Interaction of a Fluorescent Paclitaxel Analogue with Tubulin†

Suparna Sengupta,‡ Thomas C. Boge,§ Gunda I. Georg,§ and Richard H. Himes*,‡

Departments of Biochemistry and Medicinal Chemistry, University of Kansas, Lawrence, Kansas 66045

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ABSTRACT: To study the mechanism of binding of the antitumor agent paclitaxel to microtubules and tubulin, we have synthesized a fluorescent analogue of the drug. A dimethylamino group was introduced onto the 3′-*N*-benzoyl group of paclitaxel. This compound was synthesized from *N*-debenzoylpaclitaxel and 3-(dimethylamino)benzoyl chloride in 67% yield. *N*-Debenzoyl-*N*-[3-(dimethylamino)benzoyl]-paclitaxel has activity similar to paclitaxel in inducing microtubule assembly and binds to tubulin at the paclitaxel-binding site. Under assembly conditions, binding of this paclitaxel analogue to tubulin occurs in a time-dependent manner and is accompanied by a large increase in fluorescence intensity, as well as a large blue shift in the emission maximum. In addition, evidence is presented to show that this compound also binds to tubulin in the dimeric state, but the binding affinity is much lower ($K_d = 49 \pm 8$ μM at 25 °C) than that reported for polymeric tubulin. The fluorescent paclitaxel analogue, with a high quantum yield, will be a useful tool in studying the mechanism of paclitaxel binding to tubulin and the environment of the paclitaxel-binding site on tubulin.

The diterpene paclitaxel (Taxol) has received a great deal of attention in the last several years because of its potential as a new cancer chemotherapeutic agent. It has been approved as a drug in the treatment of ovarian and breast cancers and shows effectiveness against several other cancers (Spencer & Faulds, 1994; Holmes et al., 1995). Paclitaxel is a unique member of the group of antimitotic agents, most of which inhibit tubulin self-assembly and depolymerize microtubules. Paclitaxel, however, has the ability to stabilize microtubules and to induce microtubule assembly under conditions where assembly otherwise is not observed, e.g., at low temperatures or in the absence of exogenous GTP. Paclitaxel binds to polymerized tubulin *in vitro* with a stoichiometry of about 1 mol/mol of tubulin dimer (Parness & Horwitz, 1981) at a site different from the colchicine or vinblastine sites (Kumar, 1981; Schiff & Horwitz, 1981). Stabilization of microtubules can be achieved *in vitro* and in cultured cells at substoichiometric concentrations of paclitaxel (Jordan et al., 1993; Deery et al., 1995), presumably because paclitaxel, bound to a tubulin dimer at the end of a microtubule, decreases the rate of dissociation of the dimer from the microtubule.

Because of the clinical importance of paclitaxel as an antitumor agent, as well as its unique property of inducing microtubule formation, there is considerable interest in determining its mechanism of action and mode of interaction with tubulin. With the use of paclitaxel photoaffinity analogues, it has been shown that paclitaxel binds predominantly to β-tubulin (Rao et al., 1994; Dasgupta et al., 1994; Combeau et al., 1994) and that the N-terminal 31 amino acids of β-tubulin are probably involved in the binding (Rao et al., 1994). Fluorescent analogues of drugs are widely used as important tools in studies of the elucidation of structures and the mechanisms of drug binding to proteins and nucleic acids. In order to obtain more information about the mechanism of action of paclitaxel and the environment of the paclitaxel-binding site, we have synthesized the fluorescent paclitaxel analogue, *N*-debenzoyl-*N*-[3-(dimethylamino)-benzoyl]paclitaxel (Figure 1). Our studies in this report show that this compound is biologically active with a potency similar to that of paclitaxel. Its binding to polymerized tubulin is accompanied by a large blue shift in fluorescence and a substantial increase in fluorescence intensity. The analogue also appears to bind to dimeric tubulin, but with much lower affinity.

