Taxol Stabilization of Mitotic Spindle Microtubules: Analysis Using Calcium-Induced Depolymerization

E.D. Salmon and Stephen M. Wolniak

University of North Carolina, Chapel Hill, North Carolina (E.D.S.), University of Maryland, College Park, Maryland (S.M.W.) and Marine Biological Laboratory, Woods Hole, Massachusetts (E.D.S., S.M.W.)

Taxol stabilizes or promotes the assembly of microtubules. In this report we characterize the rate, extent, and reversibility of taxol stabilization of calcium-labile microtubules in isolated mitotic spindles, principally from embryos of the sand dollar Echinarchaenus parma. The intense depolymerizing action of 100 μM Ca²⁺ was used to assess the extent of stabilization by taxol. Changes in spindle microtubule assembly were evaluated and recorded by measuring changes in spindle birefringent retardation (BR). Membrane-free mitotic spindles, isolated with a calcium-chelating, nonionic detergent buffer, were stored in an EGTA-glycerol storage buffer to prevent microtubule depolymerization. When perfused with an EGTA-buffer without glycerol, microtubules in these isolated spindles depolymerized gradually over 60–120 min; but in isolated spindles perfused with buffer that contained 100 μM Ca²⁺, BR decreased by 90% within 2–5 sec. In contrast, spindles that were pretreated for 3 min with 1 μM taxol, or for about 30 sec with 10 μM taxol, lost less than 10% of their initial BR when perfused with buffer containing 100 μM Ca²⁺. The rate and extent of microtubule stabilization by taxol depended on both the concentration and the duration of exposure to taxol. Taxol stabilization was reversible. After a 15 min preincubation with 1 μM or 10 μM taxol then washout, stability of spindle BR to 100 μM Ca²⁺ decreased exponentially with a time constant of 30–60 min. Thus taxol dissociates from spindle microtubules at significant rates; taxol-stabilized microtubules are not "fixed."

Key words: taxol, microtubules, mitosis, mitotic spindle, calcium

Received January 27, 1984; accepted March 8, 1984.

Address reprint requests to Dr. E.D. Salmon, Department of Biology, University of North Carolina, Chapel Hill, NC 27514.

© 1984 Alan R. Liss, Inc.
INTRODUCTION

The antimitotic drug taxol, a neutral compound extracted from the bark of the Western yew Taxus brevifolia [Wani et al., 1971], inhibits cell replication and development, apparently by stabilizing microtubules or promoting their assembly [Albertini and Clark, 1981; Brenner and Brinkley, 1982; Cande, McDonald, and Meensen, 1981; De Brabander et al., 1981a,b; Heidemann and Gallas, 1980; Schatten et al., 1982; Schiff and Horwitz, 1980]. Most other antimitotic drugs, such as colchicine, podophyllotoxin, vinblastine, nocodazole, and steganacin, inhibit the polymerization of microtubules [De Brabander et al., 1981a; Dustin, 1978; Kumar, 1981; Schiff and Horwitz, 1981]. Taxol may be a potentially valuable and novel physiological probe, because most microtubule-dependent processes have been studied previously by disrupting the structure as well as the function of the microtubules. Taxol could be used to stabilize the structure of microtubules, and thus expose any functions of the microtubules that depend on the normal rate of tubulin depolymerization or subunit flux through the microtubule. The ultimate usefulness of taxol as a physiological probe, however, depends on the specificity of its stabilizing effect on intact microtubules and on the rate, extent, and reversibility of its stabilizing effect.

Characterization of taxol's action on the structure and assembly of microtubules is just beginning. In vitro experiments with purified microtubule protein and phosphocellulose-purified tubulin from brain [Caplow and Zeeberg, 1982; Kumar, 1981; Schiff, Fant, and Horwitz, 1979; Schiff and Horwitz, 1981; Thompson, Wilson, and Purich, 1981; Vallee, 1982] have shown that taxol enhances the rate and extent of microtubule assembly. Taxol's effect was greatest at a concentration that is about stoichiometric with the tubulin dimer. In vitro, microtubules could assemble in the presence of taxol even under conditions that normally prevent assembly—e.g., 4°C, 4 mM Ca^{2+}, absence of GTP, or absence of assembly-stimulating microtubule-associated proteins [Kumar, 1981; Schiff et al., 1979; Schiff and Horwitz, 1981; Thompson et al., 1981]. Using ^3H-taxol, Parness and Horwitz [1981] have demonstrated that taxol binds stoichiometrically to purified brain tubulin that is assembled into microtubules. Taxol apparently does not affect the assembly of actin [Parness and Horwitz, 1981] or actin-dependent cellular processes [Schatten et al., 1982], nor does it seem to compete with the binding of microtubule-associated proteins to brain microtubules [Caplow and Zeeberg, 1982; Schiff and Horwitz, 1981; Vallee, 1982].

