule protein (9–23 μM) and TC (1–3.5 μM), pelleted through 50% sucrose, resuspended, and pelleted again through 50% sucrose. Y values obtained from the first pellet differed no more than 15–20% from that of the second pellet (data not shown) despite the use of a variety of microtubule protein and TC concentrations, as well as a variety of centrifugation times (30 min to 1.5 h) and sucrose cushions (4 to 7 ml). This result, which suggests no contamination by TC, was consistent with other studies using TC alone. Incubation of TC preparations (1–5 μM) at 37°C in assembly buffer for 1 h followed by centrifugation through sucrose failed to give a detectable pellet. In a further set of experiments designed to detect adventitious binding of TC to microtubules, microtubules were assembled from microtubule protein, incubated at steady state with high colchicine concentrations (50 μM) to block further assembly at their ends (13, 16), and then incubated with [^{3}H] TC for 30–60 min at 37°C. Microtubules centrifuged from these preparations had Y values that were only 5 to 10 % that of control microtubules assembled from mixtures of microtubule protein and radiolabelled TC at correspondingly similar concentration of protein and TC.

Absorbance Measurements

Microtubule assembly was monitored spectrophotometrically at 350 nm. Previous studies of the high-scattering properties of microtubule solution (23) have suggested that A_{350} values are proportional to C_W, the weight concentration of polymer formed. However, Carlier and Pantolini (24) observed deviations from a linear relationship between A_{350} and C_W at protein concentrations >2 mg/ml. Our results for protein concentrations <8 mg/ml confirmed their observations. Noting that ~15% of the microtubule mass was due to nontubulin proteins, we related $(T + TC)_{MT}$ to A_{350} by the expression $(T + TC)_{MT}\ (\mu M) = 40\ A_{350}/(1 - 0.4\ A_{350})$.

Kinetic Analysis

We noted that $Y \ll 1$ (copolymers are composed primarily of tubulin) and assumed TC and tubulin incorporation proceeded exponentially to steady state. We estimated k^T and k^{TC}, the apparent assembly rate constants for tubulin and TC, from the expressions: $k^T = [dA_{350}(t)/dt)]_{\text{initial value}}/A_{350,\infty}$ and $k^{TC} = \{d[TC(t)]_{sol}/dt\}_{\text{initial value}}/([TC]_{total} - [TC]_{sol,\infty})$, where $A_{350,\infty}$ and $[TC]_{sol,\infty}$ denote, respectively, absorbance and concentration values at steady state (9, 12). $[TC]_{sol,\infty}$ and $[TC(t)]_{sol}$ were estimated from material balance analyses based on Y and A_{350} as described above. In a number of cases, $[TC(t)]_{sol}$ was estimated directly from the protein and radioactivity content of the supernatants (Fig. 5 *D*).

RESULTS

Theory

We perceive copolymerization as a reaction that transfers subunit mass from the solution phase, where the TC mole fraction is X, to the microtubule phase, where the TC mole fraction is Y. In our theory copolymer composition depends on the composition of the reaction mixture, the affinity of the microtubule ends for tubulin and TC (affinity_T and affinity_{TC}), and the rates at which tubulin and TC incorporate into the microtubule. Our derivation was based on an analysis of the elongation phase.

Two aspects of the copolymerization reaction were vital in our formulation of the copolymerization theory. First, inhibition studies indicated that decreases in microtubule yield at steady state depended strongly on TC_{total} (9, 10), but were insensitive to microtubule protein concentration. This behavior was attributed to an increase in the tubulin dissociation constant, $\text{affinity}_{TC}^{-1}$, with increasing TC_{total} (9, 10). Affinity_T^{-1} was related to the critical (minimum) tubulin concentration (D_c) required for assembly in the presence of TC. Second, Y did not equal the TC mole fraction values in the reaction mixtures, although TC mole fractions in the copolymers and reaction mixtures were similar when TC_{total} was small (<0.25 μM) and T_{total} was large. However, when TC_{total} was large (>2 μM), copolymer compositions were relatively insensitive to initial tubulin concentrations in the reaction mixture (10). Our data therefore suggested that there were constraints on the copolymerization reaction that caused a complex relationship between Y and reaction mixture compositions.

When assembly occurs in the presence of low to moderate TC concentrations, the number of assembly-competent microtubules during elongation remained constant and equal to the number of microtubules (9). Microtubule growth during the elongation phase of the copolymerization reaction could be represented as a pseudobimolecular reaction. Assembly occurred at a rate that could be taken as the difference between the rate for a subunit to add to microtubule ends and the rate for a subunit to dissociate from microtubule ends (9)

$$-d[\text{subunit}]_{sol}/dt = k_+\, m[\text{subunit}]_{sol} - k_-m, \qquad (1)$$

where $[\text{subunit}]_{sol} = [\text{tubulin}]_{sol} + [TC]_{sol}$; m denotes the number concentration of microtubule ends; k_+ and k_- denote the apparent association and dissociation rate constants. Although Eq. 1 is similar to the Oosawa-Kasai equation previously used to represent microtubule assembly (18, 25), our theory interprets Eq. 1 differently from previous work. In typical expressions of Oosawa-Kasai equations, k_+ and k_- denote apparent association and dissociation rate constants for a single species undergoing a bimolecular reaction with microtubule ends, while $[\text{subunit}]_{sol}$ denotes the solution concentration of that species (17). In our derivation (Eq. 1), however, each of these terms represents the combined values for two species, tubulin and TC (26). Furthermore k_+ and k_- values for the copolymerization reaction depended on TC_{total} and the copolymer TC mole fraction (9, 12). Under MAP-depleted conditions both k_+ and k_- decreased as TC_{total} and Y increased. Eq. 1 could be obtained as a sum of the

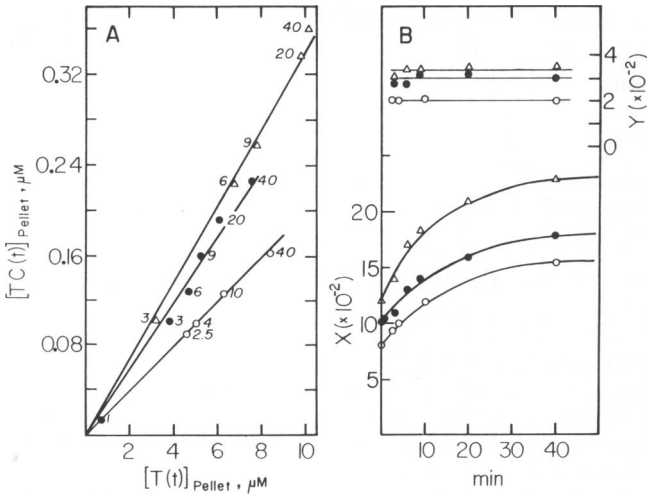

FIGURE 2 TC mole fractions in the polymer and solution phases during assembly. Microtubule protein preparations (T_{total} ~ 14.5 μM) were assembled at 37°C in the presence of 1.2-μm (-O-), 1.6-μm (-●-), and 2-μm (-Δ-) (^{3}H)TC. Numbers adjacent to the data points in *A* indicate the time (minutes after the start of assembly) when 50-μM colchine was added to abruptly arrest assembly (Materials and Methods). (*A*) Copolymer TC concentration vs. copolymer tubulin concentrations. Y during assembly, estimated from the slopes, had values of $1.9 \pm 0.2 \times 10^{-2}$, $3.0 \pm 0.3 \times 10^{-2}$, and $3.3 \pm 0.3 \times 10^{-2}$ for $[TC]_{total}$ of 1.2, 1.7, and 2.0 μM, respectively. (*B*) TC mole fractions in the copolymer and solution phases, $Y(t)$ and $X(t)$, respectively, plotted against time. $Y(t)$ was obtained from the data displayed in *A* while $X(t)$ was calculated from a material balance based on $Y(t)$ values (Materials and Methods).