EXPERIMENTAL PROCEDURES

Materials. Bovine brain tubulin was prepared by three cycles of a temperature-dependent assembly−disassembly procedure (Tiwari & Suprenant, 1993) followed by phosphocellulose−Biogel P-10 chromatography (Algaier & Himes, 1988). Tubulin was drop-frozen in liquid nitrogen and stored at −80 °C. Prior to use, it was thawed and centrifuged at 27000*g* for 10 min to remove aggregated protein. Paclitaxel was a gift from Hauser Chemical Research, Inc. (Boulder, CO). [^{3}H]Paclitaxel was purchased from Moravek Biochemicals. Podophyllotoxin and ultrapure urea were purchased from ICN Chemical Co. Urea was recrystallized from water before use.

Synthesis of N-Debenzoyl-N-[3-(dimethylamino)benzoyl]-paclitaxel (DMAT). 3-(Dimethylamino)benzoyl chloride was synthesized first. 3-(Dimethylamino)benzoic acid (99 mg, 0.6 mmol) was suspended in an acetone (1.0 mL) solution of cyanuric chloride (55 mg, 0.3 mmol) and triethylamine (0.083 mL, 0.6 mmol). After 4 h, the mixture was filtered and the filtrate concentrated. The residue was treated with CCl_4 and the particles were filtered. Evaporation of the solvent gave crude 3-(dimethylamino)benzoyl chloride, which was used without further purification.

To a vigorously stirred solution of *N*-debenzoylpaclitaxel (Dasgupta et al., 1994) (7.7 mg, 0.01 mmol) in EtOAc (0.5

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* Address correspondence to this author.
‡ Department of Biochemistry.
§ Department of Medicinal Chemistry.

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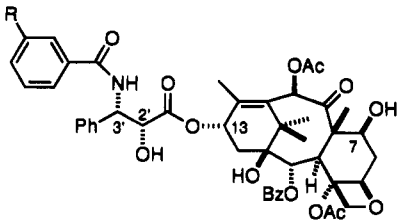

FIGURE 1: Structures of paclitaxel and DMAT: R = H, paclitaxel; R = NMe_2, *N*-debenzoyl-*N*-[3-(dimethylamino)benzoyl]paclitaxel (DMAT).

mL) and saturated aqueous $NaHCO_3$ was added 3-(dimethylamino)benzoyl chloride (2.2 mg, 1.2 mmol) in EtOAc (0.2 mL). After 30 min, the reaction mixture was extracted with CH_2Cl_2 (5 mL) and the organic layer was dried with Na_2SO_4. Following silica gel flash chromatography (hexane–EtOAc, 1:1), the desired compound, an amorphous solid, was isolated.

Polymerization Assays. Tubulin polymerization induced by paclitaxel or DMAT was performed in PEM buffer [0.1 M Pipes (pH 6.9) containing 1 mM $MgSO_4$ and 1 mM EGTA]. Two methods were used to follow the reaction. In one, the turbidity at 350 nm was measured in a thermostated Shimadju UV 2100U spectrophotometer. A centrifugation assay was also used to determine the effectiveness of DMAT and paclitaxel in promoting tubulin assembly. Different concentrations of paclitaxel or DMAT were incubated with 10 μM tubulin and 0.5 mM GTP at 37 °C for 15 min, followed by centrifugation at 40000*g* in a Beckman TL-100 ultracentrifuge for 4 min. The protein concentration in the supernatant was measured by the Bradford procedure (Bradford, 1976), and the drug concentration causing 50% retention of protein in the supernatant was taken as the ED_{50} value.

Competition of [^{3}H]Paclitaxel Binding by DMAT. Tubulin (10 μM) was incubated at 37 °C for 15 min with 5 μM [^{3}H]-paclitaxel, 0.5 mM GTP, and varying concentrations of DMAT. The reaction mixtures were then centrifuged at 40000*g* in a Beckman TL-100 ultracentrifuge, pellets were dissolved in 0.1 M NaOH, and the radioactivity and protein concentration were measured.

Fluorescence Measurements. All fluorescence spectra were recorded in a Perkin-Elmer MPF-44B spectrofluorometer attached to a circulating temperature-controlled bath. Excitation and emission bandpasses were 5 nm each, and all fluorescence values reported are uncorrected unless otherwise stated. Excitation spectra of DMAT were recorded from 250 to 400 nm keeping the emission wavelength at 465 nm, and emission spectra were recorded from 410 to 570 nm using an excitation wavelength of 350 nm.