In this report, we show that taxol reversibly stabilizes the calcium-labile microtubules of isolated mitotic spindles, principally those of the sand dollar Echinarchaenius parma. We used the intense depolymerization action of 100 μM Ca^{2+} to analyze the rate, extent, and reversibility of stabilization using a pulse-chase, perfusion protocol. Relative changes in microtubule assembly of individual spindles were quantitated by measurements of spindle birefringent retardation (BR) made by sensitive polarization microscopy and video recording techniques.

MATERIALS AND METHODS

Taxol was provided by Dr. John Douros, the National Products Branch, Division of Cancer Treatment of the National Cancer Institute. It was dissolved in dimethyl sulfoxide (DMSO) at a concentration of 10 mM and stored at −20°C. The
final concentration of DMSO in the experiments, 0.1% or less, had no effects in control experiments.

**Biological Material**

Gametes from the sand dollar Echinarchnus parma (collected by the Marine Biological Laboratory, Woods Hole, MA) were obtained by injecting 0.3 M KCl into the body cavity, as described by Costello and Henley [1971]. Dry sperm were stored at 4°C. The eggs were washed twice in filtered seawater before fertilization.

Mitotic cytoskeletons and spindles were isolated and stored at 4°C as described in detail elsewhere [Salmon, 1982a,b; Salmon and Segall, 1980]. The eggs were fertilized, washed in 1 M glycerol, 5 mM Tris HCl, pH 8.0, to remove the fertilization membranes, then incubated in filtered seawater for several minutes before spindles were isolated. At the desired stage for isolation, determined by monitoring spindle BR, the developing embryos were rinsed once in 0.55 M KCl, 0.026 M NaCl, 5 mM Tris, pH 6.8, to remove the seawater salts. The embryos were then resuspended in an EGTA-lysis buffer that contained 5 mM EGTA, 0.5 mM MgCl₂, 20 mM PIPES, pH 6.8, plus 15% glycerol (vol/vol) and 1% Nonidet P40 at about 20°C. After 15 min of lysis, the extracted embryos were pelleted at 500g for 10 min. Then they were resuspended in an EGTA-glycerol storage buffer that contained 5 mM EGTA, 0.5 mM MgCl₂, 10 mM PIPES, pH 6.8, and 0.1 mM dithiothreitol (DTT) in 50% glycerol (vol/vol) and stored at 4°C. After two or three days, the settled pellet was resuspended in fresh EGTA-glycerol storage buffer at 4°C. As described previously for Lycotechnus variegatus embryos, the EGTA lysis procedure yielded a whole-embryo mitotic cytoskeleton—the mitotic spindle suspended within the cell cortex [Salmon, 1982a,b; Salmon and Segall, 1980]. Although the spindles could be freed from the cortex by pipetting the preparation in the EGTA-lysis buffer, this was not done routinely because spindles surrounded by the cortex behaved the same as completely isolated spindles. Second- and third-division mitotic embryos were usually used because more spindles could be isolated at once than from first-division embryos. The size and BR of second- and third-division spindles were similar to those of first-division spindles.

**Experimental Protocol**

Isolated mitotic spindles or mitotic cytoskeletons, suspended in a drop of glycerol storage buffer, were sandwiched between an ethanol-cleaned slide and a coverslip that was supported by parallel ridges of silicone grease (Dow Corning, Midland, MI). The isolated spindles were allowed to settle, and some adhered to the slide. Spindles in isolated mitotic cytoskeletons were more convenient to use because the surrounding cortex, firmly anchored in the slide—coverslip sandwich, held them in place.

