Kinetic Analysis of Tubulin Exchange at Microtubule Ends at Low Vinblastine Concentrations†

Mary Ann Jordan* and Leslie Wilson
Department of Biological Sciences, University of California, Santa Barbara, California 93106
Received August 15, 1989; Revised Manuscript Received November 6, 1989

ABSTRACT: We have investigated the effects of vinblastine at micromolar concentrations and below on the dynamics of tubulin exchange at the ends of microtubule-associated-protein-rich bovine brain microtubules. The predominant behavior of these microtubules at polymer-mass steady state under the conditions examined was tubulin flux, i.e., net addition of tubulin at one end of each microtubule, operationally defined as the assembly or A end, and balanced net loss at the opposite (disassembly or D) end. No dynamic instability behavior could be detected by video-enhanced dark-field microscopy. Addition of vinblastine to the microtubules at polymer-mass steady state resulted in an initial concentration-dependent depolymerization predominantly at the A ends, until a new steady-state plateau at an elevated critical concentration was established. Microtubules ultimately attained the same stable polymer-mass plateau when vinblastine was added prior to initiation of polymerization as when the drug was added to already polymerized microtubules. Vinblastine inhibited tubulin exchange at the ends of the microtubules at polymer-mass steady state, as determined by using microtubules differentially radiolabeled at their opposite ends. Inhibition of tubulin exchange occurred at concentrations of vinblastine that had very little effect on polymer mass. Both the initial burst of incorporation that occurs in control microtubule suspensions following a pulse of labeled GTP and the relatively slower linear incorporation of label that follows the initial burst were inhibited in a concentration-dependent manner by vinblastine. Both processes were inhibited to the same extent at all vinblastine concentrations examined. If the initial burst of label incorporation represents a low degree of dynamic instability (very short excursions of growth and shortening of the microtubules at one or both ends), then vinblastine inhibits both dynamic instability and flux to similar extents. The ability of vinblastine to inhibit tubulin exchange at microtubule ends in the micromolar concentration range appeared to be mediated by the reversible binding of vinblastine to tubulin binding sites exposed at the polymer ends. Determination by dilution analysis of the effects of vinblastine on the apparent dissociation rate constants for tubulin loss at opposite microtubule ends indicated that a principal effect of vinblastine is to decrease the dissociation rate constant at A ends (i.e., it produces a kinetic cap at A ends), whereas it has no effect on the D-end dissociation rate constant.

Vinblastine, widely used for the treatment of several forms of malignancy, is thought to inhibit cell proliferation, in significant part through an action on microtubules (DeConti & Creasey, 1975; Gerzon, 1980). Interestingly, the effects of vinblastine on microtubules in cells vary with the concentration of drug employed. When HeLa cells are incubated with vinblastine or its congeners in the nanomolar drug concentration range, the cells become arrested at the metaphase stage of mitosis. Metaphase arrest appears to occur without significant depolymerization of spindle microtubules, and the spindles appear to be unable to progress to anaphase (M. A. Jordan, D. Thrower, and L. Wilson, unpublished experiments). Spindle microtubules become destroyed as the vinblastine concentration is raised beyond the nanomolar level (George et al., 1965). At relatively higher concentrations, >10 μM, vinblastine induces formation of paracrystals composed of tubulin complexed with vinblastine (Schochet et al., 1968; Wilson et al., 1978).

The range of effects of vinblastine on microtubules in vitro appears to parallel the range of effects on microtubules in cells. At the lowest effective drug concentrations in vitro, vinblastine inhibits addition of tubulin to microtubule ends (Wilson et al., 1982a). Inhibition is associated with binding of the drug to a small number of high-affinity binding sites (mean K_d, 1.9 $\times 10^{-6}$ M; 16.8 ± 4.3 sites per microtubule), which appear to be located at one or both ends of the polymers. Approximately one molecule of vinblastine bound per microtubule inhibits tubulin addition by 50% (Wilson et al., 1982a). Above 2 μM, vinblastine binds to large numbers of tubulin binding sites along the microtubule surface with low affinity. Binding to this class of sites depolymerizes the microtubules by protofilament unraveling at both ends (Warfield & Bouck, 1974; Jordan et al., 1986; Singer et al., 1989). This phenomenon may be related to the ability of vinblastine to induce formation of paracrystals in cells.

In the present study, we have used MAP[1]-rich bovine brain microtubules, differentially radiolabeled at their opposite ends, to examine the effects of vinblastine in the submicromolar concentration range on tubulin exchange at microtubule ends. It is in this range that the activity of vinblastine on microtubules may be most relevant to the ability of the drug to inhibit cell proliferation. Our results indicate that, in this concentration range, vinblastine inhibits tubulin exchange at microtubule ends while producing little reduction of total polymer

†Supported by Grant CH-381 from the American Cancer Society and USPHS Grant NS13570.

[1] Abbreviations: MAP, microtubule-associated protein; GTP, guanosine 5′-triphosphate; GXP, guanine nucleotide in microtubules; MES, 2-(*N*-morpholino)ethanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid.

0006-2960/90/0429-2730$02.50/0

mass. At one microtubule end, the operationally defined assembly or A end, the apparent rate constant for tubulin dissociation is reduced, whereas the apparent dissociation rate constant at the disassembly (D) end is not affected. Thus, A ends are kinetically stabilized. Kinetic stabilization of tubulin exchange at microtubule ends may play an important role in the ability of vinblastine to inhibit mitosis.

Materials and Methods

Preparation of Bovine Brain Microtubule Suspensions. Bovine brain microtubule protein consisting of 60–70% tubulin and 30–40% MAPs was isolated without glycerol (Farrell & Wilson, 1984) and stored frozen as three times cycled microtubule pellets at −70 °C until used. For reassembly, pellets were resuspended by Dounce homogenization at 0 °C in 100 mM MES, 1 mM EGTA, and 1 mM $MgSO_4$, pH 6.8 (MEM buffer), and after 15 min, solutions were centrifuged at 48500*g* for 10 min at 4 °C (SS-34 rotor, Sorvall RC5). To assemble microtubules, GTP (0.1–0.2 mM) and a GTP-regenerating system consisting of 10 mM acetyl phosphate and 0.1 IU/mL acetate kinase were added to the supernatants to give microtubule protein in reassembly buffer, and the temperature was raised to 30 °C.

Preparation of Microtubules with Assembly and Disassembly Ends Differentially Labeled with [^3H]- and [^{14}C]-Guanine Nucleotides. Microtubules differentially labeled at their opposite ends with [^{3}H]- and [^{14}C]guanine nucleotides (Farrell & Jordan, 1982; Jordan & Farrell, 1983) were used to determine the extent of vinblastine-induced depolymerization at A and D ends of steady-state microtubules and to determine the rates of net tubulin addition and loss at the ends of microtubules that had been polymerized to steady state in the presence of vinblastine. Use of the labeling procedure requires that net tubulin addition to the microtubules occur predominantly at the operationally defined A ends and that tubulin loss occur predominantly at the operationally defined D ends. Significant label loss and gain by dynamic instability behavior would complicate interpretation of the data [see Wilson and Farrell (1986)]. That the MAP-rich microtubules used in the present work exchange label predominantly by flux and not by dynamic instability behavior has been well documented (Wilson & Farrell, 1986; Farrell et al., 1987). For example, no changes in the length distributions of these microtubules are detectable at polymer-mass steady state by electron microscopic analysis of negatively stained polymer suspensions. In addition, growth and shortening at the ends of the microtubules are not detectable by video-enhanced dark-field microscopy (K. W. Farrell, B. Matsumoto, M. A. Jordan, and L. Wilson, unpublished experiments).

Microtubules were initially labeled throughout their lengths with [^{14}C]GXP by polymerizing microtubule protein (2.6–4.1 mg/mL) to steady state in the presence of [^{14}C]GTP (final GTP concentration, 0.1–0.2 mM; final specific activity, 9.1–10.7 Ci/mol). Polymer-mass steady state was reached 25–28 min after initiation of polymerization as determined by turbidimetry using a Gilford 2400 spectrophotometer. Fifty to ninety minutes after initiation of polymerization, a trace quantity of [^{3}H]GTP (final specific activity, 137–276 Ci/mol; negligible change in the GTP concentration) was added to initiate a pulse to differentially label the A ends.