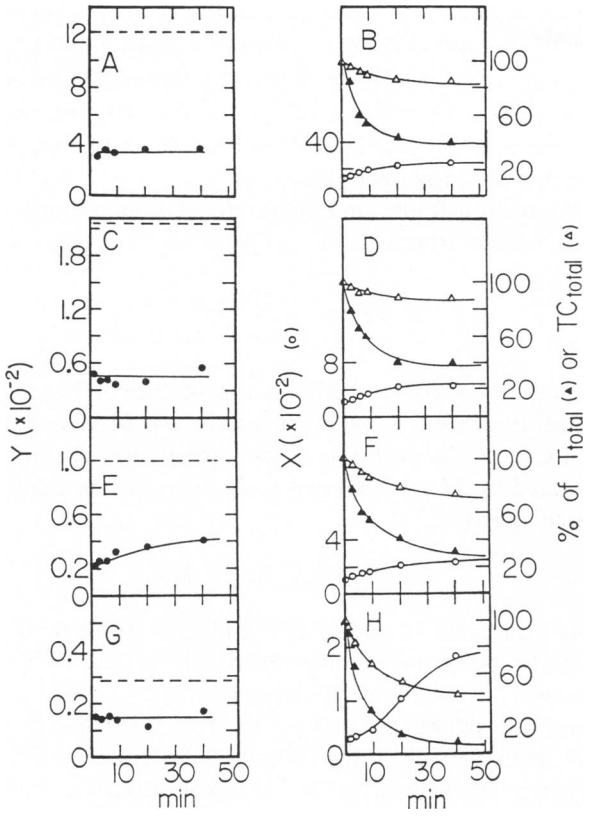

FIGURE 3 Copolymer and solution-phase compositions as a function of time. Microtubule protein was polymerized in the presence of various (^{3}H) TC concentrations. Y (-●-) and X (-○-) values during assembly were estimated as described in Fig. 2. $[T]_{sol}$ (-▲-) and $[TC]_{sol}$ (-△-) were estimated as described in Materials and Methods, and are expressed as percentages of the initial tubulin and TC concentrations in the solution phases (i.e., as percentages of $[T]_{total}$ and $[TC]_{total}$, respectively). The TC mole fraction values of the reaction mixtures (---) correspond to the TC mole fraction values of the solution phases at the start of assembly, and equal $[TC]_{total}/([T]_{total} + [TC]_{total})$. In (*A*) and (*B*) $[T]_{total} = 14.5$ μM and $[TC]_{total} = 2$ μM. In the remaining panels, $[TC]_{total}$ had the constant value of ~0.15 μM, while $[T]_{total}$ was 6.8 μM (*C* and *D*), 14.5 μM (*E* and *F*), and 42 μM (*G* and *H*).

Oosawa-Kasai equations for tubulin and for TC incorporation (Eqs. 2a and b).

$$-d[T]_{sol}/dt = k_+^T m[T]_{sol} - k_-^T m_T; \quad (2a)$$

$$-d[TC]_{sol}/dt = k_+^{TC} m[TC]_{sol} - k_-^{TC} m_{TC}. \quad (2b)$$

Tubulin or TC dissociation rates from the microtubule copolymers are proportional to m_T or m_{TC}, the number concentration of microtubules having a tubulin or TC at their ends. Assuming random TC incorporation, we equated the probability of finding a tubulin or TC at the microtubule end to their respective mole fractions and set

$$m_T = (1 - Y)m; \quad (3a)$$

$$m_{TC} = Ym. \quad (3b)$$

Adding Eqs. 2a and b and substituting the terms from Eqs. 3a and b, one obtains Eq. 1 with k_+ and k_- as stoichiometric averages of tubulin and TC rate constants. Heparin studies (9) done under conditions where X and $Y \ll 1$ such that $k_+ \sim k_+^{TC}$ and $k_- \sim k_-^{TC}$, indicated large changes in rate constant values as $[TC]_{total}$ increased ($k_-^T/k_+^T = D_c$ and increased as $[TC]_{total}$ increased). We assume the apparent rate constants k_+^T, k_-^T and k_+^{TC}, k_-^{TC}, as well as their ratios, depend on $[TC]_{total}$ and vary with copolymer composition.[2] Thus in our model, the dependence of k_+ and k_- on [TC] does not arise exclusively from the X and Y terms, but also involves contributions from the apparent rate constants.

Experimental studies indicated that copolymer composition during assembly (Figs. 2 and 3) was approximately equal to the steady-state composition (Table I). This lack of detectable variation in Y during assembly occurred at high [T] and [TC]. At low [TC] intermediate [T], Y was somewhat more variable (Fig. 3 *E*). (On the other hand, variations in solution-phase composition during assembly

TABLE I
COMPARISON OF THE OBSERVED AND PREDICTED VALUES FOR COPOLYMER COMPOSITION AND ASSEMBLY INHIBITION AS A FUNCTION OF REACTION MIXTURE COMPOSITION

T_{total}	TC_{total}	Percent inhibition		Mol % TC	
		Observed*	Calculated‡	Observed	Calculated§
μM	μM				
6.2	0.25	25	22	1.1	1.3
—	1.0	50	68	3.3	3.3
—	3.5	90	100	7.0	7.9
15.5	0.25	7‖	7	0.6‖	0.8
—	1.0	16	22	1.9	2.5
—	3.5	32	43	5.4	6.6

*Percent inhibition = 100% [$A_{350,\infty}$ (no TC) − $A_{350,\infty}$ (with TC)]/$A_{350,\infty}$ (no TC).

‡From Eqs. 7 and 8, percent inhibition = 100% × 4.3 $[TC]_{total}/(1 + 0.45\ [TC]_{total}) \times (T_{total} - 1.8)^{-1}$. (This expression is valid only if $T_{total} > D_c$, otherwise percent inhibition = 100%. See comments concerning Eq. 8 in Theory section.)

§From Eq. 9:
$Y = ([TC]_{total} + 0.45\ [TC]_{total}^2)/(15 + 28\ [TC]_{total})$, when $T_{total} = 6.2$ μM;
$Y = ([TC]_{total} + 0.45\ [TC]_{total}^2)/(25 + 32\ [TC]_{total})$, when $T_{total} = 15.5$ μM.

‖Sternlicht et al. (10).