Preparations were perfused initially with EGTA-perfusion buffer to wash out the glycerol and to lower the EGTA concentration before experimental buffers were added. It is easy to tell when the glycerol has been washed out, because the spindle BR rises abruptly as the refractive index of the medium drops [Salmon, 1982a,b]. The EGTA perfusion buffer contained 0.2 mM EGTA, 0.5 mM MgCl₂ and 10–50 mM PIPES, pH 6.8. The 2 μM Ca²⁺ buffer contained 1 mM EGTA, 0.8 mM CaCl₂, 0.5 mM MgCl₂, 10–50 mM PIPES, pH 6.8. The 100 μM Ca²⁺ buffer contained 1 mM EGTA, 1.08 mM CaCl₂, 0.5 MgCl₂, and 10 mM PIPES, pH 6.8. The basic pulse-chase protocol began with the perfusion with EGTA buffer containing various
Concentrations of taxol followed by perfusion with 100 μM Ca\(^{2+}\) buffer (with or without taxol, depending on the experiment) to depolymerize rapidly microtubules or possibly regions of a microtubule not stabilized by bound taxol.

**Spindle BR as a Measure of Microtubule Assembly**

Changes in spindle microtubule assembly were evaluated and recorded by measuring changes in spindle BR with polarizing microscopy and video recording techniques. Changes in spindle BR were usually normalized by the measured value of BR in EGTA perfusion buffer just before addition of 100 μM Ca\(^{2+}\) to give the value BR. For photography and BR measurements, mitotic spindles were oriented with the interpolar spindle axis at ±45 degrees to the analyzer-polarizer directions and normal to the principal axis of the microscope. BR was measured by eye using a Brace-Koehler λ/30 compensator [Salmon and Ellis, 1976] at a spot in the central half-spindle region about 4 μm from the chromosomes.

For mitotic spindles isolated from echinoderm eggs, the majority of BR can be accounted for by the form birefringence of microtubules and a smaller percentage by the aligned non-microtubular material along the spindle fibers [Hiramoto et al., 1981a,b; Salmon and Segall, 1980]. A detailed analysis is given by Hiramoto et al. [1981a,b] and Sato, Ellis, and Inoue [1975]. In our spindle depolymerization experiments, the spindles lost BR without any significant changes in spindle width in 100 μM Ca\(^{2+}\) buffer. Consequently, decreases in the number of half-spindle microtubules and decreases in the assembled tubulin concentration in the central half-spindle region are proportional to decreases in BR [Hiramoto et al., 1981a, b; Sato et al., 1975]. Stephens has also found that the total tubulin in isolated spindle-aster complexes is correlated with the magnitude of BR measured in the central half-spindle region [Stephens, 1972]. For E. parma metaphase isolates, the central half-spindle region had an initial BR of 3.2 ± 3 nm and a diameter of about 6 μm in EGTA buffer without glycerol. Based on the form BR of spindle microtubules, this value of initial BR corresponds to 3,000–3,500 microtubules in the central half-spindle region [Hiramoto et al., 1981b; Sato et al., 1975].

**Instrumentation**

Instruments for polarization microscopy, 35-mm photography, measurement of spindle BR, and calculation of the free Ca\(^{2+}\) concentration of the Ca-EGTA perfusion buffers were as described elsewhere [Salmon, 1982a; Salmon and Segall, 1980]. Video recordings were obtained using a DAGE-MTI Model 65S Newvicon video camera (Michigan City, IN), a Vicon Model V240T time-date generator, and a Sanyo Model VTR-1375 time-lapse video tape recorder. Video illustrations were made from 35-mm photographs of a Sanyo model VM4209, 9-inch monitor.