Quantitation of Vinblastine-Induced Depolymerization at A and D Ends. Vinblastine (0.1–6 μM) was added to double-labeled microtubules 68–110 min after initiating the [^{3}H]GTP pulse. Aliquots were stabilized for determination of radiolabeled GXP uptake between 0.2 and 60 min after addition of the drug, and label uptake into the polymers was analyzed by glass fiber filter assay (Wilson et al., 1982b).

Determination of the Effect of Vinblastine on Rates of Tubulin Uptake and Loss at Opposite Ends of Steady-State Microtubules. Vinblastine (0.15 μM) was added to half of a microtubule protein suspension before initiating polymerization. The other half of the suspension served as a drug-free control. Polymerization was initiated in the presence of [^{14}C]GTP to label the microtubules uniformly with [^{14}C]GXP. A [^{3}H]GTP pulse was initiated after steady state was attained to determine the initial burst uptake and the A-end incorporation rate. A 25-fold excess of unlabeled GTP was added 50–60 min after initiating the [^{3}H]GTP pulse as a chase for both isotopes in order to determine initial burst loss and the D-end loss rate. Microtubules were stabilized and collected on glass fiber filters for analysis of radiolabel incorporation.

Steady-state rates of tubulin incorporation at A ends were calculated by least-squares analysis of [^{3}H]GTP uptake data between 6 and 50 min after addition of the pulse (after completion of the initial burst phase of incorporation). Similarly, steady-state rates of tubulin loss from A ends during the chase were calculated from linear regression analyses of [^{3}H]GXP loss data after the initial burst phase of loss was completed. Steady-state D-end loss rates were determined from the loss of tubulin dimers during the chase that were labeled with [^{14}C]GXP after subtracting the loss from the A end of [^{14}C]GXP; i.e., (total loss) − (A-end loss) = (D-end loss). Both A- and D-end steady-state loss rates were calculated beginning after completion of the initial burst loss during the chase, i.e., between 6 and 50 min in the control suspension and between 6.5 and 52 min in the suspension that was polymerized in the presence of vinblastine. Addition and loss rate data were corrected for microtubule number concentration, and the results were expressed either as tubulin dimers gained or lost per microtubule per second or as micrometers of microtubule length gained or lost per hour. No microtubules were short enough to become labeled completely during a pulse or to lose label completely during a chase.

Determination of Whether Vinblastine Can Incorporate into the Microtubule Lattice. Microtubule protein (1.7–6.2 mg/mL) was polymerized to steady state in reassembly buffer in the presence of [^{3}H]vinblastine at either 0.11 μM (specific activity, 10.7 Ci/mmol) or 1.1 μM (specific activity, 5.8 Ci/mmol). A similar suspension of microtubule protein was assembled to steady state in the absence of vinblastine, and [^{3}H]vinblastine at 0.11 or 1.1 μM final concentration was added to the microtubules after attainment of steady state, 36–60 min after initiation of polymerization. Aliquots of the microtubule suspensions were stabilized 52–170 min after initiation, and the number of molecules of vinblastine bound or incorporated per microtubule was determined. Bound vinblastine was determined by glass fiber filter assay or after stabilization of the microtubules in 50% MEM-buffered sucrose (30 °C) followed by centrifugation through sucrose (4 mL, 50% MEM-buffered sucrose, 96000*g*, 90 min, SW 50.1 rotor, 30 °C). Microtubule pellets were then assayed for protein and radiolabeled vinblastine incorporation (Jordan et al., 1986). The microtubule number concentration was determined as described by Farrell et al. (1987).

Determination of Apparent Dissociation Rate Constants and Calculation of Apparent Association Rate Constants at Microtubule A and D Ends. Apparent dissociation rate constants were determined by dilution-induced disassembly (Farrell & Jordan, 1982; Jordan & Farrell, 1983). Double-labeled microtubules were prepared by assembling microtubule protein (2.3 mg/mL) in reassembly buffer containing [^{14}C]-

GTP (0.1 mM; final specific activity, 7.2 Ci/mol) at 30 °C for 49 min. The suspension was split in half, and both halves were pulsed with [^{3}H]GTP (164 Ci/mol) for 84 min. Vinblastine (either 0.15 or 0.30 μM final concentration) was added to one portion of the suspension, and incubation of both portions was continued for an additional 36–60 min. Aliquots (0.5 mL) of the suspensions were diluted 21-fold with rapid mixing with 10 mL of reassembly buffer (30 °C) containing either no vinblastine (control suspension) or the same concentration of vinblastine that was present during incubation of drug-treated microtubule suspensions. Samples (1 mL) were removed at 6–15-s intervals during the next 90 s and stabilized immediately in 14 mL of stabilization buffer at 30 °C. Label incorporation was determined by glass fiber filter assay.

Tubulin loss rates at A ends were calculated by determining the amount of [^{3}H]GXP remaining in microtubules at various times after dilution. Tubulin loss at D ends was calculated by subtracting A-end loss from total loss at both polymer ends (determined from the ^{14}C data). Apparent association rate constants (k^+) were calculated according to the equation $k^+ = (k^- + \phi)/C_c$, with the apparent dissociation rate constants (k^-), the determined flux rates (ϕ), and the determined critical subunit concentrations (C_c) measured in both the presence and absence of 0.15 or 0.3 μM vinblastine.

Critical Concentration Determinations. Microtubule protein (2.4 mg/mL) was polymerized to steady state in reassembly buffer in the presence of different vinblastine concentrations. After 68 min, aliquots (180 μL) were centrifuged (Beckman Airfuge, 20 psi, 10 min, 30 °C; Beckman Instruments, Inc., Palo Alto, CA). The uppermost 150 μL was collected and the protein content determined by the method of Lowry et al. (1951), using bovine serum albumin as a standard. The value obtained was reduced by 11% to correct for overestimation of tubulin (Bamburg et al., 1973). Alternatively, the new critical concentration attained after adding vinblastine to steady-state microtubules (2.4 mg/mL microtubule protein) was determined by measuring the loss of total microtubule polymer (loss of [^{14}C]GXP–tubulin from fully labeled microtubules) 60 min after addition of vinblastine. The amount of tubulin loss was added to the concentration of soluble tubulin prior to addition of vinblastine to obtain the new critical subunit concentration. Both methods used did not correct for any inactivated tubulin.

Reagents and Miscellaneous Procedures. [8-^{3}H]GTP was obtained from ICN, Irvine, CA, and [^{14}C]GTP was obtained from Research Products International, Mt. Prospect, IL. [G-^{3}H]Vinblastine was obtained from Amersham Corp., Arlington Heights, IL, and vinblastine was a generous gift of Eli Lilly and Co., Indianapolis, IN. All other chemicals were reagent grade. Protein was determined by the method of Bradford (1976) or Lowry et al. (1951), using bovine serum albumin as the standard.

Results

We wanted to examine the effects of vinblastine on the kinetics of tubulin addition and loss at the opposite ends of microtubules at vinblastine concentrations between 0.1 and approximately 2 μM. In this concentration range, the drug binds to one and perhaps both microtubule ends and inhibits net tubulin uptake at A ends when it is added to microtubule suspensions at polymer-mass steady state (Wilson et al., 1982a; Jordan et al., 1985). Our approach was to use microtubules differentially labeled at their opposite ends with [^{3}H]- and [^{14}C]guanine nucleotides (1) to determine the effects of vinblastine on the kinetics of tubulin exchange at the two ends of the microtubules at polymer-mass steady state and (2) to determine the effects of the drug on the apparent dissociation rate constants at the two ends by analysis of radiolabel loss following dilution-induced disassembly.

Two strategies were used to react the vinblastine with radiolabeled microtubules. In one strategy, vinblastine was added to solutions of microtubule protein prior to initiation of polymerization. Then, the characteristics of label addition to the microtubules at their opposite ends were examined in the continuous presence of the drug by polymerizing the protein to polymer-mass steady state with [^{14}C]GTP, followed by pulsing with [^{3}H]GTP. In the second strategy, microtubules were polymerized to polymer-mass steady state with [^{14}C]GTP and pulsed with [^{3}H]GTP to label them differentially at the two ends prior to adding the drug.

