Volume 234, number 1, 177–180 FEB 06041 July 1988

Interaction of 7-acetyltaxol with different tubulin assemblies

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Received 16 April 1988

Equilibrium microdialysis of [^{3}H]acetyltaxol against different tubulin assemblies showed that: (i) the binding capacity of tubulin does not depend on the temperature; (ii) two classes of 'polymers' exist, with respect to Ac-taxol binding. Some of them (plaques, complex cylinders induced with some polycations and spirals made with rhazinilam) bound Ac-taxol, as do normal microtubules. In contrast, spirals formed with vinblastine and griseofulvin, rings made with polycations and complex cylinders induced with spermine do not bind Ac-taxol as is the case with free tubulin.

Taxol; Tubulin; Equilibrium dialysis

1. INTRODUCTION

Taxol stabilizes microtubules in vivo and in vitro [1]. In agreement with this property, Parness and Horwitz [2] have shown, using a sedimentation procedure, that [^{3}H]taxol binds to microtubules. This result has been confirmed, using equilibrium microdialysis and 7-[^{3}H]acetyltaxol [3]. The binding parameters obtained with both compounds are quite similar: dissociation constants (0.9 and 0.3 μM), maximum binding capacity (0.6 and 1.0 mol/mol, respectively) and a Hill coefficient of 0.8 for Ac-taxol. In contrast, we have demonstrated that [^{3}H]Ac-taxol does not bind to unassembled tubulin either free, at 0°C, or complexed with podophyllotoxin or maytansine, at 0 and 37°C [3]. This result suggested that the binding site for Ac-taxol was not accessible on unassembled tubulin and was present only after tubulin assembly into microtubules. Here, we investigated whether this property could be extended to all forms of tubulin assemblies, such as plaques, spirals, rings, complex cylinders, which are induced either by a modification of the incubation medium or by the presence of various microtubule poisons.

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Abbreviations: Ac-taxol, 7-acetyltaxol; Me_2SO, dimethyl sulfoxide; DMF, dimethyl formamide

2. MATERIALS AND METHODS

Microtubule proteins were obtained from sheep brain by two (used with vinblastine only) or three cycles (80% pure, 1.2 mg/ml) of assembly/disassembly [4]. Equilibrium microdialysis [5] was performed as described [3]. The amount of sedimentable protein present at the end of dialysis was determined using a Beckman airfuge (rotor type 30°, 27 lb/inch^2 for 15 min at 0 or 37°C) and expressed as the percentage of protein sedimented from a parallel control at 37°C. Assembled tubulin was observed by electron microscopy, using negative staining with 2% uranyl acetate.

3. RESULTS

3.1. *Binding of Ac-taxol to tubulin assemblies with Me_2SO and/or zinc*

The amount of microtubules assembled at 37°C in less than 10% Me_2SO was normal and no

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00145793/88/$3.50

tubulin assembly took place at 0°C. Ac-taxol binding occurred at 37°C but not at 0°C. In higher concentrations of Me_2SO, tubulin assembled at both 0 and 37°C [6,7] and bound Ac-taxol. Microtubules and plaques were formed in 20% Me_2SO [8]. Only plaques were observed in 30% Me_2SO: 109 and 100% of the control value for sedimentable protein were observed at 0 and 37°C, respectively. In 30% Me_2SO (fig.2A), the K_d value was increased (6.5 and 3.6 μM) while the Hill coefficient (1.0 and 0.7) and B_{max} (0.8 and 0.8 mol/mol at 0 and 37°C, respectively) were not significantly altered. In 0.1 mM $ZnSO_4$/1.5% Me_2SO no structures were observed at 0°C but tubulin assembled into plaques at 37°C (fig.1A). Ac-taxol binding only occurred at 37°C (Hill coefficient = 1.0, K_d = 16 μM, B_{max} = 1.9 mol/mol). In 0.1 mM $ZnSO_4$/10% Me_2SO and 2 mg/ml tubulin, plaques were made at both 0 (fig.1B) and 37°C (91 and 109% of the control sedimentable protein, respectively). Ac-taxol binding was observed at both temperatures (K_d = 3 and 6 μM and B_{max} = 1.0 and 0.9 mol/mol at 0 and 37°C, respectively) while no binding was observed to bovine serum albumin (2 mg/ml). The possibility of assembling tubulin at 0 and 37°C in high concentrations of Me_2SO offered the opportunity to test whether bound [^{3}H]Ac-taxol could be displaced with an excess of unlabelled taxol. In 30% Me_2SO without (fig.2A) or with 0.1 mM $ZnSO_4$, Ac-taxol binding was inhibited by 0.1 mM taxol at both 0 and 37°C although the amount of sedimentable material remained unchanged (96 and 88% of the control without taxol; 88 and 73% of the control in 0.1 mM taxol at 0 and 37°C, respectively in 30% Me_2SO).