**RESULTS**

**Calcium Lability of E. parma Isolated Spindles**

In EGTA perfusion buffer at 22°C the BR of isolated E. parma spindles decayed slowly, reaching 50% of the initial value in 45 min and 10% of the initial value in about 120 min. In contrast, perfusion with 2 μM Ca\(^{2+}\) buffer caused an 80% decrease in spindle BR within 2–3 min, and Ca\(^{2+}\) at 100 μM diminished the spindle BR by 90% in less than 2–5 sec; this rate appeared limited by the rate of perfusion (Fig. 1). In general, the higher the Ca\(^{2+}\) concentration, the more complete and the more abrupt
was the decrease in spindle BR. The pattern of BR change with 100 μM Ca²⁺, however, was distinctly different than was observed for lower Ca²⁺ concentrations. When isolated spindles were perfused with buffer that contained 100 μM Ca²⁺, then the BR disappeared as a wave moving across the spindle-aster complex apparently coincident with the front of 100 μM Ca²⁺ passing the spindle (Fig. 1). The pattern of BR loss was independent of spindle morphology and depended principally on the direction of perfusion. At lower Ca²⁺ concentrations, the rate of depolymerization was slower, and the pattern of BR loss depended on the morphology of the spindle. The pattern was similar to that described in detail previously for the calcium-labile spindles isolated from embryos of Lytechinus variegatus [Salmon and Segall, 1980]. Regardless of the concentration of Ca²⁺, about 10% of spindle BR persisted (0.2–0.3 nm), even under the most extreme conditions. This persistent BR may represent stable kinetochore fiber microtubules [Hiramoto et al, 1981b; Salmon, 1982a; Salmon and Segall, 1980], or non-microtubular components of the spindle matrix that are aligned in the spindle remnant.

**Stabilization by Taxol**

In general, treating isolated spindles with taxol increased their stability immensely. For spindles maintained in EGTA buffer with 1 μM taxol, spindle BR remained constant over 240 min, at 22° or 4°C. We would have seen the BR disappear almost completely during the same period without taxol in the buffer. After isolated spindles were perfused for 5 min with EGTA buffer that contained as little as 1 μM

![Fig. 1. Video records of the disappearance of spindle fiber birefringent retardation (BR) induced by perfusion with 100 μM Ca²⁺ buffer. In both (a) and (b), a metaphase spindle in a third-division mitotic cytoskeleton isolated from an E. parma embryo was perfused, in a direction indicated by the arrow, first with EGTA buffer for 3 min and then with 100 μM Ca²⁺ buffer. The spindle interpolar axis is oriented at 45 degrees to the analyzer-polarizer direction of the polarization microscope. The compensator is set for 7 nm positive retardation with respect to the spindle interpolar axis. Astral fibers aligned perpendicular to the spindle interpolar axis appear in dark contrast because of negative compensation. Time is indicated in hours:minutes:seconds on each frame. Scale = 10 μm.](image-url)
taxol, spindle BR was stable to the addition of 100 μM Ca^{2+} or 0.5 M KCl. (Without taxol treatment, concentrations of KCl above about 0.3 M induced rapid loss of spindle BR, within about 10 sec or less, but we have not explored the action of KCl in detail.)

A pulse-chase procedure was used to measure the rate at which spindle microtubules were stabilized in the presence of 1 μM or 10 μM taxol (Fig. 2). Spindles pretreated for 2 min with 1 μM taxol in EGTA buffer retained about 50% of the initial BR (BR_i) after perfusion with 100 μM Ca^{2+} buffer; spindles pretreated for 4 min with 1 μM taxol retained greater than 90% BR_i. The rate of stabilization also depended on the taxol concentration. A 1-min pretreatment with 1 μM taxol produced no stabilization (Fig. 2), but spindles pretreated for 1 min (or even 30 sec) with 10 μM taxol retained more than 90% of BR_i after addition of 100 μM Ca^{2+}. The BR of astral and central spindle fibers appeared equally stabilized by taxol (Fig. 3).

Further experiments were performed to explore the relationship between time, concentration of taxol, and spindle stability at lower concentrations of taxol. In Figure 4, isolated spindles were pretreated for 15 min with various concentrations of taxol. Stability was assessed from the BR remaining after the pretreatment period with taxol and after the taxol-treated spindles were perfused with 100 μM Ca^{2+} buffer. Taxol concentrations greater than 0.1 μM for 15 min (Fig. 4) noticeably reduced the