[2]In contrast to other models (14) that treat rate constants (k_+^T, k_-^T, k_+^{TC}, k_-^{TC}) as being independent of $[TC]_{total}$, we believe the rate constants are dependent on $[TC]_{total}$ and correlated with copolymer composition. There is prior precedence from other microtubule studies that allow rate constants to depend on composition. Since MAP copolymerize with tubulin and facilitate assembly, Johnson and Borisy (18) allowed k_+^T and k_-^T to vary with MAP content of the reaction mixture. This reflects the fact that the Oosawa-Kasai representation is only an approximation for a complex assembly process where all the kinetic intermediates are not known. Thus there is no reason to regard the rate constants that appear in the right-hand side of Eq. 2 as true constants. Rather they should be regarded as effective or apparent rate constants that can in principle vary with composition.

were generally detectable [Figs. 2 and 3].) These results suggest that the copolymer composition during assembly should be represented as an expansion series in which the dominant or leading term is time independent. A time-independent approximation (Y°) to the solution could be obtained by appropriate integration of Eq. 2 with the substitutions in Eq. 3. At this level of approximation, we treated k_+^T, k_-^T, and k_+^{TC}, k_-^{TC} as constants (see footnote 2). Because nucleation and elongation reactions are reasonably well separated in time such that the major portion of the elongation reaction occurs after nucleation is completed, m and consequently m_T and m_{TC} were also assumed constant. Farrell and Wilson (12) reported that k_+^T and k_+^{TC} have similar values, a finding consistent with our study of tubulin and TC incorporation (Fig. 3 and Fig. 5 below), consequently we set k_+^T equal to k_+^{TC}. We found this approach was self-consistent, i.e., setting Y (Eq. 2) constant, $k_+^T = k_+^{TC}$, and integrating, leads to a solution (Y°) that is time independent during assembly

$$\frac{[TC]_{MT}}{[T]_{MT}} = \frac{Y^\circ}{1 - Y^\circ} = \left[\frac{TC_{total} - Y^\circ(k_-^{TC}/k_+^{TC})}{T_{total} - (1 - Y^\circ)(k_-^T/k_+^T)}\right] \left[\frac{1 - \exp(-k_+^{TC}mt)}{1 - \exp(-k_+^T mt)}\right]. \quad (4a)$$

With $k_+^T = k_+^{TC}$, the extreme right-hand side bracket in Eq. 4a reduces to 1. Collecting terms, one obtains

$$Y^\circ = \frac{[TC]_{total}}{\left\{\begin{array}{l}[T]_{total} + [TC]_{total} \\ + [(k_-^{TC}/k_+^{TC}) - (k_-^T/k_+^T)](1 - Y^\circ)\end{array}\right\}}. \quad (4b)$$

Noting that TC incorporates substoichiometrically ($Y \ll 1$), and that TC_{total} is generally much less than T_{total} we approximate Y° by Y^∞ and substitute $affinity_T^{-1}$ and $affinity_{TC}^{-1}$ (the apparent dissociation constants for tubulin and TC bound to microtubule ends) for k_-^T/k_+^T and k_-^{TC}/k_+^{TC}, respectively.

$$Y^\infty = \frac{[TC]_{total}}{T_{total} + [affinity_{TC}^{-1} - affinity_T^{-1}]}. \quad (5a)$$

Our derivation implicitly assumes that $[T]_{total}$ is greater than $affinity_T^{-1}$, otherwise no assembly occurs (9, 10). Previous studies (10) suggested that affinity $_{TC}^{-1} \ll affinity_T^{-1}$. Thus

$$Y^\infty = \frac{[TC]_{total}}{[T]_{total} + affinity_{TC}^{-1}}. \quad (5b)$$

We conclude from Eq. 5b and the right-hand side numerator of Eq. 4a that $[TC]_{MT,\infty}/[TC]_{total}$, the fraction of the total TC incorporated into the copolymer at steady state is

$$[TC]_{MT,\infty}/[TC]_{total} = 1 - \frac{affinity_{TC}^{-1}}{[T]_{total} + affinity_{TC}^{-1}}. \quad (6)$$

We have previously suggested that tubulin facilities TC incorporation (9, 10), and have observed that at constant $[TC]_{total}$ copolymer TC content increases as $[T]_{total}$ increases (Figs. 3 *D*, *F*, *H*). This result is modeled by Eq. 6 that predicts for constant TC_{total} that as T_{total} increases, increasing larger fractions of the total TC should incorporate into the copolymer phase.

We relate $affinity_T^{-1}$ to D_c (the critical tubulin concentration), which varies with TC_{total} (10).

$$D_c \simeq D_c^\circ + \frac{\omega[TC]_{total}}{1 + \beta[TC]_{total}}. \quad (7)$$

D_c°, the critical concentration in the absence of TC, and β are MAP-dependent parameters while ω is MAP independent (10). *I*, the extent to which microtubule assembly is inhibited by TC, was related to D_c and could be approximated as (10)

$$I = (D_c - D_c^\circ)/(T_{total} - D_c^\circ). \quad (8)$$

This expression is valid only if $[T]_{total} > D_c$ (10). If the critical tubulin concentration increases sufficiently in the presence of TC, and D_c becomes $>T_{total}$, assembly is completely inhibited (i.e., $I = 1$ for $T_{total} \leq D_c$). We believe that both $affinity_T^{-1}$ and $affinity_{TC}^{-1}$ increase as TC_{total} increases. Although $affinity_{TC}^{-1}$ may not increase as rapidly as $affinity_T^{-1}$ with increasing TC_{total}, to illustrate the theory's quantitative aspects we assume that $affinity_{TC}^{-1}$ is proportional to $affinity_T^{-1} = F\,affinity_T^{-1}$ and use a value of 6 for F obtained earlier from copolymerization reactions[3] at various TC, tubulin, and MAP concentrations (10). Setting $affinity_T^{-1} = D_c$ (Eq. 7), we can again express Eq. 5a as

$$Y^\infty = \frac{[TC]_{total} + \beta[TC]_{total}^2}{\left\{\begin{array}{l}[T]_{total} + (F - 1)D_c^\circ \\ + \beta\{[T]_{total} + (F - 1)[D_c^\circ + (\omega/\beta)]\}[TC]_{total}\end{array}\right\}}. \quad (9)$$

Eqs. 5–9 can be algebraically manipulated to yield Eqs. 10 and 11, which interrelate a number of copolymer properties. In Eq. 10 we relate the steady-state values for Y, $[T]_{MT}$, $[TC]_{MT}$, and $[TC]_{total}$ to assembly inhibition

$$[T]_{MT} = T_{total} - D_c = (T_{total} - D_c^\circ)(1 - I); \quad (10a)$$

[3] F was approximated by X_∞/Y_∞, the ratio of the solution and copolymer-phase TC mole fractions at steady state (10). The identity between X_∞/Y_∞ and $affinity_T/affinity_{TC}$ follows from Eq. 2 and allows approximation of Eq. 5. If the copolymer TC mole fraction is $<\sim 0.2$, then $[TC]_{sol,\infty}$ is approximated by $Y_\infty(k_-^{TC}k_-^{TC})$. Since $[T]_{sol,\infty} = affinity_T^{-1} = k_-^T/k_-^{TC}$ (10), $[TC]_{sol,\infty}/[T]_{sol,\infty} = Y_\infty(k_-^{TC}/k_+^{TC})/(k_-^T/k_+^T)$, or $X_\infty/Y_\infty = affinity_T/affinity_{TC}$. If copolymer affinities for tubulin and TC were identical, Y_∞, X_∞, and the TC mole fraction in the reaction mixture should be equal. Differences in the copolymer affinities for tubulin and TC would be reflected in the X_∞/Y_∞ ratio. Thus, if microtubules have no affinity for TC, both X_∞/Y_∞ and $affinity_T/affinity_{TC}$ should be infinitely large. The TC deficiency in the copolymers relative to the solution phase (e.g., Fig. 3, reference 10) suggests that $affinity_T > affinity_{TC}$.