The quantum yield of DMAT was calculated by comparing the absorbance at the excitation wavelength and the area of the corrected emission spectra with those of quinine sulfate in 5 M H_2SO_4, whose quantum yield was taken as 0.7 at 25 °C (Scott et al., 1970).

The dissociation constant of DMAT binding to tubulin in the dimeric state, either at 4 °C or complexed with podophyllotoxin, was determined from a double-reciprocal plot (Mas & Colman, 1985) using the equation:

$$(F_o/F - F_o) = [K_d/(Q - 1)]1/P_T + 1/(Q - 1)$$

where F_o is the fluorescence at a particular concentration of DMAT, F is the fluorescence of the same concentration of DMAT in the presence of different concentrations of protein, and P_T is the total concentration of protein. The protein concentration was always in molar excess over DMAT, and an approximation was made that the total protein concentration is equal to the free protein concentration. Q is the fluorescence enhancement factor, the fluorescence at a saturating protein concentration relative to the fluorescence of the free drug.

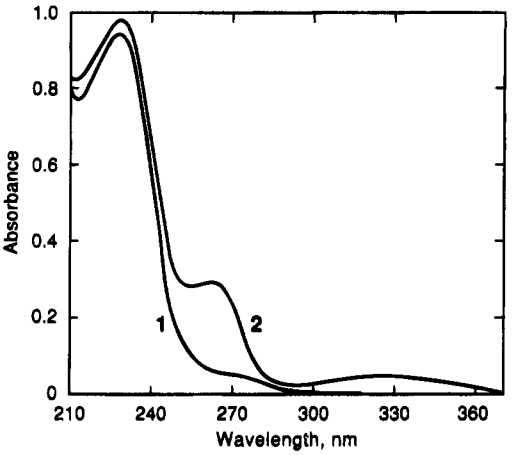

FIGURE 2: Absorption spectra of DMAT and paclitaxel in methanol: curve 1, paclitaxel; curve 2, DMAT.

Electron Microscopy. Aliquots of tubulin samples were treated with 0.25% glutaraldehyde. Glutaraldehyde-fixed tubulin structures were allowed to adhere to grids coated with Formvar and carbon for 45 s, and the grids were rinsed with water and then negatively stained with 2% uranyl acetate.

RESULTS

Properties of DMAT. DMAT was prepared in a 67% yield from *N*-debenzoylpaclitaxel. The ^{1}H NMR, ^{13}C NMR, and mass spectra were consistent with the structure. HPLC analysis of the product showed only one peak. The absorption spectra of DMAT and paclitaxel in methanol are compared in Figure 2. DMAT has absorption bands at 228 and 263 nm and a smaller absorption maximum at 330 nm. The 263 and 330 nm maxima, which are absent from paclitaxel, are due to the (dimethylamino)benzoyl group. The molar extinction coefficients of DMAT at 330, 263, and 228 nm are 1370, 8430, and 29 280 M^{-1} cm^{-1}, respectively. These compare to the values for paclitaxel of 1700 and 27 900 M^{-1} cm^{-1} at 273 and 227 nm, respectively.

Although paclitaxel does not possess fluorescence properties when excited at any of its absorption maxima, the introduction of the dimethylamino moiety at the 3-position of the 3′-*N*-benzoyl group makes the compound fluorescent. The corrected excitation and emission spectra of DMAT in methanol are shown in Figure 3A,B. The emission maximum at 465 nm and excitation maxima at 263 and 330 nm correspond to the values of 465 nm for emission and 270 and 334 nm for the excitation maxima of the uncorrected spectra of DMAT in PEM. The quantum yield was found to be 0.35. The nature of the fluorescent spectra is similar to that of 3-(dimethylamino)benzoic acid (data not shown).