Fig. 2. The normalized amount of spindle BR stable in 100 μM Ca^{2+} (BR) following pretreatment for various periods with 1 μM taxol in EGTA perfusion buffer. Each kinetic curve represents BR measurements on a single spindle. The duration of taxol pretreatment, in minutes, is indicated on each curve. BR data following Ca^{2+} perfusion is normalized (BR) by the BR in EGTA perfusion buffer (range 2.9–3.5 nm) just before Ca^{2+} treatment. The 100 μM Ca^{2+} buffer contained 1 μM taxol. The path of the solid lines drawn through the data was determined by eye.
expected loss of spindle BR in EGTA buffer; after 15 min in EGTA buffer, isolated spindles ordinarily lost about 15% of BR₉. Taxol concentrations greater than 0.1 μM also reduced the calcium lability of the spindles. After perfusion with 100 μM Ca²⁺, spindles pretreated for 15 min with 0.1 μM taxol retained 30% BR₉, and spindles pretreated with 0.5 μM taxol retained 60% BR₉. Spindles pretreated with taxol concentrations of 0.05 μM or less were not different from untreated spindles. Results following a longer taxol pretreatment (210 min; data not shown) were complicated by the competition between the rate of taxol stabilization and the intrinsic rate of tubulin dissociation from the spindle microtubules in EGTA buffer. In response to 100 μM Ca²⁺, spindles pretreated for 210 min with 0.01-0.05 μM taxol did not differ from untreated spindles. From 0.05 μM to 0.5 μM taxol, the amount of BR that remained was an increasing function of taxol concentration, and spindles pretreated with 0.5 μM taxol retained over 90% of BR₉.

Fig. 3. Polarization micrographs taken just before (a) and 4 min after (b) addition of 100 μM Ca²⁺ buffer containing 1 μM taxol. Spindles were pretreated with 1 μM taxol in EGTA buffer for durations indicated in minutes in each frame. All spindles are metaphase except the 6-min pretreatment example, which is an early anaphase spindle. Spindles are oriented and photographed as described for Figure 1, except a bias compensation of 3.5 nm was used. Scale = 10 μm.
Fig. 4. Stability of spindle BR to 100 μM Ca\(^{2+}\) as a function of taxol concentration for a pretreatment duration of 15 min. The pretreatment EGTA perfusion buffers and the 100 μM Ca\(^{2+}\) buffers contained taxol at concentrations indicated. Solid-circle data were derived from kinetic curves similar to Figure 2 and represent the normalized BR remaining 1 min after addition of 100 μM Ca\(^{2+}\). Open-circle data represent the relative decrease in spindle BR that occurs in the taxol perfusion buffers during the 15-min pretreatment period.

**Reversal of Taxol-Induced Stability**

To determine the rate at which stability was lost because of the dissociation of taxol from stabilized spindle microtubules, we treated spindles for 15 min with 1 μM or 10 μM taxol, then chased with taxol-free EGTA buffer or 100 μM Ca\(^{2+}\) buffer (Fig. 5). The spindles were either chased immediately with 100 μM Ca\(^{2+}\) buffer, or first chased with EGTA buffer then by 100 μM Ca\(^{2+}\) buffer. When the spindles were perfused immediately following the 15-min taxol pretreatment with 100 μM Ca\(^{2+}\) buffer that contained no taxol, the BR of taxol-stabilized spindles decayed exponentially. The rate of decay in 100 μM Ca\(^{2+}\) buffer after treatment with 1 μM taxol was about twice as fast as the rate of decay after treatment with 10 μM taxol: the BR decayed to 0.37 times the initial value in about 31 min after 1 μM taxol, and in about 63 min after 10 μM taxol. In some experiments calcium was not added until some time after taxol was removed from EGTA perfusion buffer. No decrease in BR was observed over 120 min when spindles were perfused with EGTA buffer after pretreatment with taxol. When 100 μM Ca\(^{2+}\) was eventually exchanged for the EGTA perfusion buffer, the BR dropped rapidly to a level determined by the time that had elapsed since the removal of taxol, the same level of BR that would have been reached if Ca\(^{2+}\) had been added as soon as the taxol was removed. Therefore, 100 μM Ca\(^{2+}\) does not affect the rate of dissociation of taxol from the spindle microtubules.
Fig. 5. Taxol dissociation from spindle microtubules measured by the decrease in normalized spindle BR in 100 μM Ca²⁺ buffer following a 15-min pretreatment with EGTA perfusion buffer containing (A) 1 μM taxol and (B) 10 μM taxol. Time = 0 at the end of the taxol pretreatment. Note different time scales in (A) and (B). Solid circles (●) indicate results of addition of 100 μM Ca²⁺ directly following taxol pretreatment. Solid triangles (▲) indicate results for EGTA buffer chase without Ca²⁺ or taxol. In (A), after a 26-min chase with EGTA buffer, 100 μM Ca²⁺ was added (open circles). In (B), after 45-min (○) and 130-min (□) chases with EGTA buffer, 100 μM Ca²⁺ was added. Solid lines through closed circles were calculated from the equation, BR = e⁻kt. For (A) k = 5.4 × 10⁻⁴ sec⁻¹ and (B) k = 2.7 × 10⁻⁴ sec⁻¹.
DISCUSSION