$Y \sim Y^{\infty}$

$$= \frac{(T_{total} - D_c^o)I}{[(T_{total} - D_c^o) + FD_c^o + (F-1)(T_{total} - D_c^o)I][\omega - \beta I(T_{total} - D_c^o)]} \quad (10b)$$

$$[TC]_{MT,\infty} = [T]_{MT,\infty}Y^{\infty},$$

$$= \frac{(T_{total} - D_c^o)^2(1-I)I}{[(T_{total} - D_c^o) + FD_c^o + (F-1)(T_{total} - D_c^o)I][\omega - \beta I(T_{total} - D_c^o)]}; \quad (10c)$$

$$[TC]_{total}^{-1} = \left[\frac{\omega}{T_{total} - D_c^o}\right] I^{-1} - \beta. \quad (10d)$$

In Eq. 11 we relate the steady-state values for $[T]_{MT}$ and $[TC]_{MT}$ to $[T]_{total}$ and $[TC]_{total}$

$$[T]_{MT,\infty} = T_{total} - D_c,$$

$$= \frac{(T_{total} - D_c^o)(1 + \beta[TC]_{total}) - \omega[TC]_{total}}{1 + \beta[TC]_{total}}; \quad (11a)$$

$$[TC]_{MT,\infty} = [T]_{MT,\infty}Y^{\infty} \quad \text{(Eq. 9)},$$

$$= \frac{[TC]_{total}\{(T_{total} - D_c^o)(1 + \beta[TC]_{total}) - \omega[TC]_{total}\}}{\left\{\begin{array}{c}[T_{total} + (F-1)D_c^o]1 \\ + \beta[TC]_{total} + (F-1)\omega[TC]_{total}\end{array}\right\}}. \quad (11b)$$

In the subsequent section we compare the predicted values from these expressions to the experimental values (Figs. 6–9).

The absence of microtubules composed primarily of TC suggested there may be a limiting value for $Y < 1$ that when exceeded does not allow assembly (11). Our theory predicts a maximal value for Y that is appreciably <1 when $\beta = 0$. If TC_{total} is held constant and T_{total} is varied, a local maximum for $Y(Y_c)$, dependent on TC_{total}, should occur at D_c.

$$Y_c \simeq \frac{[TC]_{total}}{FD_c^o + F\omega[TC]_{total}}. \quad (12)$$

As TC_{total} increases, Y_c increases and approaches a limiting value for the copolymer TC mole fraction equal to $(F\omega)^{-1}$. Eq. 12 was derived by substituting D_c (Eq. 7, $\beta = 0$) for T_{total} in Eq. 9. In the case of PC tubulin (no MAP) where β was estimated to be small (10), we obtain a critical ratio of ~ 0.05 ($\beta = 0$). Assembly should not occur at high TC_{total} if the TC mole fraction in the solution phase is initially $> \omega^{-1}$ (Eq. 7). These results may be related to Farrell and Wilson's finding that copolymerization ceases at certain critical TC mole fraction values (12). When $\beta \neq 0$, theory predicts that copolymers that have Y values near 1 should form at high TC_{total}.[4]

Applications

Parameter values of $D_c^o \simeq 1.8\ \mu M$, $\omega \simeq 4.3$, $\beta \simeq 0.45\ \mu M^{-1}$, and $F = 6$ were used for microtubule protein studies (10) while values $D_c^o \simeq 7\ \mu M$, $\omega \simeq 4$, $\beta \simeq 0$, $F = 4$ to 6 were used for MAP-depleted and MAP-free tubulin (10).

Copolymer Composition as a Function of TC. Eq. 5b predicts that copolymers assembled from microtubule protein and TC have Y values that can be approximated by

$$[TC]_{total}/([T]_{total} + C), \quad (13)$$

where C is 55 at $TC_{total} > 2\ \mu M$, or 11 at $TC_{total} < 0.2\ \mu M$. The constant terms correspond to the high and low TC limit values for affinity$_{TC}^{-1}$, assuming affinity$_{TC}^{-1} \simeq 6$ affinity$_T^{-1}$. These expressions reproduce the general behavior predicted by the complete expression (Eq. 9). When [TC] is sufficiently large ($>2\ \mu M$) and T_{total} sufficiently small ($<\sim 60\ \mu M$), Y values should depend on TC_{total} and be relatively insensitive to T_{total}. However, when [TC] is low and $[T]_{total} > 11\ \mu M$, Y should depend on both TC_{total} and T_{total}. At sufficiently high $[T]_{total}$ (i.e., $[T]_{total} \gg$ affinity$_{TC}^{-1}$) Y should approach the TC mole fraction in the reaction mixture. These predictions appear to agree with our experimental results ([10], Fig. 3).

Comparisons between predicted and observed Y values are displayed in Fig. 4 and Table I. Predicted Y values coincided well with observed values and were relatively insensitive to $[T]_{total}$ when $[TC]_{total}$ was large (Fig. 4 *A*). When $[TC]_{total}$ was held constant, observed and predicted Y values were also in good agreement, although discrepancies were noted at high $[T]_{total}$ (Fig. 4 *B*). When $[T]_{total}$ was $<$ affinity$_{TC}^{-1}$, both predicted and observed Y values were less than the TC mole fraction in the reaction mixture. Copolymerization studies with phocellulose chromatographed (PC)-tubulin or heparin-treated tubulin (i.e., preparations depleted of MAP) also gave good agreement between predicted and observed Y values (Table II).

Solution Phase Composition as a Function of Time. As a further test of our theory, we modeled tubulin and TC incorporation into copolymer with time (Fig. 5). This was done by assuming a pseudobimolecular reaction of tubulin and TC with a constant number of microtubule ends (m) in accord with

$$\frac{[TC(t)]_{sol}}{[TC]_{total}} = \frac{\text{affinity}_{TC}^{-1}}{[T]_{total} + \text{affinity}_{TC}^{-1}} + \left[1 - \frac{\text{affinity}_{TC}^{-1}}{[T]_{total} + \text{affinity}_{TC}^{-1}}\right] \exp(-k_+^{TC} m t) \quad (14a)$$

[4] Our analysis was based on Eqs. 4b and 7. If one attempts to use Eq. 9 to determine the limiting value of Y_c at high TC_{total} when $\beta \neq 0$, one would paradoxically find $Y_c \gg 1$, a physically unmeaningful result since the TC mole fraction in the copolymer cannot exceed 1. As noted, Eq. 9 was derived from Eq. 4b and 5a, and should only be used for cases where substoichiometric incorporation of TC is considered (TC mole fractions <0.2). At high levels of $[TC]_{total}$ Eq. 9 breaks down for $\beta \neq 0$. However, when $\beta = 0$, Eq. 9 can be used and gives meaningful values for Y_c (Eq. 12).