In order to use the first strategy and to compare the results obtained to those obtained with the second strategy, it was necessary to verify that the characteristics of the microtubules polymerized in the continuous presence of vinblastine were similar to those of the microtubules to which the drug had been added after polymerization to polymer-mass steady state. For example, it was necessary that the microtubules polymerized in the presence of vinblastine have the drug bound only at the ends of the polymers and not incorporated into the polymers. Also with this strategy, in order for the [^{3}H]guanine nucleotide to add predominantly at the A ends of the microtubules, the [^{3}H]GTP pulse had to be performed when the microtubules were at a polymer-mass steady state and treadmilling and not undergoing net depolymerization or significant dynamic instability.

The second strategy was used when a large quantity of [^{3}H]GXP was required at A ends. However, it was necessary to characterize the duration and extent of the depolymerization that occurs initially after addition of vinblastine to steady-state microtubules, and it was necessary to ensure that the drug did not induce appreciable dynamic instability.

Microtubule Polymerization in the Continuous Presence of Vinblastine: Characteristics of the Polymers. When low concentrations of vinblastine are added to assembled microtubules at polymer-mass steady state, inhibition of polymerization occurs by the reversible binding of small numbers of vinblastine molecules at one or both microtubule ends (Wilson et al., 1982a). However, polymerization of microtubules in the presence of vinblastine might alter the interaction of the drug with the microtubule lattice, and if so, the mechanism of inhibition of polymerization in the two conditions could be different. Such is the situation with colchicine. When microtubules are polymerized in the presence of tubulin–colchicine complexes, the drug complexes become incorporated along with tubulin into the microtubule (Sternlicht & Ringel, 1979; Farrell & Wilson, 1984). Thus, we determined the stoichiometry of vinblastine binding to microtubules polymerized in the continuous presence of the drug and compared the binding results with those obtained when vinblastine was added to previously polymerized microtubules.

We found that the binding of vinblastine to microtubules was essentially the same when microtubule protein was polymerized to polymer-mass steady state in the presence of vinblastine or when the drug was added to microtubule suspensions after attaining steady state (Table I). Similar to results of earlier experiments in which vinblastine was added to polymerized microtubules (Jordan et al., 1982), approximately one molecule of vinblastine was bound per microtubule at 0.11 μM vinblastine when the vinblastine was added to previously polymerized microtubules. Similar stoichiometries were obtained when microtubules were polymerized in the

Table I: Stoichiometry of Vinblastine Bound per Microtubule When Vinblastine Is Present during Polymerization and When Vinblastine Is Added to Previously Polymerized Microtubules[a]

	microtubule protein concn (mg/mL)	microtubule no. concn[b] (nM)	mean length[b] (μm)	$\bar{r}$[c]
0.11 μM vinblastine				
steady state	1.7	0.29	13.2	0.97
initiation	1.7	0.31	12.1	0.92
initiation	3.2	1.1	8.4	0.53
initiation	6.2	3.2	6.5	0.38
1.1 μM vinblastine				
steady state	2.4		8.8	17.9
initiation	2.4		10.6	13.9

[a] Microtubule protein was polymerized to steady state in reassembly buffer at 30 °C. [^{3}H]Vinblastine (0.11 μM, 1320 Ci/mol, or 1.1 μM, 5800 Ci/mol) was added just prior to initiation or at steady state (36–60 min after initiation). Samples were stabilized 44 min after drug addition when 0.11 μM vinblastine was used, and at 52 min they were centrifuged through MEM-buffered 50% sucrose and assayed for microtubule protein content and bound [^{3}H]vinblastine. Samples were stabilized 110 min after drug addition when 1.1 μM vinblastine was used, and bound [^{3}H]vinblastine was determined by glass fiber filter assay. [b] Mean microtubule lengths were determined as described under Materials and Methods. Microtubule number concentration is the molar concentration of microtubules, assuming 1690 dimers/μm and that microtubule protein contains 70% tubulin. [c] Molecules of vinblastine bound per microtubule.

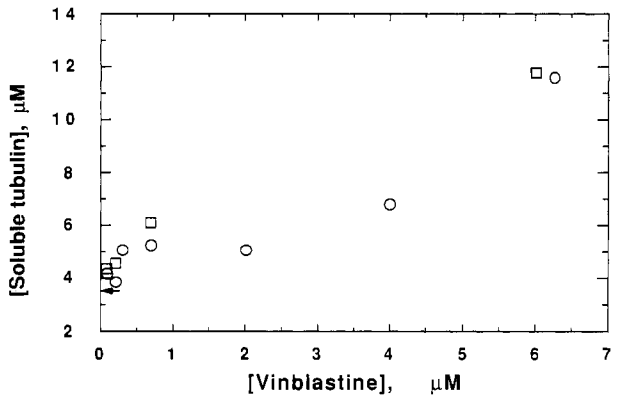

FIGURE 1: Soluble tubulin dimer concentration at different vinblastine concentrations: polymerization in the continuous presence of vinblastine (squares); polymerization to steady state in the absence of vinblastine, followed by incubation in vinblastine for 60 (circles) (see Materials and Methods). The arrow denotes soluble tubulin concentration in the absence of the drug.

continuous presence of the drug (Table I). Increasing the microtubule protein concentration, which increased the rates of polymerization, did not increase the final vinblastine binding stoichiometry in the microtubules. The stoichiometry of vinblastine binding per microtubule was also similar in the two experimental strategies when 1.1 μM vinblastine was used (Table I). Thus, unlike colchicine–tubulin complexes, vinblastine does not coassemble with tubulin. The data indicate that vinblastine binds in a highly reversible fashion at one or both microtubule ends.

Another indication that the mechanism of inhibition by vinblastine was similar in the two experimental strategies was that the critical subunit concentrations at polymer-mass steady state at all vinblastine concentrations were indistinguishable when the microtubules were polymerized in the presence of the drug and when the drug was added to previously polymerized microtubules (Figure 1). The critical subunit concentration increased linearly with vinblastine concentration with a positive slope of 1.1 mol of tubulin/mol of vinblastine

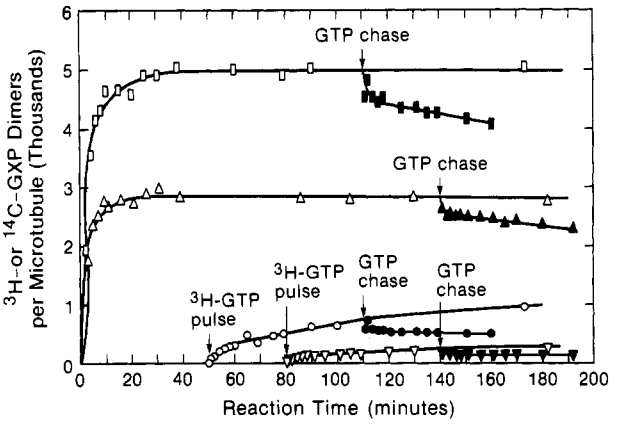

FIGURE 2: Effect of vinblastine on the incorporation of tubulin at A ends and loss of tubulin at D ends of steady-state microtubules: double-label protocol. Two portions of a microtubule protein solution, 4.1 mg/mL total protein, which were identical except that one portion contained 0.15 μM vinblastine, were polymerized to steady state in reassembly buffer containing [^{14}C]GTP. Control microtubules (rectangles); microtubules polymerized in the presence of vinblastine (triangles). The stoichiometry of radiolabeled GTP to tubulin in both microtubule samples was 0.59. A [^{3}H]GTP pulse was added to the control sample at 50 min and to the sample polymerized in the presence of vinblastine at 80 min after initiation. [^{3}H]GXP data: control microtubules (circles); vinblastine-polymerized microtubules (inverted triangles). An unlabeled GTP chase (5 mM) was initiated 60 min after the start of the pulse for each sample (closed symbols denote chase conditions). The ordinate is presented as radiolabeled subunits per microtubule; this value was derived by dividing the concentration of incorporated GTP by the concentration of microtubules in suspension as determined from the length data of Table II.

added (r = 0.95). Significantly, with both strategies, the reduction in total polymer mass at the concentration of vinblastine that reduced tubulin exchange at the microtubule ends by approximately 50% (see below) was less than approximately 7%.