Our analysis of how taxol stabilizes isolated spindle microtubules provides
criteria for the use of taxol to stabilize microtubules in other physiological studies.
When time is not a factor, complete stabilization should result at taxol concentrations
greater than 0.5 \( \mu \text{M} \). Taxol concentrations as low as 0.5–1.0 \( \mu \text{M} \) will eventually
stabilize microtubules in vivo, but the rate of stabilization may be too slow to capture
microtubules in dynamic processes such as mitosis. For rapid stabilization of microtubules
(within 30 sec), taxol concentrations near 10 \( \mu \text{M} \) will be required. These
criteria are based on stabilization of 100 \( \mu \text{M} \) Ca\(^{2+} \) lability; less potent microtubule
depolymerization agents may require lower concentrations of taxol for equivalent
rates and degrees of stabilization. In developing embryos of Arbacia punctulata and
E. parma, astral and spindle microtubules were augmented and stabilized by 1.0–10.0
\( \mu \text{M} \) taxol if it was added 15–25 min before mitosis [Salmon and Wolniak–unpublished
observation; Schatten et al, 1982]. Similar concentrations have been found to promote
assembly or to stabilize labile cytoplasmic microtubules in other cell types [Albertini
and Clark, 1981; Brenner and Brinkley, 1982; Cande et al, 1981; De Brabander et al,
1981a,b; Heidemann and Gallas, 1980; Schatten et al, 1982; Schiff and Horwitz,
1980].

Theoretically, taxol could stabilize spindle microtubules by a) binding directly
to exposed tubulin sites on the spindle microtubules; b) binding to microtubule-
associated proteins and inducing conformational changes in these molecules which
stabilize intertubulin bonds; and c) binding to exogenous tubulin to form a complex
that binds stably to the tubulin-exchange sites. Our experimental protocol eliminates
the third possibility. No significant concentration of tubulin–taxol complexes could
occur, because no exogenous tubulin was included in our perfusion buffers and the
isolated spindles were washed extensively with EGTA lysis and storage buffers. The
stabilizing action of taxol, then, must result from taxol binding to components of the
isolated spindles, which are not freely diffusible. Taxol may enhance the stability of
microtubules by binding to microtubule-associated proteins but, for the in vitro
assembly of either brain tubulin [Caplow and Zeeberg, 1982; Kumar, 1981; Schiff et
al, 1979; Schiff and Horwitz, 1981; Vallee, 1982] or sea urchin egg tubulin [Scholey
and Salmon, unpublished observation], taxol stabilizes microtubules in the absence of
microtubule-associated proteins. Taxol probably binds to exposed tubulin sites on the
spindle microtubules [Manfredi, Parness, and Horwitz, 1981; Parness and Horwitz,
1981]. It is apparent that taxol binding to a microtubule substantially increases the
bond strength between tubulin subunits within the wall of the microtubule, but the
mechanism of action of taxol is unknown.

Analysis of the taxol stabilization data presented here in terms of the binding of
taxol to exposed sites along the spindle microtubules is not possible at this time
without several assumptions, in part because the mechanism by which 100 \( \mu \text{M} \) Ca\(^{2+} \)
induces depolymerization of the spindle and astral microtubules is not certain. As
shown in Figure 1, 100 \( \mu \text{M} \) Ca\(^{2+} \) enormously increases the tubulin dissociation rate
from spindle microtubules; at least 540–1,350 times the rate in EGTA buffer without
calcium. Since no tubulin is contained in our perfusion buffers, calcium must be
binding to sites on the microtubule. Some evidence suggests that the molecule binding
Ca\(^{2+} \) may be the spindle tubulin itself [Keller et al, 1982; Nishida and Kumagai,
1980; Salmon, 1982b]. Where along the spindle (and astral) microtubules tubulin
dissociation takes place is also not clear. For microtubules assembled in vitro from
Taxol Stabilization of Spindle Microtubules