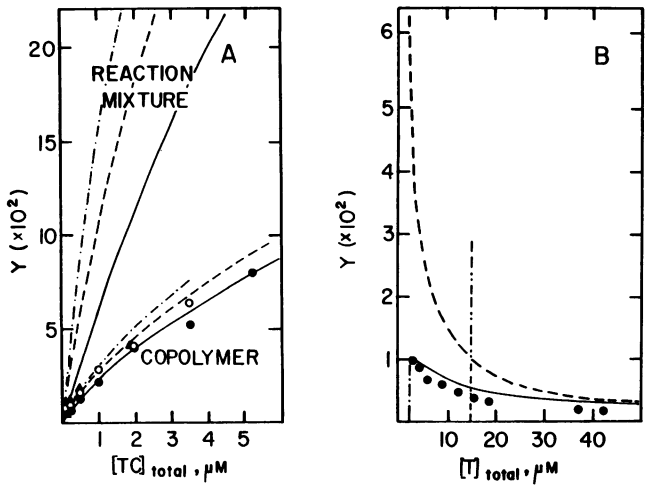

FIGURE 4 Comparison of observed and predicted Y values at steady state. Predicted values were calculated from Eqs. 5a and 9. (A) Predicted and observed Y values plotted against $[TC]_{total}$. Microtubule protein preparations containing fixed tubulin concentrations were polymerized in the presence of various $[TC]_{total}$. Predicted Y values (——), (---), and (·--) for T_{total} = 15.5, 9.3, and 6.2 μM, respectively, were in good agreement with the corresponding observed values (●), (○), and (▲). (B) Predicted (——) and observed (●) Y values plotted against $[T]_{total}$. Microtubule protein preparations consisting of various concentrations of $[T]_{total}$ were polymerized in the presence of 0.13 μM TC. Predicted Y values were calculated from Eq. 5a with affinity$_T^{-1}$ (·--) estimated as ≈2.5 μM (Eq. 7) and affinity$_{TC}^{-1}$ (---·) estimated as ≈15 μM. The TC mole fraction in the reaction mixtures (---) is displayed for comparison.

TABLE II
COMPARISON OF OBSERVED AND PREDICTED COPOLYMER COMPOSITIONS FOR MAP-DEPLETED AND MAP-FREE PREPARATIONS

	TC_{total}	Mol % TC	
		Observed*	Calculated‡
Microtubule protein plus 0.13-mM heparin	μM		
	0.17	0.4	0.36
	0.34	0.6	0.67
	0.68	0.9	1.1
	1.2	1.4	1.7
PC-tubulin	0.82	1.0	1.4
	1.8	2.6	2.6
	2.9	3.8	3.5

*Heparin data from Sternlicht and Ringel (9), T_{total} = 12 μM in PB-2.5 M. Phosphocellulose chromatographed (PC) tubulin data from Sternlicht et al. (10), T_{total} = 27 μM in PB-4M.
‡Eq. 9. Parameters in heparin study (9, 10): $F \sim 6$, $D_c^o \sim 6.5$ μM, $\omega \sim 4$, $\beta \sim 0$ (T_{total} = 12 μM). $Y = [TC]_{total}/(44.5 + 20\,[TC]_{total})$; parameters in PC-tubulin study (10): $F \sim 4$, $D_c^o \sim 7$, $\omega \sim 4$, $\beta \sim 0$ (T_{total} = 27 μM). $Y = [TC]_{total}/(48 + 12\,[TC]_{total})$. (Concentrations are in micromolars.)

and

$$\frac{[T(t)]_{sol}}{[T]_{total}} = \frac{\text{affinity}_T^{-1}}{[T]_{total}} + \left[1 - \frac{\text{affinity}_T^{-1}}{[T]_{total}}\right] \exp(-k_+^T mt). \quad (14b)$$

Eq. 14 was derived from Eqs. 4a and 5, affinity$_T^{-1}$ was estimated from Eq. 7, and affinity$_{TC}^{-1}$ was estimated to be equal to 6 affinity$_T^{-1}$. $k_+^{TC}m$ and k_+^Tm were estimated from the observed TC and tubulin incorporation rates (Fig. 3, Materials and Methods with $k_+^Tm = k^T$ and $k_+^{TC}m = k^{TC}$). k^T/k^{TC} and k^T varied with reaction mixture conditions but had average apparent values of 1.5 ± 0.5, and 0.14 ± 0.05 min^{-1}, respectively. Overall agreement between observed and simulated values was good. Discrepancies noted in the TC simulations at low TC (Figs. 5 *B–D*) may indicate affinity$_{TC}^{-1}$ values somewhat larger than our estimated values (see Discussion).

This kinetic analysis suggests that k_+^T and k_+^{TC} have similar values within a factor of ~1.5 to 2. Since our best estimates of affinity$_{TC}$ and affinity$_T$ also suggested that affinity$_{TC}^{-1}$ > 6 affinity$_T^{-1}$, we concluded that $k_-^{TC} > 4\,k_-^T$. Thus, differences in the microtubule affinities for TC and tubulin reflect primarily differences in the apparent dissociation rate constants.

Other Comparisons. We also tested the above theory by comparing predicted values from Eqs. 9–11 with

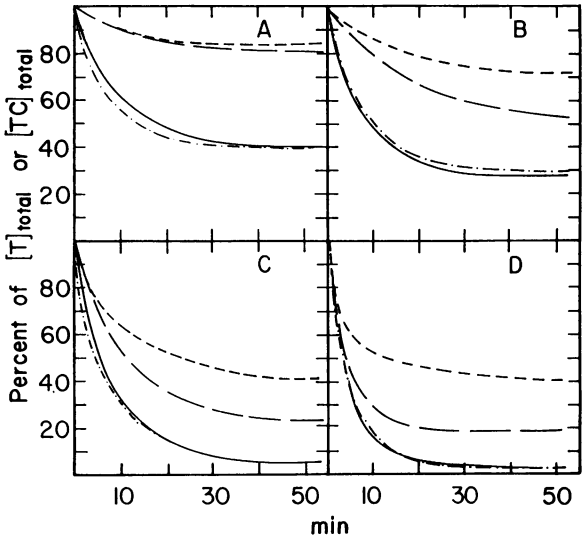

FIGURE 5 Simulated and observed copolymerization kinetics. Predicted TC (— —) and tubulin (——) concentrations in the solution phase during assembly, expressed as percentages of TC_{total} and T_{total}, are compared with the corresponding observed values (---), (·--). Observed valued in *A–C* are reproduced from data displayed in Fig. 3, and were derived from material balance considerations (Materials and Methods). Observed values in *D* were derived using a more direct approach based on turbidity measurements and analyses of supernatants during assembly (Materials and Methods). TC_{total} was 2 μM in *A* and ~0.15 μM in *B–D*. T_{total} was 14.5 μM in *A* and *B*, 42 μM in *C*, and 59 μM in *D*. Affinity$_{TC}^{-1}$ was taken to be 37 μM in *A* and ~15 μM in *B–D*.

measured steady-state values from copolymerization reactions at various microtubule protein and TC concentrations. Observed values were superimposed on the calculated plots (Figs. 6–9) and were generally in good agreement with predicted values. However, several aspects of this comparative study (i.e., Figs. 6, 8, and 9) require clarification and discussion.