Effect of Vinblastine on Net Exchange of Tubulin at Opposite Ends of Steady-State Microtubules. The effects of vinblastine on the exchange of tubulin at microtubule ends at polymer-mass steady state were examined by polymerizing microtubules to steady state in the continuous presence of 0.15 μM vinblastine. Polymerization as monitored by incorporation of [^{14}C]GTP into microtubules in the presence (open triangles) and absence (open rectangles) of vinblastine is shown in Figure 2. Both suspensions reached stable polymer-mass plateaus approximately 20 min after polymerization was initiated. Plateaus were maintained for the duration of the experiment (200 min). Vinblastine caused a 6.8% reduction in polymer mass at the plateau compared with the control microtubule suspension as determined from [^{14}C]GXP incorporation data. The reduction was 6.9% as determined by turbidimetry (data not shown).

There were no changes detectable in the mean lengths of the control microtubules or those polymerized in the presence of vinblastine once the suspensions attained polymer-mass steady state (Table II). Further, the length distributions of the microtubules polymerized in the presence of vinblastine did not change detectably for the duration of the experiment (Table II). Similar results were obtained for the control microtubules. The mean length of the microtubules in the control suspension was 8.2 μm. Although polymerization in the presence of the vinblastine reduced the polymer mass only by 6.8%, it reduced the mean length of the microtubules by 43%, to 5.2 μm. Thus, the number concentration was higher when polymerization was carried out in the presence of the drug, indicating that the initiation phase of the polymerization

Table II: Means and Distributions of Microtubule Lengths with Time for Microtubules Polymerized in the Absence (Control) and in the Continuous Presence of 0.15 μM Vinblastine[a]

	time[b] (min)	mean length (μm)	standard deviation (μm)	N[c]
control	41	8.2	3.8	397
	71	8.5	3.7	280
	170	8.1	3.7	316
	198	8.2	3.7	418
vinblastine	41	5.2	2.2	528
	71	4.9	2.1	525
	170	4.9	1.9	412
	198	4.9	2.0	553

[a] Data from the experiment shown in Figures 2–4. [b] Time from initiation of polymerization. [c] Number of microtubules measured.

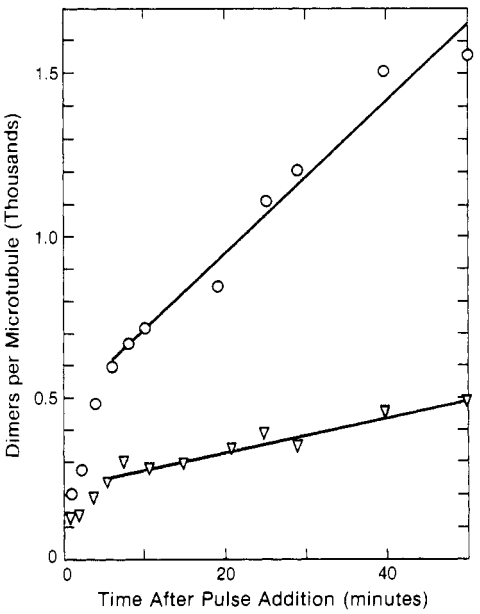

FIGURE 3: Time course for incorporation of [^{3}H]GTP into control microtubules (circles) and microtubules polymerized in the presence of vinblastine (inverted triangles) at steady state. Data represent the pulse portion of the experiment shown in Figure 2.

reaction was stimulated by the drug.

Addition of a [^{3}H]GTP pulse to the control microtubule suspension 50 min after initiation of polymerization resulted in an initial burst of nucleotide incorporation into the polymers that lasted several minutes. This was followed by a slower, approximately linear incorporation of label that continued for the duration of the experiment (Figure 2, open circles; shown in expanded form in Figure 3). The slower, approximately linear rate of incorporation is considered to reflect the flux rate (i.e., net addition of tubulin to A ends) and was 0.85 μm/h (0.4 tubulin dimer/s). This value was calculated from the linear region of the data, from 6 to 50 min after initiation of the pulse. The size of the burst in the control microtubule suspension was approximately 595 molecules of tubulin per microtubule (equivalent to approximately 0.36 μm of microtubule length). These results are similar to those described previously with a similar preparation of MAP-rich microtubules (Farrell et al., 1987).

Addition of a [^{3}H]GTP pulse to the microtubules polymerized in the presence of vinblastine 80 min after initiation of polymerization, as with control microtubules, resulted in an initial burst of [^{3}H]GXP incorporation that lasted several minutes, followed by a slower, approximately linear incorporation that continued for the duration of the experiment

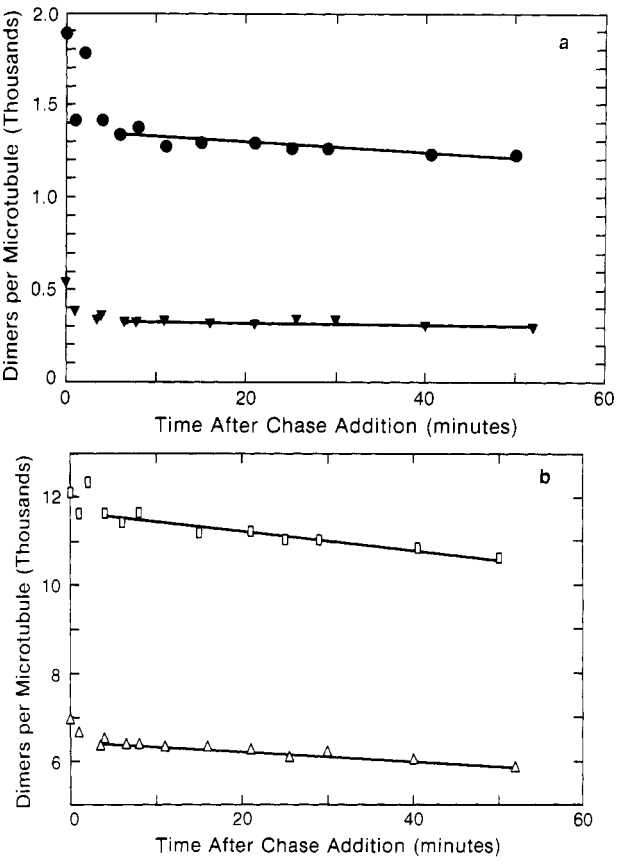

FIGURE 4: Time course for loss of radiolabeled GXP–tubulin from A and D ends of control microtubules and microtubules polymerized in the presence of vinblastine. Data are from the chase portion of the experiment shown in Figure 2. (a) [^{3}H]GXP–tubulin; A-end label loss for control microtubules (circles) and for microtubules polymerized in vinblastine (inverted triangles). (b) [^{14}C]GXP–tubulin after subtracting [^{14}C]GXP–tubulin loss from the A end; D-end label loss for control microtubules (rectangles) and for microtubules polymerized in vinblastine (triangles).

(Figures 2 and 3). The magnitudes of both phases were reduced approximately to the same extent by the drug. The burst was reduced by 58%, to 248 tubulin dimers per microtubule (0.15 μm of microtubule length). If the initial burst of incorporation represents dynamic instability at the ends of the microtubules (i.e., very short excursions of growth and shortening extending into the polymer for a total distance at both ends of 0.36 μm in control microtubules and 0.15 μm in microtubules assembled in the presence of the drug), then incubation with vinblastine inhibited dynamic instability by approximately 58%.

By analysis of the retention of [^{3}H]GXP during a chase (see below), it was clear that the microtubules polymerized to polymer-mass steady state in the presence of vinblastine were treadmilling (fluxing). The linear rate of uptake of [^{3}H]GXP between 6 and 50 min following the initial burst, which is considered to represent the flux rate, was reduced by 78% by vinblastine, to 0.19 μm/h (0.09 tubulin dimer/s).