3.2. *Effect of griseofulvin*

In 0.1 mM griseofulvin [9] and 2.6% DMF, tubulin assembled into sheets at 37°C (fig.1C) but not at 0°C (85 and 23% of the control). DMF (2.5%) did not perturb normal microtubule assembly (11 and 77% of the control at 0 and 37°C, respectively). No difference in Ac-taxol binding was observed between tubulin assembled at 37°C in 0.1 mM griseofulvin/2.5% DMF and in 2.5% DMF alone (Hill coefficient = 0.92 and 0.89; K_d = 0.46 and 0.55 μM; B_{max} = 1.08 and

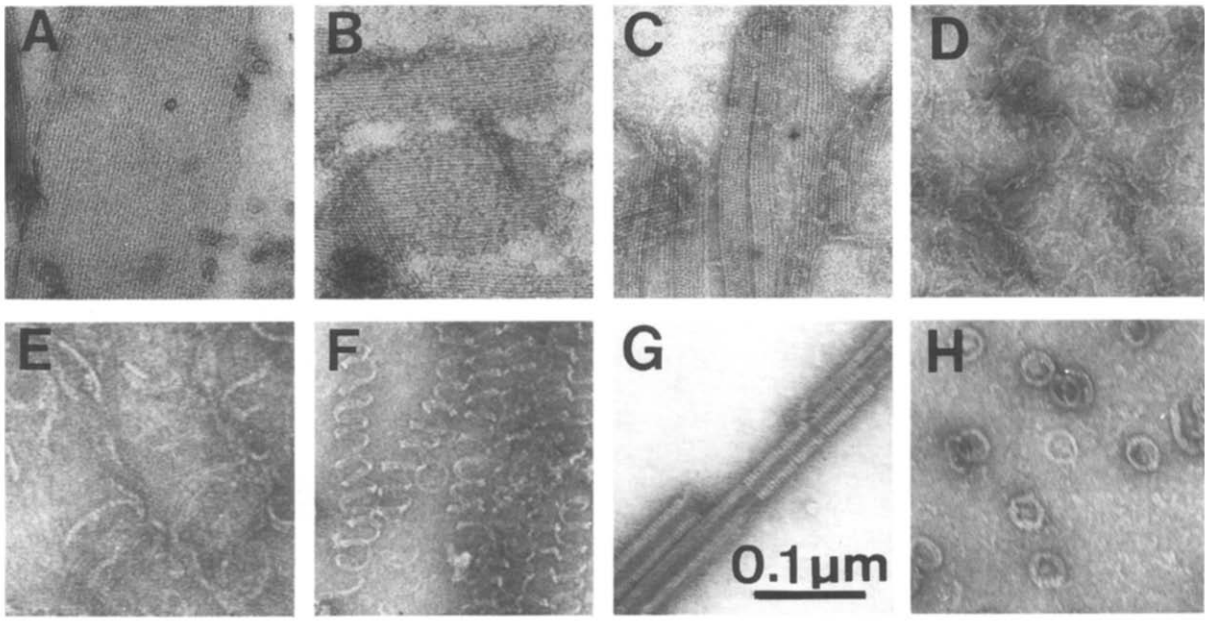

Fig.1. Electron micrographs of tubulin assemblies after 6 h dialysis vs 32 μM [^{3}H]Ac-taxol. Magnification, × 98000. (A) Plus 0.1 mM $ZnSO_4$, 37°C. (B) Plus 0.1 mM $ZnSO_4$/10% Me_2SO, 2 mg tubulin/ml, 0°C. (C) Plus 0.1 mM griseofulvin/2.6% DMF, 37°C. (D) Plus 0.1 mM griseofulvin/10% Me_2SO, 0°C. (E) Plus 50 μM rhazinilam, 0°C. (F) Two-cycle tubulin, plus 200 μM vinblastine, 37°C. (G,H) Plus 10 μM protamine, 37 and 0°C, respectively.

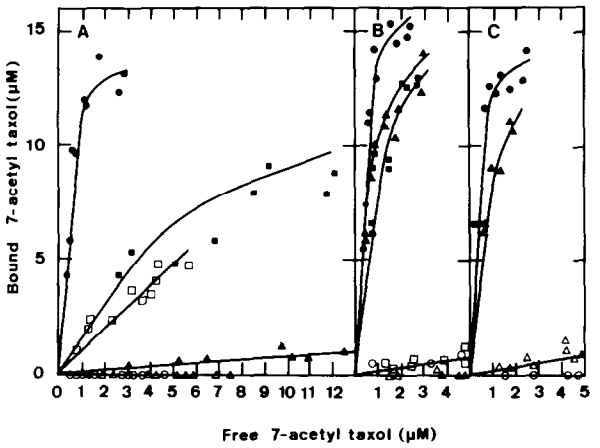

Fig.2. Binding of [^{3}H]Ac-taxol measured by equilibrium dialysis. Solid symbols, 37°C; open symbols, 0°C. (A) Effect of 30% Me_2SO and displacement of Ac-taxol with unlabelled taxol. (●,○) Control, (■, □) 30% Me_2SO, (▲, △) 30% Me_2SO/0.1 mM unlabelled taxol. (B) Effect of griseofulvin. (●,○) Control, (▲, △) 2.5% DMF, (■, □) 0.1 mM griseofulvin/2.5% DMF. (C) Effect of DEAE-dextran. (●,○) Control, (▲, △) 0.1 mg/ml DEAE-dextran.