The analogue was compared to paclitaxel in its effectiveness in inducing tubulin self-assembly using a centrifugation assay. This assay measures the amount of unassembled tubulin remaining after incubation with the drug. The data

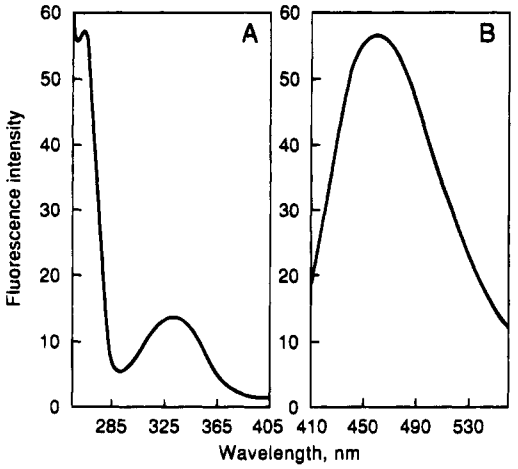

FIGURE 3: Fluorescence spectra of DMAT. Spectra of 20 μM DMAT were recorded at 25 °C in methanol. (A) Corrected excitation spectrum using an emission wavelength of 465 nm. (B) Corrected emission spectrum using an excitation wavelength of 350 nm.

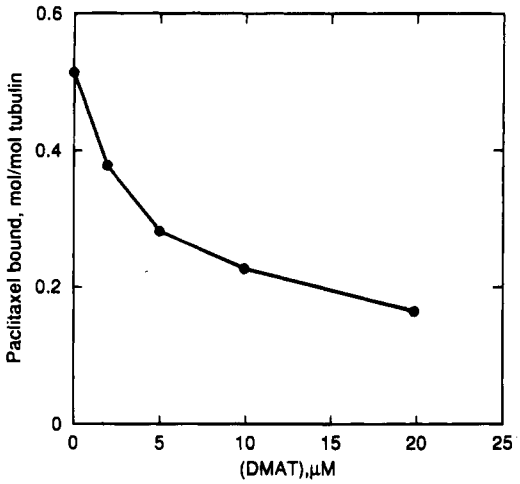

FIGURE 4: Competition of [^{3}H]paclitaxel binding to tubulin by DMAT. Tubulin (5 μM) was incubated with 5 μM [^{3}H]paclitaxel, 0.5 mM GTP, and varying concentrations of DMAT in PEM at 37 °C for 15 min. After centrifugation, the pellets were treated as described under Experimental Procedures.

showed that the concentration of DMAT that resulted in 50% of the tubulin being assembled was 1 μM, which is slightly higher than the corresponding value for paclitaxel of 0.6 μM.

That the analogue binds to tubulin at the same site as paclitaxel is shown by the ability of DMAT to inhibit paclitaxel binding to polymerized tubulin. Results of a competition study shown in Figure 4, using 5 μM [^{3}H]-paclitaxel and varying DMAT concentrations, demonstrate that the fluorescent analogue effectively inhibits paclitaxel binding, with 50% inhibition achieved at a concentration of 7 μM.

Changes in DMAT Fluorescence upon Binding to Tubulin. When 5 μM tubulin was incubated with 5 μM DMAT at 25 °C, the fluorescence emission maximum underwent an instantaneous blue shift of 20 nm and a 5-fold increase in intensity at 465 nm. This was followed by a slow increase in intensity until, after 60 min, the intensity had increased another 2-fold (Figure 5A). The results suggest that DMAT bound instantaneously to tubulin and that this was followed by a slow polymerization. To determine whether the slower increase was indeed due to polymer formation, the reaction was done in the presence of GTP and at 10 μM DMAT to increase the rate of microtubule formation (Figure 5B). The data show that an immediate increase in fluorescence intensity again occurred, followed by a further increase that coincided with polymer formation, as measured by the increase in light scatter at 350 nm (Figure 5C). When 10 μM paclitaxel was also present, the total fluorescence increase was reduced by 60% (Figure 5B), and the increase associated with the slower process was decreased by 80%. Thus, paclitaxel effectively competes with the fluorescence change associated with microtubule formation. The decrease in fluorescence intensity in the presence of paclitaxel also demonstrates that the fluorescence intensity that develops during the assembly reaction is not due to an artifact resulting from the turbidity of the solution. In fact, the turbidity in the presence of DMAT and paclitaxel was actually higher than that developed with DMAT alone (Figure 5C). Further evidence that turbidity was not responsible for the observed change in fluorescence intensity was the fact that the apparent fluorescence after polymerization of 5 μM tubulin with paclitaxel was about 10% of the value obtained with DMAT. As an additional control, we measured the fluorescence of DMAT in a glycogen solution that had the same A_{350} value as 5 μM polymerized tubulin. The intensity of the DMAT solution was increased by 10% in the glycogen solution.