Addition of a 25-fold excess of unlabeled GTP as a chase for both isotopes was performed 60 min after initiation of the [^{3}H]GTP pulse. In control microtubules (closed circles, Figures 2 and 4a), an initial rapid burst loss of [^{3}H]GXP–tubulin occurred during the first 6 min, equivalent to approximately 560 tubulin dimers per microtubule; this is approximately the same as the burst incorporation of [^{3}H]GXP during the pulse for these microtubules. This was followed by a stable plateau during which almost no additional [^{3}H]-GXP was lost (equivalent to 0.09 μm/h or 0.044/s per mi-

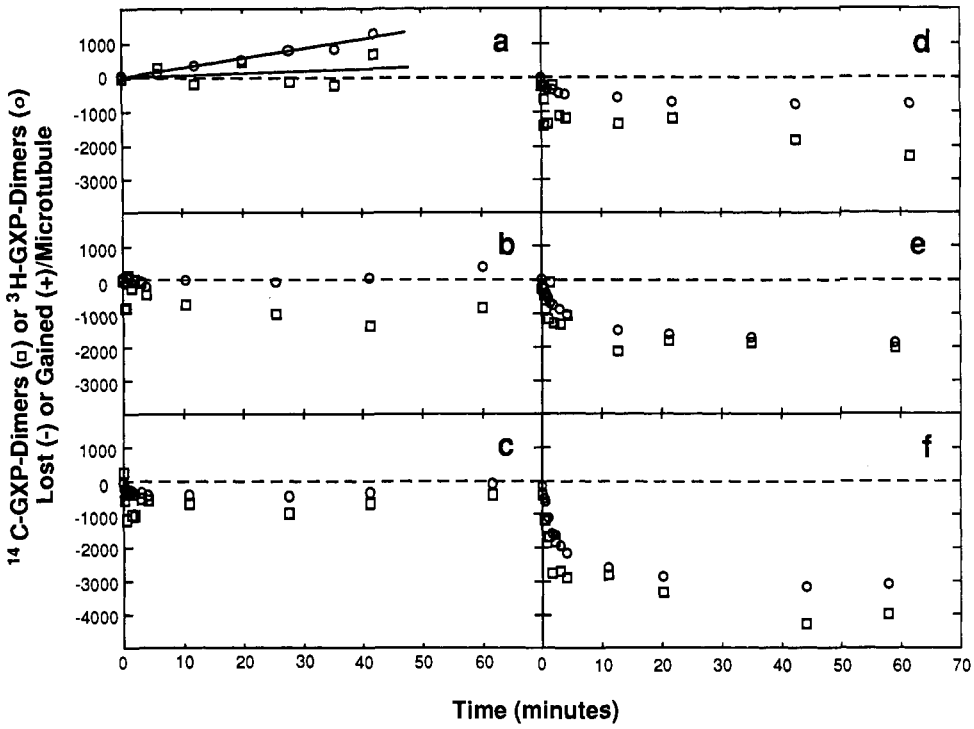

FIGURE 5: Effect of addition of vinblastine to steady-state double-labeled microtubules: (a) control, no vinblastine; (b) 0.1 μM; (c) 0.2 μM; (d) 0.3 μM; (e) 2 μM; (f) 4 μM. Bovine brain microtubule protein (2.9 mg/mL) was polymerized at 30 °C in reassembly buffer containing [^{14}C]GTP (mean length of microtubules 10.6 μm or approximately 18 000 dimers). A pulse of [^{3}H]GTP was added 90 min after initiation to label the A ends for a distance of between 2.0 and 2.7 μm (see Materials and Methods). Vinblastine was added 68–110 min after pulse initiation. Aliquots were stabilized at the indicated times and radiolabel retention was determined by filter assay. Zero time on the abscissa is the time of vinblastine addition (circles = [^{3}H]GXP; squares = [^{14}C]GXP).

crotubule). These results are similar to those reported previously for the MAP-rich microtubules used in this work and indicate that most of the [^{3}H]GXP incorporated during the linear portion of the pulse represents treadmilling incorporation [see Zeeberg et al. (1980) and Wilson and Farrell (1986)].

In microtubules polymerized in the presence of vinblastine, as in control microtubules, there was an initial rapid loss of [^{3}H]GXP equivalent to approximately 220 dimers per microtubule. This was approximately equal to the burst of label incorporation that occurred during the pulse in the presence of the drug. The burst loss was inhibited 61% in the vinblastine-treated microtubule suspension as compared with the control suspension. The burst was followed by a stable plateau in which almost no additional [^{3}H]GXP was lost (0.016 μm/h or 0.007/s per microtubule). Thus, greater than 88% of the label incorporated during the pulse was retained following the burst loss in a chase of equal duration in the microtubules polymerized in the presence of vinblastine. These data indicate that labeled tubulin incorporation in the presence of vinblastine occurred predominantly by a flux mechanism [see Zeeberg et al. (1980) and Wilson and Farrell (1986)].

In order to examine the exchange of tubulin at D ends during a chase with excess unlabeled GTP, it is necessary to subtract the [^{3}H]GXP–dimer loss (which represents primarily A-end loss) from [^{14}C]GXP–dimer loss (which represents both A- and D-end losses). D-end loss during the chase with unlabeled GTP is shown in Figure 4b. There was a rapid and approximately similar initial loss from both microtubule suspensions of 527 dimers per microtubule, which lasted for approximately 4 min, followed by a slow, approximately linear loss from both the drug-treated and control microtubules that continued for the remainder of the experiment (50 min).

The slow, linear D-end loss rate is an independent measure of the flux rate as well as further confirmation that the microtubules were treadmilling. The flux rate as determined by D-end loss in the control microtubule suspension was 0.86 μm/h (0.40/s per microtubule), which is indistinguishable from the rate obtained with the [^{3}H]GXP pulse. Similarly, the flux rate as determined by D-end loss in the microtubule suspension polymerized in the continuous presence of vinblastine was 0.41 μm/h (0.19/s), essentially the same as the rate determined from the [^{3}H]GXP pulse for this suspension of microtubules. Thus, the microtubules were treadmilling because the incorporation of tubulin at A ends of the vinblastine-treated microtubules was balanced by a similar loss of tubulin at the D ends. Calculation of the inhibition of flux using the [^{14}C]GXP data gave 53% inhibition, reasonably similar to the value calculated by using the [^{3}H]GXP data.

Addition of Vinblastine to Previously Assembled Double-Labeled Microtubules. The flux rate of microtubules polymerized in the presence of vinblastine was too slow to permit adequate labeling of A ends with [^{3}H]GXP for analysis by dilution-induced disassembly. Thus, vinblastine was added to previously formed double-labeled microtubules for this purpose. Prior to performing the dilution-induced disassembly studies, we characterized the depolymerization that occurs when vinblastine is added to microtubules constructed in this way.

In the experiment shown in Figure 5, the [^{3}H]GXP data reflect tubulin gain or loss predominantly at A ends, while the [^{14}C]GXP data reflect total tubulin gain or loss. As can be seen by examining the [^{14}C]GXP loss data, addition of vinblastine to the microtubules resulted in a concentration-dependent depolymerization and relaxation to a new, lower polymer-mass plateau. Depolymerization was biphasic, with an initial rapid rate of depolymerization that lasted approximately 5–10 min followed by a slower rate of depolymerization. Initial rates of depolymerization at each vinblastine concentration were calculated from linear regression lines of the radiolabel retention data during the initial 4 min after drug

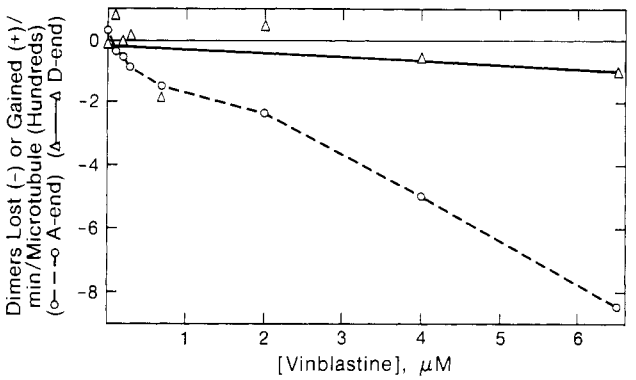

FIGURE 6: Initial rates of depolymerization upon addition of vinblastine to steady-state double-labeled microtubules. The initial rate of loss from A ends (circles) was calculated by linear regression of the [^{3}H]GXP–dimers lost from the microtubules of Figure 5 during the first 4 min of vinblastine treatment. The initial rate of loss from D ends (triangles) was calculated by linear regression during the same time period of [^{14}C]GXP–dimers lost from both ends after subtracting the loss of dimers labeled with [^{3}H]GXP (i.e., loss from D ends = loss from both ends − loss from A ends).

addition and are shown in Figure 6. The initial rapid depolymerization increased linearly with increasing vinblastine concentration. Eighty-nine percent of the label loss represented A-end loss (subunits that had been added during the pulse); only 11% was lost from D ends (subunits added prior to the pulse).