Predicted Y values as a function of inhibition were in good agreement with experiments in most cases (Table I, Fig. 6 *A*). The increase in Y (at a given level of inhibition) as T_{total} increased (Fig. 6 *A*) reflected the requirement for higher TC levels to compensate for higher T_{total}. Discrepancies were noted at high TC_{total} (>10 μM) (data not shown). Although our model (Eq. 10b) predicts Y values >0.1 at high TC_{total} (high levels of inhibition), we have not been able to produce Y values >~0.1 (11).

In Fig. 8 we plot predicted $[TC]_{MT}$ vs. $[T]_{MT}$ for various $[T]_{total}$ and $[TC]_{total}$ values. Calculated values are displayed as contour plots at constant $[T]_{total}$ and $[TC]_{total}$. Contour plots are a powerful way to summarize copolymerization. Whether a particular line of constant $[T]_{total}$ does or does not intersect a particularly line of constant $[TC]_{total}$

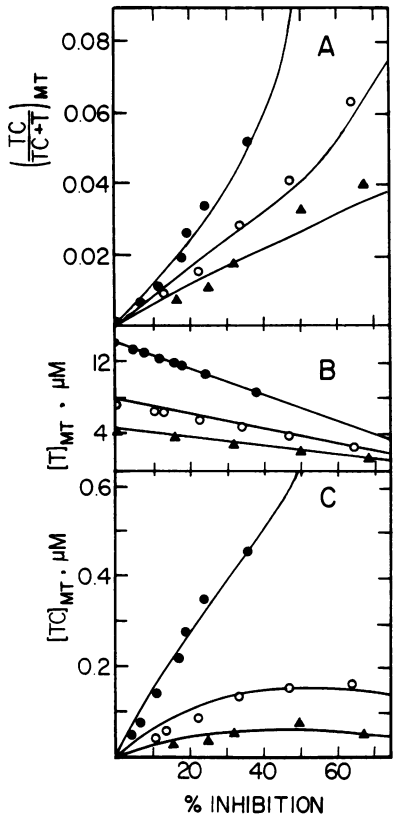

FIGURE 6 Steady-state values of Y, T_{MT}, and TC_{MT} vs. percent inhibition for three concentrations of $[T]_{total}$: 6.2 (▲), 9.3 (O), and 15.5 (●) μM. The theoretical curves in *A* and *B* and *C* were calculated from Eqs. 10 a, b, c, respectively. Percent inhibition refers to the percent decrease in microtubule yield in the presence of TC relative to the TC-free controls, and were estimated from turbidity measurements (10).

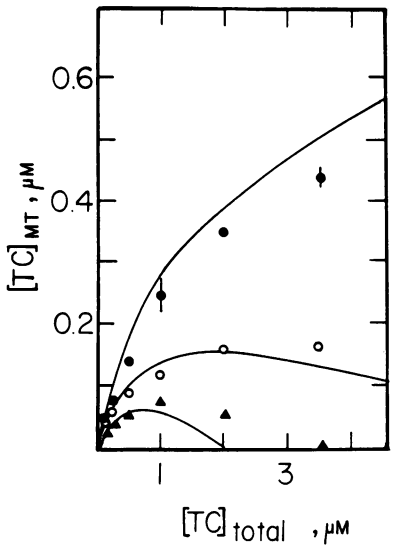

FIGURE 7 $[TC]_{MT}$ at steady state as a function of TC_{total}. Co-assembly studies were done at constant T_{total}, and the results of three such studies T_{total} = 6.2 (▲), 9.3 (O), and 15.5 (●) μM are shown together with the predicted values (—) calculated from Eq. 11 b.

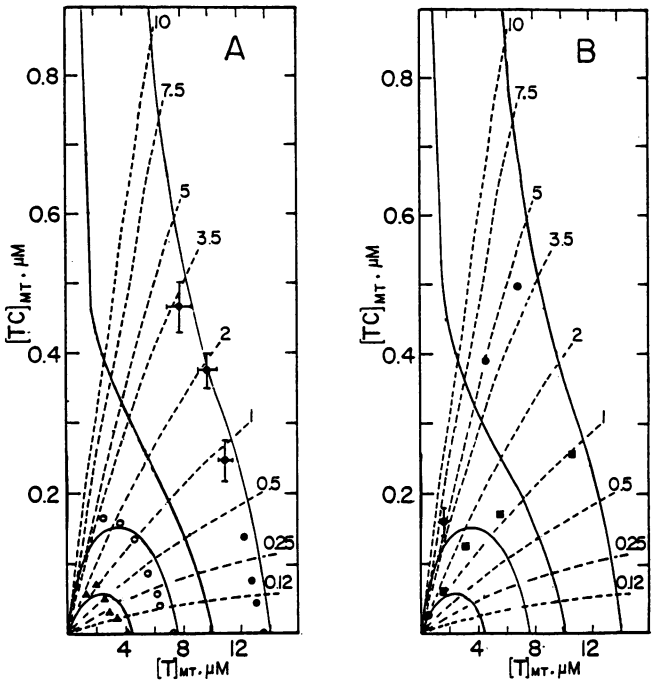

FIGURE 8 Contour plots relating the steady-state values for $[TC]_{MT}$ and $[T]_{MT}$ to $[T]_{total}$ and $[TC]_{total}$. Lines of constant T_{total} (—) and constant TC_{total} (---) were calculated with the aid of Eq. 11. T_{total} values increase as one proceeds from the lower left-hand to the upper right-hand corner of each panel and have the values of 6.2, 9.3, 12, and 15.5 μM. TC_{total} values ranged from 0 to 10 μM as indicated. (*A*) Observed $[TC]_{MT}$ and $[T]_{MT}$ values from copolymerization reactions carried out at constant T_{total} (T_{total} = 6.2 [▲], 9.3 [O], and 15.5 [●] μM) and various TC_{total}. Vertical and horizontal bars represent standard deviation estimates based on several repeat experiments. (*B*) Observed $[TC]_{MT}$ and $[T]_{MT}$ values from copolymerization reactions carried out at constant TC_{total} (TC_{total} ≡ ~1.2 [■] and 3.5 [●] μM) and various T_{total}.