Changes in DMAT Fluorescence under Nonassembly Conditions. The data presented in Figure 5 show an immediate change of DMAT fluorescence when it was added to tubulin, suggesting that DMAT binds to tubulin in the unpolymerized state. To determine whether this was the case, we examined the interaction of DMAT with tubulin under various nonassembly conditions: at 4 °C and in the presence of podophyllotoxin or urea.

Paclitaxel is known to induce a slow polymerization of tubulin into polymorphic structures at low temperatures (Thompson et al., 1981; Hamel et al., 1981). By using a low tubulin concentration and short time periods, this assembly process could be avoided. The increase in fluorescence of a 3 μM DMAT solution was measured in the presence of increasing concentrations of tubulin at 4 °C (Figure 6). At any specific tubulin concentration, fluorescence increased immediately upon the addition of protein and remained constant. There was no evidence of microtubule formation as judged by the absence of an increase in turbidity for a period of 20 min and by electron microscopy.

The fluorescence of DMAT also increased in a time-independent manner when added to tubulin previously saturated with podophyllotoxin (Figure 7). Treatment of 5 μM tubulin–podophyllotoxin complex with 5 μM DMAT led to an instantaneous increase in fluorescence that did not change with time (Figure 7, curve 2). The change with tubulin alone was higher due to slow polymerization under these conditions. Under the conditions used, no assembly of the tubulin–podophyllotoxin complex could be observed by following light scattering, even after 45 min. To determine whether podophyllotoxin caused the formation of nonspecific aggregates that might bind DMAT, we examined the aggregate content by size exclusion HPLC. No significant difference was found between tubulin and tubulin incubated with podophyllotoxin and DMAT.

Sackett et al. (1994) studied the effect of urea concentrations on various properties of tubulin and found that paclitaxel-induced assembly is completely inhibited after a

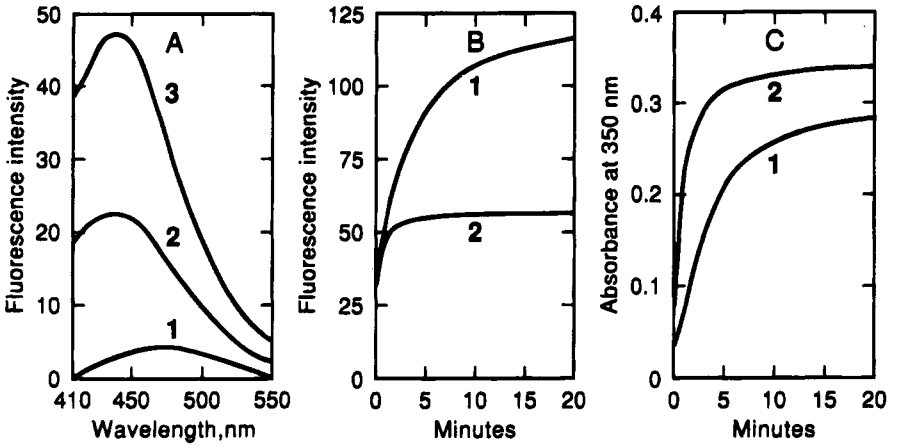

FIGURE 5: Fluorescence spectra of DMAT upon binding to tubulin. (A) DMAT (5 μM) was incubated with 5 μM tubulin at 25 °C in PEM, and spectra were recorded using 350 nm as an excitation wavelength: DMAT (curve 1), DMAT with tubulin at 0 min (curve 2), and DMAT with tubulin after 60 min (curve 3). (B) Time course of fluorescence intensity changes at 465 nm under polymerizing conditions. Tubulin (5 μM) in PEM containing 0.5 mM GTP and 10 μM DMAT (curve 1) or 10 μM DMAT and 10 μM paclitaxel (curve 2) was incubated at 22 °C and the fluorescence intensity changes were followed. (C) Time course of light-scattering changes at 350 nm. Conditions were identical to those in (B), with 10 μM DMAT alone (curve 1) and containing both 10 μM DMAT and 10 μM paclitaxel (curve 2).