We also examined the length distributions of the microtubules before addition of vinblastine and after a 1-h incubation in the presence of the drug to determine whether vinblastine induced any dynamic instability behavior. This would be evident as spreading of the length distributions with time on either side of the mean length as the microtubules were shrinking in response to the drug. The length distributions of the microtubules at a number of vinblastine concentrations used in the experiment of Figure 5 are shown in Figure 7. The mean lengths decreased with increasing concentrations of vinblastine, but the shapes of the distributions did not change significantly. These data indicate that vinblastine depolymerized all length classes of microtubules equivalently and did not induce detectable dynamic instability behavior.

The extent of depolymerization after 1 h induced by different vinblastine concentrations as determined by direct electron microscopic measurement was essentially the same as that determined from analysis of radiolabeled guanine nucleotide retention in the polymers (data not shown). These results indicate that analysis of radiolabeled guanine nucleotide retention after addition of vinblastine is a quantitatively accurate reflection of the extent of depolymerization induced by vinblastine.

Estimate of the Dissociation Rate Constants at Ends of Vinblastine-Treated Microtubules. Molecular rate constants at A and D microtubule ends can be estimated by dilution-induced disassembly of double-labeled microtubules. However, the initial rate of tubulin loss is not a linear function of the initial free tubulin concentration, as would be expected for a simple bimolecular reaction (Farrell et al., 1983; Carlier et al., 1984; Wilson & Farrell, 1986). Thus, the values do not necessarily represent the true steady-state rate constants. The measured apparent dissociation rate constants must reflect the rate-limiting reactions under dilution conditions. Although the reactions giving rise to the measured initial rates of tubulin loss are not understood, apparent dissociation rate constants measured in the presence of vinblastine can be used to examine the effects of the drug at each microtubule end in a quantitative manner [see Wilson et al. (1985)]. By contrast with determination of apparent dissociation rate constants, apparent association rate constants cannot be measured directly. They are calculated by using an equation derived for a steady-state situation and they employ apparent dissociation rate constants measured under dilution conditions. Thus, calculated association rate constants probably do not represent the true association rate constants at steady state.

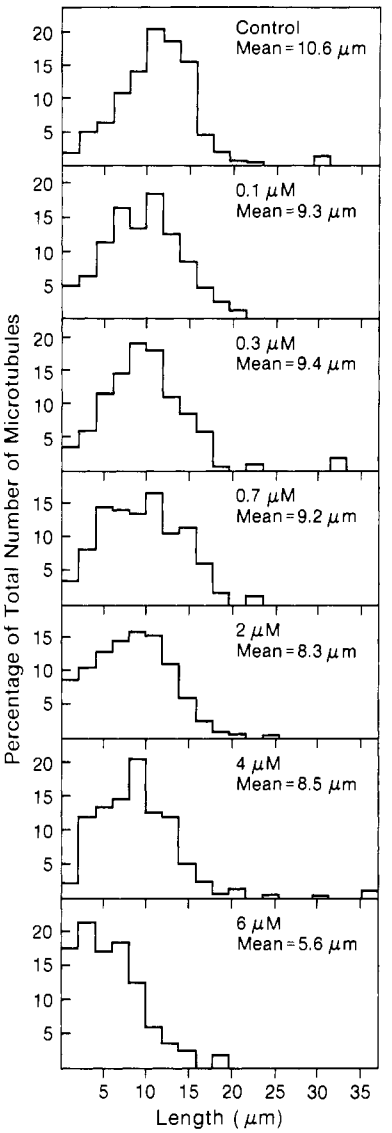

FIGURE 7: Effect on the mean microtubule length and length distribution of addition of vinblastine to steady-state microtubules. The histograms represent the lengths of the microtubules in the experiment of Figures 5 and 6, 60 min after addition of vinblastine.

Results of a typical dilution experiment appear in Figure 8. Depolymerization kinetics at A ends were linear during the first 0.5 min after dilution (correlation coefficients >0.98). From the length distributions of the microtubules fixed just prior to dilution, a decrease in number concentration of less than 2% would be expected during this time. D-end loss was linear for at least 1.5 min (correlation coefficients about 0.8, probably due to the scatter produced by small observed changes in a large total [^{14}C]GXP signal). During this time a 5–7% decrease in number concentration would be expected.

The apparent dissociation rate constants for control and vinblastine-treated microtubules for two different vinblastine concentrations appear in Table III. Also shown are calculated association rate constants for the two ends (see Materials and Methods) as well as the flux rates and critical concentrations

Table III: Steady-State Parameters and Apparent Rate Constants of Control and Vinblastine-Treated Microtubules

	A ends		D ends			
	k^- (s^{-1})	k^+ (M^{-1} s^{-1} × 10^{-7})	k^- (s^{-1})	k^+ (M^{-1} s^{-1} × 10^{-7})	flux rate (s^{-1})	critical subunit concn[a] (μM)
control	42.2 ± 1.5	1.7	14.4 ± 1.0	0.55	0.64	2.5
0.15 μM vinblastine	31.0 ± 2.6	1.0	13.5 ± 1.1	0.44	0.32	3.0
change (%)	−27	−41	[b]	−20	−50	+20
control	37.0 ± 0.5	1.6	28.1 ± 1.0	1.2	0.46	2.4
0.3 μM vinblastine	14.3 ± 0.8	0.3	30.5 ± 0.9	0.7	0.12	4.6
change (%)	−61	−81	[b]	−43	−74	+92

[a] Total subunit concentration 15 μM. [b] Statistically insignificant.

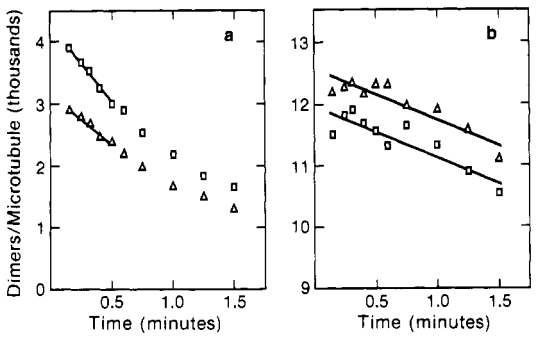

FIGURE 8: Effect of vinblastine on the rates of dilution-induced diassembly from (a) microtubule A ends and (b) microtubule D ends. Double-labeled control microtubules (rectangles) and vinblastine-treated microtubules (triangles) at steady state were constructed as described under Materials and Methods (mean length of control microtubules 11.5 ± 0.3 μm and vinblastine-treated microtubules 10.1 ± 0.3 μm). Control microtubules were labeled with ^{3}H for a distance of 2.5 μm, and vinblastine-treated microtubules were labeled for a distance of 1.9 μm (microtubules totally labeled with ^{3}H comprised 6–8% of the total population). Microtubules were diluted 21-fold into reassembly buffer containing the same concentration of vinblastine (0 for control microtubules, 0.15 μM for vinblastine-treated microtubules), and aliquots were stabilized at the indicated times for analysis of retained radiolabel by filter assay. D-end data represent dimers labeled with [^{14}C]GXP after subtracting A-end loss of [^{14}C]GXP.

for the microtubules just prior to dilution. A ends of control microtubules exhibited apparent rate constants that were similar to those reported earlier from this laboratory (Farrell & Wilson, 1984; Wilson et al., 1985). However, D ends of these microtubules were kinetically slower than A ends, rather than being 2–3 times faster as was found previously. The quantity of MAPs, which was higher in the preparation used here than in the previous studies, may be responsible for the observed difference in the D-end kinetics.