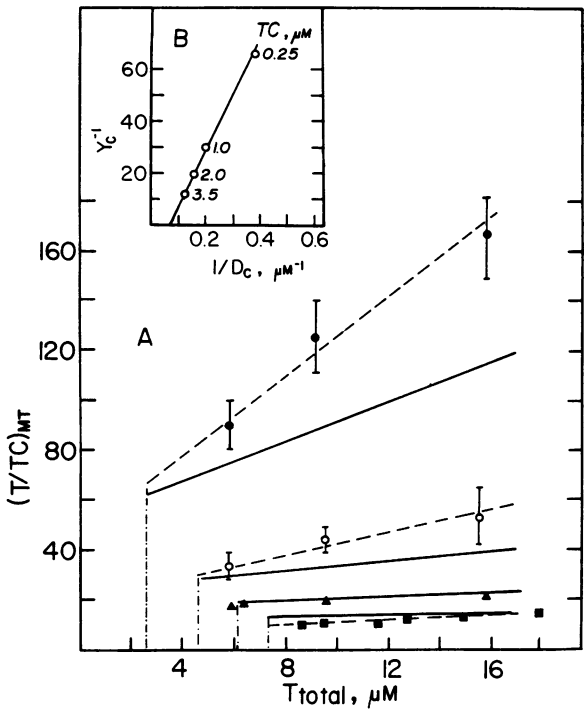

FIGURE 9 (*A*) $(T/TC)_{MT}$ ratios at steady state plotted against $[T]_{total}$. Measurements were done at a series of constant TC concentrations: 0.25 (●), 1.0 (○), 2.0 (▲), and 3.5 (■) μM. The corresponding predicted values (—) were calculated from Eq. 9, which predicts a linear dependence on T_{total}. Observed and predicted values extrapolate to similar values (Y_c^{-1}) at the tubulin critical concentrations, $T_{total} = D_c$ (---). (*B*) Y_c^{-1} plotted against D_c^{-1}. Y_c^{-1} varied linearly with D_c^{-1} when $[TC]_{total} > 0.25$ μM, and extrapolated to 1 at high $[TC]_{total}$. The vertical bars denote standard deviation estimated based on three or more determinations.

depends on D_c in the presence of TC_{total}. (If $T_{total} > D_c$, the two lines intersect indicating copolymer formation; if $T_{total} < D_c$, the lines do not intersect indicating total inhibition.) Intersection of a constant T_{total} line with a constant TC_{total} line defines a pair of predicted $[TC]_{MT}$ and $[T]_{MT}$ values. Theory and experiment agree at low to intermediate TC_{total} but not at high TC_{total}. We noted that constant T_{total} lines can be divided into two groups. The first group, which intersects the abscissa twice (once at the origin), defines a range of tubulin concentrations ($T_{total} < 11$ μM) that can be totally inhibited from polymerizing by sufficiently high TC concentrations; the second group, which intersect the absicssa once, defines a range of tubulin concentrations ($T_{total} > 11$ μM) that cannot be totally inhibited even at very high TC concentrations. However, contrary to this prediction, high concentrations of colchicine totally inhibit microtubule assembly even if microtubule protein concentrations are high. This process (capping) occurs with colchicine abruptly blocking assembly, a mechanism qualitatively different from the one we have considered, although inhibition is still presumed to occur via the TC complex (13) (see Discussion).

In Fig. 9 *A* we plot predicted and observed Y^{-1} values vs. $[T]_{total}$ for several $[TC]_{total}$ values. The observed Y^{-1} values were extrapolated to $[T]_{total} = D_c$, permitting us to estimate Y_c, the Y value at the critical tubulin concentrations. We have previously suggested that an understanding of Y_c may be essential for a comprehensive understanding of the inhibition process (11). In Fig. 9 *B* we display a double reciprocal plot of Y_c vs. D_c that extrapolates to a limit of $D_c^{-1} \sim 0.08$ μM^{-1} when $Y_c^{-1} = 1$, suggesting that copolymers composed primarily of TC should exist at sufficiently high TC_{total} and that tubulin should bind to these copolymers with an apparent dissociation constant of ~13 μM. Despite these predictions we have not obtained copolymers with Y values >0.1 under our assembly conditions.[5] We have interpreted this failure as an indication that in copolymers for each TC there must be ~10 tubulins for assembly to occur, which is in agreement with the work by Farrell and Wilson (12) that proposed a critical ratio of ~0.1.

DISCUSSION

Our theory has predicted Y values in good agreement with those observed at moderate TC concentrations over a wide range of MAP concentrations. This model was checked against earlier copolymerization studies (9, 10) and was based on the following assumptions. (*a*) Microtubule ends are assembly competent in the presence of TC (9); (*b*) the affinity of the microtubule ends for tubulin decreases as $[TC]_{total}$ increases (9, 10); (*c*) TC incorporation is random; (*d*) the copolymerization reaction proceeds to an equilibrium endstate. However, assumption (*d*) is an approximation because the two ends of the copolymer presumably have different affinities for tubulin (different k_+ and k_-) (29). A more rigorous analysis that allows for differences between the two ends leads to conclusions (unpublished results) that are similar to our simplified derivation.

We derived Oosawa-Kasai type equations (Eqs. 2a and b) that defined tubulin and TC incorporation rates into copolymers. Estimates for copolymer composition (Y) were obtained from these equations provided k_+^T and k_+^{TC}, the apparent association rate constants had similar values ([12], Figs. 2 and 4). Copolymer composition depended both on reaction mixture composition and on affinity_T^{-1} and $\text{affinity}_{TC}^{-1}$, the apparent dissociation constants for tubulin and TC bound to copolymer ends. Affinity_T^{-1} was related to the TC content in the reaction mixture (Eq. 7), while $\text{affinity}_{TC}^{-1}$ was taken as ~6 affinity_T^{-1} (10). Application of these findings led to expressions (Eqs. 10 and 11) that successfully predicted several copolymer properties (Figs. 6–9).

Predicted Y values were sensitive to $\text{affinity}_{TC}^{-1}$ (Eq. 5) that was estimated from affinity_T^{-1} (X_∞/Y_∞), i.e., from the tubulin critical concentrations and the TC mole fraction ratio in the solution and copolymer phases at steady state

[5]Although there are reports that TC will polymerize under appropriate conditions, these TC-rich polymers appear to be different from microtubules (27, 28, 37).

(see footnote 3) (10). From composition analysis we estimated that affinity$_{TC}^{-1}$ increases with increasing TC_{total} and is approximately proportional to affinity$_{T}^{-1}$ (10). Subsequent studies (Figs. 3 and 5) suggest, however, that affinity$_{TC}^{-1}$ may not increase as rapidly with increasing TC_{total} as we originally thought. We cannot completely exclude the possibility that affinity$_{TC}^{-1}$, unlike affinity$_{T}^{-1}$, may be essentially independent of TC_{total}. Clearly a method to directly determine affinity$_{TC}^{-1}$ as a function of TC_{total} is needed. Our estimate that affinity$_{TC}^{-1} \gtrsim 6$ affinity$_{T}^{-1}$ is the opposite from Lambier and Engelborghs, who found affinity$_{TC}^{-1} \sim 1/6$ affinity$_{T}^{-1}$ (16). We are unable to reconcile our experimental results with their estimate that microtubule ends have a greater affinity for TC than for tubulin.

Although our model was derived from Oosawa-Kasai equations, the theoretical results are not a consequence of this particular formulation. We have also derived a time-independent solution, Y^{∞} (Eq. 5), without recourse to kinetic arguments or kinetic equations. This alternate derivation (unpublished results) was based on a material balance at steady state and hypothesizes that X_{∞}/Y_{∞} = affinity$_T$/affinity$_{TC}$. The kinetic approach, however, appears to be more general and useful.