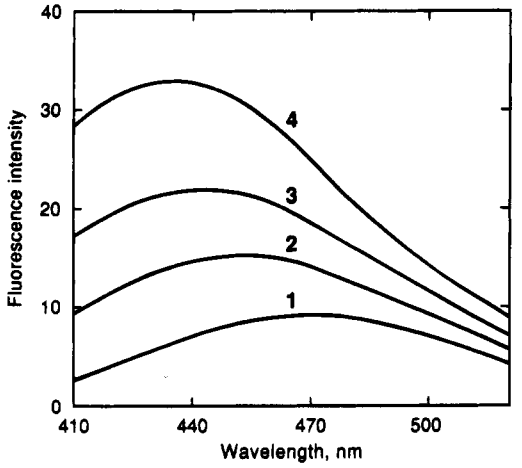

FIGURE 6: Binding of DMAT to tubulin at 4 °C. Fluorescence of 3 μM DMAT (curve 1) was observed after mixing with tubulin at 2 μM (curve 2), 3.9 μM (curve 3), or 6.5 μM (curve 4) in PEM. Fluorescence was observed at 465 nm with excitation at 350 nm. The excitation and emission bandpasses were both 5 nm.

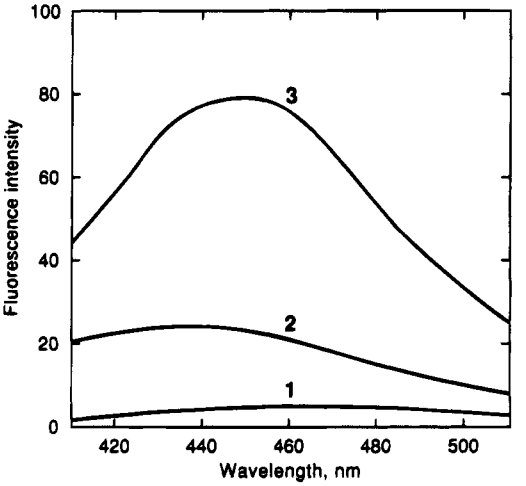

FIGURE 7: Binding of DMAT to tubulin and the tubulin–podophyllotoxin complex. The fluorescence of 5 μM DMAT (curve 1) was observed in PEM after 60 min at 25 °C with 5 μM tubulin–podophyllotoxin complex (curve 2) and 5 μM tubulin (curve 3). The tubulin–podophyllotoxin complex was prepared by incubating 5 μM tubulin with 50 μM podophyllotoxin for 30 min at 25 °C.

15 min incubation with 0.5 M urea. Functions such as binding of the antimitotic colchicine analogue 2-methoxy-5-(2′,3′,4′-trimethoxyphenyl)tropone and the widely used hydrophobic fluorophore ANS were found to be less sensitive than polymerization to urea denaturation, and indeed, their binding increased as the urea concentration was increased to a limiting value (Sackett et al., 1994). If DMAT binds to tubulin in a urea concentration higher than that that prevents polymerization, binding to dimeric tubulin would be suggested. Incubation of tubulin for 15 or 30 min with urea, followed by the addition of DMAT, showed that the fluorescence intensity increased in urea concentrations up to 3 M, but higher concentrations reduced the extent of the intensity change (Figure 8), evidently because of the loss of tertiary structure of the protein.

In the experiment described in Figure 5, paclitaxel did not prevent the instantaneous increase in fluorescence intensity that occurred when DMAT was added to tubulin, suggesting that the two compounds do not compete for the same site in dimeric tubulin. To investigate this in more detail, we included up to 40 μM paclitaxel in a solution containing 5 μM tubulin and either podophyllotoxin or urea and 5 μM DMAT. No decrease in the fluorescence intensity could be detected by the inclusion of paclitaxel. As will be explained later, this apparent lack of competition is simply due to the fact that the binding affinity is low and the paclitaxel site is not saturated under the conditions used. 3-(Dimethylamino)-benzoic acid was also tested for binding to tubulin, but no increase in fluorescence nor any shift in emission maxima was observed, eliminating the possibility of a nonspecific attachment by the dimethylamino group of DMAT. The preceding experiments clearly demonstrate that DMAT binds to dimeric tubulin as well as to polymeric tubulin.