The most pronounced effect of vinblastine was at A ends, where it stabilized those ends strongly. At D ends, the data, which are somewhat less precise than A-end data due to increased scatter, indicate that vinblastine had no effect on the measured apparent dissociation rate constant. The calculated apparent association rate constants were reduced at A ends, and to a lesser extent at D ends, but these results should be regarded with caution until the reactions at the microtubule ends are understood.

Discussion

Properties of MAP-Rich Microtubules. We have used MAP-rich bovine brain microtubules with the opposite ends differentially labeled with [^{3}H]- and [^{14}C]guanine nucleotides to examine the effects of low concentrations of vinblastine on the addition and loss of tubulin at opposite ends of the microtubules in vitro. Once polymerized to polymer-mass steady state, MAP-rich microtubules are stable relative to MAP-free or MAP-depleted microtubules, and when unperturbed, they treadmill slowly and no dynamic instability behavior is detectable by analysis of length distributions or by direct visualization of individual microtubules by computer-enhanced video dark-field microscopy (Farrell et al., 1987; K. W. Farrell, B. Matsumoto, M. A. Jordan, and L. Wilson, unpublished experiments).

When such microtubules are polymerized to polymer-mass steady state in the presence of [^{14}C]GTP, they become labeled uniformly throughout their lengths with [^{14}C]GXP. When the microtubules are pulsed with a trace quantity of [^{3}H]GTP, there is an initial burst of incorporation that may be due to growth and shortening at both microtubule ends (Horio & Hotani, 1986), which is undetectable by computer-enhanced video dark-field microscopy. Alternatively, the burst may represent direct nucleotide exchange into tubulin already assembled into microtubules (Farrell et al., 1987). [^{3}H]GXP incorporated into the microtubules during this phase of a pulse may be at one or both ends of the microtubules. The burst phase is followed by a slower, relatively linear incorporation of [^{3}H]GXP at the operationally defined A ends that we have interpreted to predominantly reflect the rate of treadmilling. An initial burst loss of [^{14}C]GXP that may reflect either visually undetectable dynamic instability at one or both microtubule ends or direct nucleotide exchange, as well as a slower, relatively linear rate of [^{14}C]GXP–tubulin loss that reflects the net loss of tubulin at D ends, occurs upon addition of a large excess of unlabeled GTP as a chase for both isotopes.

Similarity of the Mechanism of Action of Vinblastine Regardless of the Strategy Used for Addition of the Drug. Microtubule protein polymerized in the presence of vinblastine attained a stable polymer-mass plateau. The final extent of polymerization was reduced linearly as the vinblastine concentration was raised from 0.1 to 6.0 μM (Figure 1).

When vinblastine was added to microtubules that previously had been polymerized to polymer-mass steady state, the microtubules depolymerized until a new, lower polymer-mass steady state was attained (Figure 5). Depolymerization was biphasic, with an initial rapid loss of polymer followed by a slower rate of loss. The extent of depolymerization was concentration dependent, increasing linearly as the concentration of vinblastine was raised from 0.1 to 6 μM. Tubulin labeled with [^{3}H]GXP was lost preferentially at all vinblastine concentrations examined (Figures 5 and 6). As already indicated, the location in the microtubule of the approximately 600 [^{3}H]GXP–dimers which comprise the region that is labeled rapidly during a pulse may be at one or both ends of the microtubules due to dynamic instability at the ends or to nucleotide exchange. However, loss of [^{3}H]GXP–dimers in excess of 600 must represent predominantly loss of tubulin that had been incorporated into the interior of the microtubule at A ends by treadmilling. Thus, despite the fact that the A ends are kinetically stabilized by vinblastine (see below), the depolymerization that occurs upon addition of the drug to preformed steady-state microtubules appears to be predominantly at the A ends. This may be due to the greater kinetic activity

of A ends as compared with D ends for this microtubule preparation.

The critical subunit concentrations at final polymer-mass plateaus for the experimental strategy in which the drug was present continuously from initiation of polymerization and for the strategy in which the drug was added to microtubules previously polymerized to steady state were indistinguishable for all vinblastine concentrations examined (Figure 1).

Using concentrations of vinblastine of 0.1 and 1 μM, we found that vinblastine did not copolymerize with tubulin. At both vinblastine concentrations, extremely small numbers of vinblastine molecules were bound per microtubule, most likely at the ends (Table I). The stoichiometries obtained with microtubules polymerized in the continuous presence of vinblastine were similar to those obtained with microtubules that were assembled prior to the addition of the drug. Increasing the microtubule protein concentration, which should have favored copolymerization by increasing the rate of polymerization, resulted in a decrease in the stoichiometry rather than an increase. The decrease probably reflects the increased number of microtubule ends in solution under these conditions (experiment 1, Table I). Thus, we conclude that the mechanism of interaction of vinblastine with microtubules polymerized in the presence of vinblastine is the same as with microtubules to which vinblastine was added after polymerization. The data here, along with previous data (Wilson et al., 1982a), are most consistent with a mechanism in which vinblastine, uncomplexed with tubulin, binds rapidly and reversibly to a small number of high-affinity tubulin binding sites located at one or both microtubule ends.

Inhibition of Dynamics at Microtubule Ends at Polymer-Mass Steady State by Vinblastine. Microtubules polymerized in the continuous presence of 0.15 μM vinblastine attained a stable polymer-mass plateau (Figure 2) and a stable mean length (Table II). No evidence of dynamic instability was detectable by analysis of microtubule length distributions (Table II). Thus, the polymerization of MAP-rich microtubules in the presence of vinblastine did not appear to induce dynamic instability.

Analysis of uptake and loss rates at opposite ends of the microtubules along with analysis of retention of [^{3}H]GXP from the pulse indicated that the microtubules were treadmilling. The rate of tubulin addition at A ends was similar to the rate of tubulin loss at D ends (Figures 2–4, Table III). Further, the loss of [^{3}H]GXP subsequent to the burst loss during the chase with excess unlabeled GTP was negligible in the presence of vinblastine, which further indicates that the [^{3}H]GXP incorporated during the pulse, excluding the radiolabel incorporated during the burst, was incorporated by a flux mechanism. However, the rate of flux was reduced by the drug as compared with that observed in control microtubules (Figures 2–4, Table III). This extent of inhibition of treadmilling corroborates earlier results in which 50% inhibition of net uptake of tubulin at A ends occurred at 0.14 or 0.18 μM vinblastine when the drug was added to already polymerized steady-state microtubules (Wilson et al., 1982a; Jordan et al., 1985).

Interestingly, the initial burst of [^{3}H]GXP uptake during the pulse and the initial burst loss of [^{3}H]GXP during the chase with excess unlabeled GTP were reduced by the drug to approximately the same extent as the rate of flux (58% reduction in initial burst uptake, 61% reduction in the initial burst loss). Thus, vinblastine appeared to inhibit all forms of tubulin exchange to the same extent. If the initial burst phases of the pulse and chase represent dynamic instability (growth and shortening) that is undetectable by computer-enhanced dark-field microscopy, then vinblastine inhibits both dynamic instability and flux to approximately the same extent.

Effect of Vinblastine on the Apparent Dissociation Rate Constants at Microtubule Ends. The apparent dissociation rate constants at microtubule A and D ends were estimated by dilution-induced depolymerization of double-labeled microtubules (Figure 8). Vinblastine reduced the rate of tubulin loss at microtubule A ends in a concentration-dependent manner (27% and 61% at 0.15 and 0.30 μM vinblastine, respectively), but it neither increased nor decreased the rate of tubulin loss at D ends. Thus, vinblastine does not depolymerize microtubules by destabilizing the microtubule ends. The fact that vinblastine slowed the rate of tubulin loss at A ends strongly indicates that the drug directly stabilizes the A ends (Wilson et al., 1982a).