brain microtubule protein (tubulin plus microtubule-associated proteins), calcium at mM concentrations has been demonstrated to induce rapid tubulin dissociation in an end-dependent manner (Karr, Kristofferson, and Purich, 1980; Weisenberg and Deery, 1981). Rates of 680 dimers/sec have been measured in the presence of 5 mM Ca\(^{2+}\) [Karr et al., 1980]. At the 100 \(\mu\)M Ca\(^{2+}\) concentrations used in our experiments, little effect is observed on the assembly of brain microtubule protein in vitro [Weisenberg and Deery, 1981]. Because the Ca\(^{2+}\) sensitivity of spindle microtubules [Salmon and Segall, 1980] and microtubules assembled in vitro from egg tubulin [Keller et al., 1982; Nishida and Kumagai, 1980] is significantly greater, the pathway of tubulin dissociation at 100 \(\mu\)M Ca\(^{2+}\) concentrations or greater may not be strictly end-dependent. The visual impression from the video records of the 100 \(\mu\)M Ca\(^{2+}\) perfusion experiments (Fig. 1) is that 100 \(\mu\)M Ca\(^{2+}\) produces nearly instantaneous disruption of microtubules. A similar result has been reported for the injection of 1–5 mM Ca\(^{2+}\) into local regions of mitotic spindles in living sea urchin embryos [Kiehart, 1981]. The rate of microtubule depolymerization was estimated to be in excess of 1.6 \(\times\) 10\(^6\) dimers/sec. In contrast, at 2 \(\mu\)M Ca\(^{2+}\), the pattern of loss of isolated spindle BR does depend on the morphology of the spindle and could be end-dependent [Nishida and Kumagai, 1980; Salmon, 1982a]. If 100 \(\mu\)M Ca\(^{2+}\) disrupts microtubules all along their length, then the taxol stabilization data presented here could be analyzed in terms of taxol binding and dissociation from tubulin sites distributed along the length of a spindle microtubule.

We were particularly interested to find that taxol stabilization of spindle microtubules can be reversed. After taxol was washed out, the stability of spindle BR to addition of 100 \(\mu\)M Ca\(^{2+}\) decreased exponentially with a time constant of 35–60 min. Parness and Horwitz [1981] recently demonstrated that 90% of \(^3\)H-taxol bound to microtubules could be removed in 30 min by excess unlabeled taxol. Our results, together with those of Parness and Horwitz [1981], demonstrate that taxol dissociation from microtubules and the loss of stability occur with a time constant of 30–60 min or less (see Fig. 5). Thus taxol will inhibit substantially the dissociation of tubulin subunits, but it will not block it irreversibly, particularly in the presence of potent depolymerization agents or agents that block subunit reassociation. For example, in our experiments, spindle BR slowly decreased in the presence of 100 \(\mu\)M Ca\(^{2+}\) plus 1 \(\mu\)M taxol following taxol pretreatment (see Fig. 2). However, spindle BR did not decrease noticeably in EGTA buffer plus 1 \(\mu\)M taxol over several hours. In addition, under mild depolymerization conditions, a substantial amount of taxol would have to be removed from the spindle microtubules for the microtubules to be unstable. As shown in Figure 5, spindle BR decreased only slightly over 110 min after the removal of 10 \(\mu\)M taxol.

Several other investigations have indicated that in living cells tubulin does dissociate at low rates in the presence of 1–20 \(\mu\)M taxol [De Brabander et al., 1981a,b; Schatten et al., 1982]. De Brabander et al. [1981b] have demonstrated in PtK\(_2\) culture cells after taxol treatment that preexisting microtubule networks do disassemble and that taxol-induced microtubules are susceptible to modification by the mitotic cycle and the tubulin-binding drug nocodazole. Similarly, Schatten et al. [1982] have shown that aster microtubules, which were generated in sea urchin embryos and eggs by 30 min pretreatment with 10 \(\mu\)M taxol, depolymerized substantially in about 15 min when colcemid was added. Colcemid, like colchicine and nocodazole, prevents repolymerization of tubulin. As De Brabander [1981b] initially pointed out, the
microtubule arrays induced by taxol in living cells can exchange tubulin subunits; the microtubules are not “fixed.”

ACKNOWLEDGMENTS

We wish to thank Nancy Salmon for her usual excellent editorial assistance and M. De Brabander for his useful suggestions. This investigation was supported in part by NIH GM 24364 to E.D.S. S.M.W. was supported by NIH GM 32552 and NIH GM 25120 to Dr. P.K. Hepler.

REFERENCES

Taxol Stabilization of Spindle Microtubules