An attempt was made, therefore, to determine the binding affinity of DMAT to dimeric tubulin. A dissociation constant was calculated by titrating DMAT with tubulin either at 4 °C or complexed with podophyllotoxin at 25 °C. Data for one experiment are presented in Figure 9 for binding to the tubulin–podophyllotoxin complex. A K_d value of 49 ± 8 μM was calculated (mean of three experiments). However, it is apparent from Figure 9 that saturation of DMAT with

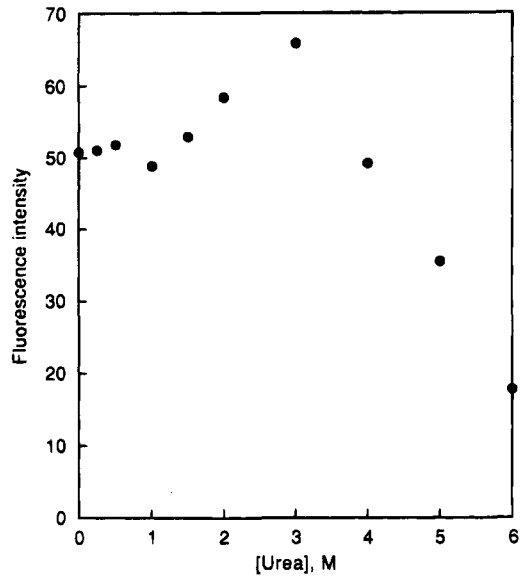

FIGURE 8: Effect of urea on DMAT binding to tubulin. The fluorescence of 5 μM DMAT in PEM was observed at 465 nm immediately after mixing with 5 μM tubulin previously incubated with different concentrations of urea for 30 min at 25 °C.

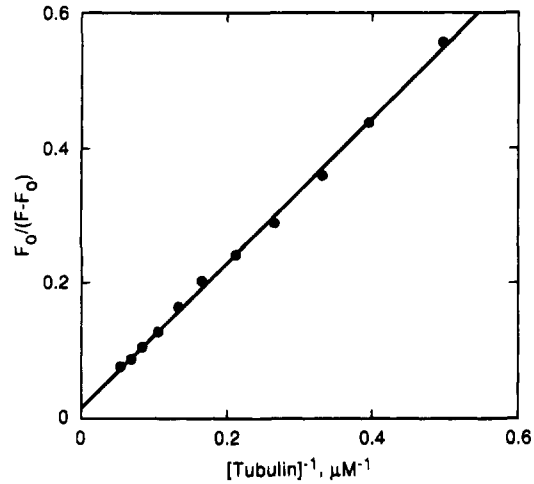

FIGURE 9: Double-reciprocal plot of tubulin binding to DMAT. DMAT (1 μM) was titrated with 2−18.5 μM tubulin−podophyllotoxin complex in PEM at 25 °C. Fluorescence was observed at 450 nm using excitation and emission bandpasses of 5 and 10 nm, respectively.

tubulin was not achieved, as the highest experimentally determined intensity value was only about 30% of the extrapolated value at infinite tubulin concentration. Higher concentrations of tubulin were not used to avoid the possibility of aggregate formation. An identical K_d value was obtained by titrating DMAT with tubulin at 4 °C.

DISCUSSION

Although a number of studies on paclitaxel-induced tubulin assembly have been done, little is known about the mechanism of this process or the tubulin−paclitaxel interaction. This interaction can be studied with a variety of paclitaxel analogues. Structure−activity relationship investigations using a variety of analogues are important in probing the interaction sites on the protein: photoaffinity analogues help to determine the sites of interaction, and fluorescent analogues can be used to determine the environment around regions of the paclitaxel molecule in the bound form.

Previous studies have shown that the *N*-benzoyl group of paclitaxel can be modified in a number of ways without causing large changes in the activity of the drug [for reviews, see Georg et al. (1994) and Hepperle and Georg (1994)]. With this in mind, we synthesized a fluorescent analogue of paclitaxel containing a dimethylamino group in the 3-position of the *N*-benzoyl group. This compound is comparable to paclitaxel in its ability to stimulate tubulin assembly and competes effectively with paclitaxel for binding to microtubules (Figures 4 and 5). The compound, DMAT, has a fluorescent emission signal at 465 nm when excited with 350 nm radiation. When bound to microtubules, the maximum shifts to 445 nm with a corresponding large increase in fluorescence intensity. Such a blue shift and intensity increase are indicative of a change from a polar to a nonpolar environment around the fluorophore upon binding. Another paclitaxel fluorescent analogue, with a 3-aminobenzoyl group at the 2-position of Taxol, has been described in a brief report (Han et al., 1994). This analogue shows properties similar to those of DMAT upon binding to microtubules, suggesting that both the *N*-benzoyl and 2-benzoyl groups are located in nonpolar environments in the tubulin molecule.