The copolymerization mechanism was a more complex reaction than we earlier envisioned. Copolymer compositions were more dependent on tubulin concentration at low $[TC]_{total}$ than high $[TC]_{total}$ (Table I). Furthermore, at constant TC_{total} tubulin facilitates TC incorporation. Our theory suggests an important factor determining TC incorporation was the $[T]_{total}$/affinity$_{TC}^{-1}$ ratio (Eq. 5b). In our model tubulin facilities TC incorporation by assembling over the TC complex and reducing the apparent TC dissociation rate constant (from k_-^{TC} to Yk_-^{TC}). Thus, while the number of microtubule ends are equal to m, the number of microtubules with a TC at the immediate end position, m_{TC}, is equal to Ym. The TC depolymerization rate ($k_-^{TC}\, m_{TC}$) (Eq. 2b) effectively equals $Yk_-^{TC}m$. When Y is small (<0.1) this depolymerization rate is actually less than the tubulin depolymerization rate. Tubulin facilitation of TC incorporation explains the relationship of Y and time (Fig. 3). We anticipate three classes of time-dependent Y behavior. (*a*) If $T_{sol} >$ affinity$_{TC}^{-1}$ during assembly, we expect nearly ideal behavior with Y relatively constant and approximately equal to the reaction mixture TC mole fraction. (*b*) If $[T]_{sol}$ decreases gradually from values > affinity$_{TC}^{-1}$ at the start of assembly to values < affinity$_{TC}^{-1}$ at the end of assembly, we expect Y to vary during assembly (Fig. 3 *E*). (*c*) If $[T]_{sol} <$ affinity$_{TC}^{-1}$, we expect Y values to be significantly less than the reaction mixture TC mole fraction, and to vary very little during assembly (Fig. 3 *A*).

Our critical ratio concept followed from tubulin's facilitation of TC incorporation (11). If TC alone cannot incorporate into microtubules under normal assembly conditions but requires tubulin, there may be a maximum value for the TC mole fraction in the copolymer (critical ratio). The critical ratio presumably reflects microtubule structure, the molecular interactions between tubulin, TC and possibly MAP, and the biochemical factors involved in assembly (guanosine triphosphate [GTP] hydrolysis). This theory predicts a critical ratio under certain conditions ($\beta = 0$, Eq. 12), and links this ratio to ω (the constant relating affinity$_{T}^{-1}$ to TC_{total}; Eq. 7) and to F (the affinity$_T$/affinity$_{TC}$). As ω increases, assembly inhibition also increases and the critical ratio decreases. Estimating ω and F, we predict a critical ratio of ~0.05 ($\beta = 0$), similar to the critical ratio of ~0.07 reported by Farrell and Wilson (12). Their perception of tubulin facilitating TC incorporation is qualitatively similar to ours, except they hypothesize a cyclical pattern, TC inhibition followed by recovery as tubulin assembles over TC, which occurs on a timescale shorter than our analysis. In terms of their model, our rate constants (k_+, k_-) and component parts (k_+^T, k_+^{TC}, and k_-^T, k_-^{TC}) represent complex events that presumably could be obtained by averaging assembly kinetics over a inhibition-recovery cycle. This concept of inhibition-recovery cycles can rationalize TC inhibition over wide ranges of TC concentration (12). A direct demonstration that such cycles exist remains to be done.

Although our copolymerization model successfully reproduced the experimental observations in most cases, discrepancies existed at high $[T]_{total}$. When $[T]_{total} \gg$ affinity$_{TC}^{-1}$ the predicted (Eq. 5b and 6) convergences of Y to the reaction mixture value, and $[TC]_{MT}$ to $[TC]_{total}$ did not occur. Although the discrepancies were not large (Fig. 4 *b* and 5 *d*) and may have arisen from a variety of factors, we believe that inadequacies in our theory may be partly responsible for the disagreement. When $T_{total} \gg$ affinity$_{T}^{-1}$, the instantaneous TC and tubulin solution phase concentrations differ significantly from TC_{total} and T_{total} during assembly. Furthermore, k_+^T appears to be ~1.5–2 times as large as k_-^{TC} ([12], Fig. 5). Consequently, we suspect that when $T_{total} \gg$ affinity$_{TC}^{-1}$ and assembly goes to completion (inhibition is small), (*a*) differences between k_+^T and k_+^{TC} need to be considered, and (*b*) the final copolymer compositions should be related to the instantaneous rather than the initial concentrations. Our rate equation (Eq. 2) explicitly allows for differences in k_+^T and k_-^{TC}, and relates the incremental changes in $[TC(t)]_{MT}$ and $[T(t)]_{MT}$ to the instantaneous solution-phase compositions.

We have been unable to confirm two other predictions of our theory, i.e., that copolymers composed primarily of TC should form at high TC concentrations, and that assembly of microtubule protein preparations, where $T_{total} > 11\ \mu M$, should not be totally inhibitable by TC (see footnote 5). These discrepancies may be a consequence of using improper parameters in our derivation. For example, D_c was estimated (Eq. 7) at high TC_{total} by extrapolating D_c values at low and moderate [TC] to high [TC] (10). The predicted limit for D_c of ~11 μM (Eq. 7) also limits the extent to which TC inhibits assembly. These discrepancies at high TC would diminish if the actual D_c values deviated

from extrapolated D_c values and increased (in excess of 11 μM) as TC_{total} increased.

Although improper estimates of certain parameters may be partially responsible for the apparent discrepancies at high TC, we believe that other factors need to be considered. High colchicine concentrations inhibit assembly by a process (capping) that is qualitatively different from copolymerization. Capping was originally thought to involve irreversible binding of colchicine to microtubule ends, however, it is now thought that colchicine inhibits microtubule assembly via the TC complex (13). Reversible, weak colchicine binding to several other sites on tubulin (30–32) is not considered important. High [TC] may adversely affect MAP function, in contrast to a negligible effect at low to moderate [TC] (10). Inhibition by high colchicine concentrations is similar to the blocking effects that high guanosine diphosphate (GDP) concentrations have on tubulin assembly (33). Zackroff et al. (34) proposed that GTP stabilizes the binding of microtubule ends and oligomers (tubulin-MAP complexes) as part of the elongation process, while high GDP (33) and colchicine (34) destabilize this binding. Our neglect of this destabilization mechanism may account for the anomolous predictions by our theory at high [TC]. Zackroff et al.'s proposal relating colchicine and GDP inhibitory effects to a common mechanism was strengthened by the finding of an endogenous GTPase activity intrinsic to tubulin that is stimulated by colchicine (35).

Discrepancies at high [TC] could also result from limitations of our simplified model based on copolymer composition that neglects the possibility that certain tubulin-TC sequences at the assembly ends may be particularly unfavorable for assembly. Farrell and Wilson suggested that when the TC-tubulin ratio at the copolymer ends equals or exceeds a critical value, the inhibition process becomes cooperative, possible because of TC-TC neighbor effects (12). The number of assembly-component ends reaches zero and assembly is completely blocked, i.e., a kinetic cap is established. Cooperative effects of the type envisioned by Farrell and Wilson have interesting implications, and may be sensitive to the types of tubulin-TC sequences present at the microtubule ends.

In summary, we have formulated a copolymerization theory that successfully models the TC inhibition process at low and moderate concentrations of TC. At high TC concentrations, the theory was inadequate. Although various explanations for this are possible (12, 34), the molecular basis for the theory's deficiency at high TC remains to be established.

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