1.15 mol/mol, respectively). In both cases no binding was observed at 0°C (fig.2B). In 0.1 mM griseofulvin/10% Me_2SO spirals (fig.1D) and plaques were assembled at 0 and 37°C, respectively (66 and 115% of the control) but Ac-taxol binding occurred at 37°C only and accounted for 58% of control binding.

3.3. *Effect of rhazinilam*

At 0°C, in the presence of 50 μM rhazinilam [3], tubulin assembled into spirals (61% of the control at 37°C) (fig.1E) which bound Ac-taxol (35% of the value observed with control microtubules at 37°C).

3.4. *Effect of vinblastine*

Using two-cycle microtubule proteins, Ac-taxol binding was inhibited by 32, 87 and 93–98% when the vinblastine concentration was 2, 5 and 10–200 μM, respectively. Spirals (fig.1F) were formed in 10 μM or more vinblastine [10] and 50 and 88% of the control protein (37°C) was sedimented at 37°C in 10 and 20–200 μM vinblastine, respectively. In 200 μM vinblastine, spirals were made in equal amounts at 0 and 37°C but no Ac-taxol binding was observed at either temperature. In agreement with these observations, in either the absence or presence of 50 μM vinblastine during dialysis, previously purified vinblastine spirals (1.2 mg/ml) did not bind Ac-taxol. Reciprocally, at 37°C, [^{3}H]vinblastine (5–15 μM) did not bind to microtubules made in the presence of 80 μM taxol (in 8% Me_2SO, 37°C) although it bound to control microtubule proteins.

3.5. *Effect of polycations*

When tubulin was incubated with various polycations [11–13], complex cylinders were formed at 37°C (fig.1G) while rings were assembled at 0°C (fig.1H). Complex cylinders were formed in 0.1 mg/ml DEAE-dextran, 10 μM protamine, 0.40 mg/ml polylysine or 0.15 mM spermine. The amounts of sedimentable protein were 118, 45, 51 and 99% of the control value, respectively. At 0°C, the aforementioned polycations led to values of 51, 47, 84 and 72% of the control for sedimentable material, respectively. With all these polycations no Ac-taxol binding occurred at 0°C, but clear Ac-taxol binding was observed at 37°C (fig.2C) except with spermine: in 2 μM free Ac-taxol 84.3, 51.3, 13.2 and 6.5% of the control Ac-taxol binding was observed, respectively. Moreover, in DEAE-dextran, the binding parameters were unchanged: Hill coefficient = 0.5, K_d = 0.19 μM and B_{max} = 0.75 mol/mol. Neither 0.1 mg/ml DEAE-dextran nor 0.18 μM sperminc alone was able to displace Ac-taxol at 0 or 37°C.

4. DISCUSSION

The ability of [^{3}H]Ac-taxol to bind several forms of tubulin assemblies has been investigated by equilibrium microdialysis. Ac-taxol binds to microtubules, but not to free tubulin [3]. It is usually assumed that the lattice spacing of ribbon-like structures (plaques, sheets) is the same as that found in the wall of microtubules [12]. In agreement, plaques and sheets are able to bind Ac-taxol at 0 and 37°C. As judged by electron microscopy (fig.1D–F) tubulin spirals constitute a heterogeneous class of polymers. Ac-taxol binds equally well to microtubules stabilized with rhazinilam [3] and to spirals made in the presence of rhazinilam. In contrast, Ac-taxol does not bind to spirals induced in the presence of vinblastine and griseofulvin. The interaction of Ac-taxol with complex cylinders depends on the polycation used.

A simple protective effect of some polycations could account for these observations. In the presence of DEAE-dextran, Ac-taxol binds to complex cylinders but not to rings. Similarly, in the presence of griseofulvin, a neutral microtubule poison, Ac-taxol binds to sheets but not to spirals. The interaction of Ac-taxol with tubulin does not depend simply on the charge and structure of the additional ligand. In the presence of DEAE-dextran and griseofulvin, Ac-taxol binds or does not bind to tubulin assemblies. It is not possible to suggest a simple explanation in order to account for these observations. The differential accessibility of the taxol-binding site could depend on differential protection of this site by the additional ligand. In the case of griseofulvin and DEAE-dextran, this difference in accessibility could be related to a conformational difference in the tubulin polymer, at the level of either the tubulin lattice or the tubulin molecule itself. Such a possibility has already been suggested in order to account for the absence of taxol binding to free tubulin [14]. Thus, despite several reports [2,15], the exact relationships between the taxol-binding site and most microtubule poisons remain elusive.

Acknowledgements: The advice of Drs J.C. Meunier and M. Adelman is greatly appreciated. This work has been supported by L'Association pour la Recherche sur le Cancer and La Ligue Nationale Française contre le Cancer.

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