In our investigations, we found evidence for the interaction of DMAT and, by implication, paclitaxel with tubulin in the dimeric form. The protein was maintained as a dimer by conducting the experiments either at 4 °C or in the presence of podophyllotoxin or urea. Under these conditions, a blue shift and an increase in the fluorescence intensity of DMAT also occurred. However, unlike the situation when studies were done under assembly conditions, the change in fluorescence properties was not time-dependent, indicating that it was not due to binding to polymer being formed in a self-assembly system. Previously, Carlier and Pantaloni (1983) presented indirect evidence for paclitaxel binding to dimeric tubulin by demonstrating that the drug stimulates the GTPase activity of the tubulin−colchicine complex. Their data indicated a K_d of 13 μM. Other studies have failed to show binding of paclitaxel (Diaz et al., 1993) or 7-acetylpaclitaxel (Takoudju et al., 1988) to dimeric tubulin, but perhaps these studies were done under conditions that would not allow the detection of weak binding.

Attempts to determine a binding affinity by titrating with protein yielded a K_d of 49 μM. Although a plot of $F_o/F - F_o$ *vs* $1/P$ was linear (Figure 9), indicating one class of sites, the data points collected were at protein concentrations far from saturation, requiring a long extrapolation. Thus, it is not known whether classes of weaker sites also exist. The inability to detect competition by paclitaxel for the dimer site probably results from the fact that saturation was not achieved and the paclitaxel simply bound to unoccupied sites. With a K_d of 49 μM and a tubulin and DMAT concentrations of 5 μM, only 8% of the sites would be occupied. Even with 40 μM paclitaxel present, only 50% saturation would be achieved, assuming a similar K_d for paclitaxel binding. Because of the low aqueous solubility of paclitaxel, higher concentrations could not be used. It is not clear whether the tubulin dimer−paclitaxel interaction is at the same site found in microtubules or at another lower affinity site.

The K_d value of 49 μM for binding of paclitaxel to unpolymerized tubulin is far lower than that for binding to polymerized tubulin. Recently Caplow et al. (1994) determined a K_d value of 10 nM for this interaction, a value about

1% of that reported earlier by Parness and Horwitz (1981). However, Caplow et al. suggest that the latter value is in error because studies were done at concentrations of polymerized tubulin that were much too high: concentrations at which all paclitaxel present would be bound. If the weaker binding site we detected is the same site as that on polymerized tubulin, the much higher affinity for the latter could be due to a conformational change in the protein produced as a result of tubulin−tubulin interactions in the microtubule wall or to the fact that a higher affinity is achieved by paclitaxel binding to two different associated dimers in the microtubule wall. If the sites in polymerized and unpolymerized tubulin are identical, it would suggest that the steps in paclitaxel binding would include first an interaction with the dimer, followed by a much stronger interaction in the polymer, which is a mechanism similar to that described for vinblastine-induced tubulin aggregate as a ligand-mediated association (Na & Timasheff, 1986). However, Diaz et al. (1993) proposed a mechanism in which paclitaxel binds first to the end of a microtubule and then participates in binding to a newly added dimer, in other words, a double-sided ligand. Our results would suggest another mechanism considered by Diaz et al., one in which the drug binds first to the dimer and upon addition to the end of a maicrotubule also binds to another dimer. If the weak binding site is different from the tighter binding site in polymerized tubulin, it should be possible to detect more than one binding site in microtubules, given that the appropriate concentrations of DMAT could be used.

Fluorescent analogues of paclitaxel can be useful tools for determining the environment of both the paclitaxel and tubulin molecules. We are currently engaged in placing fluorescent probes at different positions on the paclitaxel molecule so that the effect of binding on the different parts of the molecule can be studied. These fluorescent analogues will also be used to determine the distances from the paclitaxel-binding site to other ligand-binding sites, appropriately labeled with fluorophores.

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