Estimates of apparent association rate constants at A ends were calculated by using measured values for the apparent dissociation rate constants, flux rates, and critical concentrations in the presence of vinblastine. The data (Table III) indicate that vinblastine inhibits the apparent association rate constant at A ends and to a lesser extent at D ends. However, the calculated constants should be considered with caution (see Results). More direct evidence that vinblastine inhibits the association rate constant at A ends is that substoichiometric concentrations of the drug inhibit the net rate of tubulin addition at these ends when the drug is added to microtubule suspensions at polymer-mass steady state (Wilson et al., 1982a) and that depolymerization occurs primarily at the A ends (see Figures 5 and 6). Thus, we can conclude that vinblastine reduces both the association and dissociation rate constants at microtubule A ends (i.e., it kinetically caps A ends).

Microtubules polymerized in the continuous presence of low concentrations of vinblastine were significantly shorter than those polymerized in the absence of vinblastine. Because the polymer mass was only slightly reduced at the lower effective drug concentrations as compared with control microtubule suspensions, it appears that microtubule nucleation in the presence of the drug was stimulated significantly. This may be related to the ability of vinblastine to stabilize the microtubule ends kinetically and to induce tubulin self-association (Na & Timasheff, 1980, 1986).

Mechanism of Action of Vinblastine at Low Concentrations. At low concentrations, vinblastine significantly reduces the rate of both tubulin addition and loss at A ends. The change in the balance of rate constants results in a higher critical concentration in the presence of vinblastine than in the absence of the drug. A new steady state is attained with a stable polymer mass, and the microtubules exhibit significantly reduced exchange kinetics.

It is difficult with existing data to determine whether vinblastine also acts at the D ends. Approximately 17 high-affinity vinblastine binding sites have been titrated at the ends of the microtubules, but it has not been possible to determine whether the sites are located at one or both microtubule ends because the binding is rapidly reversible (Wilson et al., 1982a). It is clear that the drug neither increases nor decreases the stability of D ends because the apparent dissociation rate constant at this end was indistinguishable from that for control microtubules. The possibility that vinblastine inhibits tubulin addition at D ends was suggested by the calculated estimates for the apparent molecular association rate constants at this end (Table III), but as indicated (see Results), these data should be regarded with caution. This question must remain unresolved until direct evidence is obtained.

The evidence that vinblastine stabilizes microtubule A ends is consistent with data of Na and Timasheff (1980, 1986), which demonstrated that addition of vinblastine to purified tubulin in vitro induces an isodesmic indefinite self-association of tubulin. The vinblastine-induced self-association of tubulin occurs with a significant increase in the affinity between vinblastine and associated tubulin as compared with the affinity of vinblastine for tubulin dimers. It may be significant that the affinity of vinblastine for its binding sites at microtubule ends (5×10^5 M^{-1}; Wilson et al., 1982a) is 10-fold higher than the intrinsic binding constant of the tubulin dimer and is 10-fold lower than the binding constant to in vitro self-associated MAP-free tubulin (Na & Timasheff, 1986). The addition of free tubulin at microtubule ends may, in the absence of the drug, induce a conformational change that increases the affinity for vinblastine as compared with the affinity for vinblastine binding to tubulin dimers. The binding of vinblastine to tubulin at microtubule ends could further increase the forces holding the tubulin at the end or it could prevent a conformational change in tubulin at the end of the microtubule that may occur prior to dissociation of the tubulin from the end. Either mechanism would kinetically stabilize the end.

Vinblastine inhibits flux and tubulin exchange at microtubule ends at concentrations (0.1–0.2 μM) that cause only slight microtubule depolymerization (less than 7% of the total polymer mass). Recent evidence for poleward flux of tubulin in kinetochore microtubules during metaphase (Mitchison, 1989) and for vinblastine-induced accumulation of HeLa cells arrested at metaphase without accompanying microtubule depolymerization (M. A. Jordan, D. Thrower, and L. Wilson, unpublished data) suggests that kinetic stabilization of microtubule ends or inhibition of flux or both may play important roles in the ability of vinblastine to inhibit mitosis.

Finally, we note that vinblastine can produce a reversal of the apparent kinetic polarity of the microtubule (Table III). At 0.3 μM vinblastine, the D ends became kinetically more active than the A ends. Such a differential effect of a drug on the kinetic behavior at opposite ends of microtubules in vitro may provide some clues to possible regulatory mechanisms for the control of polymerization and depolymerization and control of the functions and spatial arrangements of microtubule subsets in vivo.

Acknowledgments

We thank Herbert Miller for excellent technical help. We also thank John Gathuru for assistance in the early stages of this work and Drs. Terri Burgess, Russell Stewart, and Kevin Farrell for thoughtful discussions of the results.

Registry No. Vinblastine, 865-21-4.

References

Bamburg, J. R., Shooter, E. M., & Wilson, L. (1973) *Biochemistry 12*, 1476–1482.

Bradford, M. M. (1976) *Anal. Biochem. 72*, 248–254.

Carlier, M. F., Hill, T. L., & Chen, Y.-D. (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*, 771–775.

DeConti, R. C., & Creasey, W. A. (1975) in *The Catharanthus Alkaloids* (Taylor, W. I., & Farnsworth, N. R., Eds.) pp 237–278, Dekker, New York.

Farrell, K. W., & Jordan, M. A. (1982) *J. Biol. Chem. 257*, 3131–3138.

Farrell, K. W., & Wilson, L. (1984) *Biochemistry 23*, 3741–3748.

Farrell, K. W., Himes, R. H., Jordan, M. A., & Wilson, L. (1983) *J. Biol. Chem. 258*, 14148–14156.

Farrell, K. W., Jordan, M. A., Miller, H. P., & Wilson, L. (1987) *J. Cell. Biol. 104*, 1035–1046.

George, P., Journey, L. J., & Goldstein, M. N. (1965) *J. Natl. Cancer Inst. 35*, 355–375.

Gerzon, K. (1980) in *Anticancer Agents Based on Natural Product Models* (Douros & Cassidy, Eds.) pp 271–317, Academic Press, New York.

Horio, T., & Hotani, H. (1986) *Nature (London) 321*, 605–607.

Jordan, M. A., & Farrell, K. W. (1983) *Anal. Biochem. 130*, 41–53.

Jordan, M. A., Himes, R. H., & Wilson, L. (1985) *Cancer Res. 45*, 2742–2747.

Jordan, M. A., Margolis, R. L., Himes, R. H., & Wilson, L. (1986) *J. Mol. Biol. 187*, 61–73.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951) *J. Biol. Chem. 193*, 265–275.

Mitchison, T. J. (1989) *J. Cell Biol. 109*, 637–652.

Na, G. C., & Timasheff, S. N. (1980) *Biochemistry 19*, 1355–1365.

Na, G. C., & Timasheff, S. N. (1986) *Biochemistry 25*, 6214–6222.

Schochet, S. S., Jr., Lambert, P. W., & Earle, K. M. (1968) *J. Neuropathol. Exp. Neurol. 27*, 645–658.

Singer, W. D., Jordan, M. A., Wilson, L., & Himes, R. H. (1989) *Mol. Pharmacol. 36*, 366–370.

Sternlicht, H., & Ringel, I. (1979) *J. Biol. Chem. 254*, 10540–10550.

Warfield, R. K. N., & Bouck, G. B. (1974) *Science 186*, 1219–1220.

Wilson, L., & Farrell, K. W. (1986) *Ann. N.Y. Acad. Sci. 466*, 690–708.

Wilson, L., Morse, A. N. C., & Bryan, J. (1978) *J. Mol. Biol. 121*, 255–268.

Wilson, L., Jordan, M. A., Morse, A., & Margolis, R. L. (1982a) *J. Mol. Biol. 159*, 125–149.

Wilson, L., Snyder, K. B., Thompson, W. C., & Margolis, R. L. (1982b) *Methods Cell Biol. 24*, 159–169.

Wilson, L., Miller, H. P., Farrell, K. W., Snyder, K. B., Thompson, W. B., & Purich, D. L. (1985) *Biochemistry 24*, 5254–5262.

Zeeberg, B., Reid, R., & Caplow, M. (1980) *J. Biol. Chem. 255*, 9891–9899.