the complete denaturation of proteins (Tanford, 1968) and therefore is much larger than expected for association of a subunit with a microtubule.

The large increase in the rate of disassembly with decreasing temperature leads to an apparent negative activation energy which is the basis for the nonlinear van't Hoff plot. The observation of a negative activation energy implies that the rate of depolymerization is not governed by a single unimolecular step as the simplest model would suggest. Rather, there must be at least two steps in the pathway, the second of which is rate limiting. The first step must be rapidly reversible relative to the rate of the second step, and must be exothermic. Moreover, the reaction in question must affect only the rate of disassembly and not the rate of assembly.

These data can be understood in terms of the role of microtubule associated proteins (MAPs). It has been shown that MAPs, associated with sites on the microtubule lattice, attenuate the rate of microtubule disassembly without affecting the rate of assembly (Murphy et al., 1977; Sloboda and Rosenbaum, 1979). Accordingly, the dissociation of MAPs should be exothermic and result in an increase in the rate of disassembly without affecting the rate of assembly. This model can quantitatively account for the temperature dependence.

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REFERENCES

Gaskin, F., C. R. Cantor, and M. L. Shelanski. 1974. *J. Mol. Biol.* **89**:737–758.
Inoué, S., and H. Sato. 1967. *J. Gen. Physiol.* **50**:259–288.
Johnson, K. A., and G. G. Borisy. 1977. *J. Mol. Biol.* **117**:1–32.
Johnson, K. A., and G. G. Borisy. 1979. *J. Mol. Biol.* **133**:199–216.
Lee, J. C., and S. N. Timasheff. 1977. *Biochemistry* **16**:1754–1764.
Murphy, D. B., K. A. Johnson, and G. G. Borisy. 1977. *J. Mol. Biol.* **117**:33–52.
Oosawa, F., and M. Kasai. 1962. *J. Mol. Biol.* **4**:10–21.
Sloboda, R. D., and J. L. Rosenbaum. 1979. *Biochemistry* **18**:48–54.
Sutherland, J. W. H. 1977. *Proc. Natl. Acad. Sci. U.S.A.* **74**:2002–2006.
Tanford, C. 1968. *Adv. Prot. Chem.* **23**:121.

A KINETIC MODEL FOR COLCHICINE INHIBITION OF MICROTUBULE ASSEMBLY

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Colchicine is a potent drug used to probe microtubule dependent processes (1, 2). We have recently shown that substoichiometric concentrations of colchicine-tubulin complex (CD), a 1:1 tight binding complex of drug with tubulin, copolymerizes with tubulin to form microtubule copolymers (3). The affinity of the microtubule ends for tubulin decreased as the CD mole fraction in the microtubule increased. Mole fraction ratios as small as 1 CD to ~50–100 tubulins in the copolymers were accompanied by a significant change in binding affinities and polymerization rates (3). We have further extended our investigation of the CD-tubulin copolymerization reaction. A kinetic model was derived which relates the composition of the microtubule copolymer to the composition of the reaction mixture. This model allowed a predictive correlation to be made between copolymer composition and the extent of assembly inhibition. The results of our findings are briefly presented below.

THE KINETIC MODEL

We perceive copolymerization to be a reaction which transfers subunit mass from the solution phase, where the CD mole fraction is X, to the microtubule phase, where the CD mole fraction is Y (Fig. 1). The CDs are presumed to be randomly incorporated into the microtubule lattice. The composition of the microtubule copolymer depends on the composition of the reaction mixture, and the affinity of the microtubule ends for tubulin and CD. X will differ from Y if the affinity of the microtubule ends for tubulin and CD differ. Generally, $Y < X$, i.e., the microtubule phase is observed to be enriched in tubulin relative to the solution phase. In principal both X and Y can change with time as assembly proceeds. However, our experimental data suggests that Y is essentially constant during the assembly reaction (H. Sternlicht, I. Ringel, and J. Szasz, unpublished observations). A rate equation for assembly was derived, and expressed as the difference between the rates of polymerization and depolymerization. In this model, polymerization was approximated as a "bimolecular" reaction involving microtubule ends and subunit protein (i.e., CD and tubulin). Thus, the rate of polymerization was set equal to the product of three terms: k_+, the concentration of microtubule ends, and the concentration of subunits in the solution phase. Depolymerization was approximated as a unimolecular reaction which proceeds at a rate equal to the product of k_- and the concentration of microtubule ends. k_+ was taken as the stoichiometric average of k_+^T and k_+^{CD}, the apparent association rate constants for tubulin and CD addition to the microtubule ends, while k_- was taken as the stoichiometric average of k_-^T and k_-^{CD},the apparent dissociation rate constants for tubulin and CD. Stoichiometric averaging corresponds to probability averaging for a random copolymerization reaction. Our data suggests (H. Sternlicht, et al., unpublished observations) that k_+^T is is similar to k_+^{CD}. We integrated the assembly rate equation assuming $k_+^T = k_+^{CD}$, and obtained an expression (Fig. 2) for Y^s, the CD mole fraction in he microtubule phase at steady state. If the microtubule ends had zero affinity for CD, Y^s would be zero. If the microtubule ends had identical affinities for tubulin and CD, Y^s would equal CD_{total}/T_{total}, the CD mole fraction in the reaction mixture. The actual situation is intermediate between these extremes.

RESULTS

Bovine tubulin was isolated by the Gaskin et al (4) procedure and was spontaneously assembled at 37°C, pH 6.8, in the presence of increasing concentrations of CD(0–5 μM). Assembly was monitored by turbidity changes (4). Samples were removed at steady state and centrifuged. X^s, the CD mole fraction in the supernate, and Y^s, the CD mole fraction in the

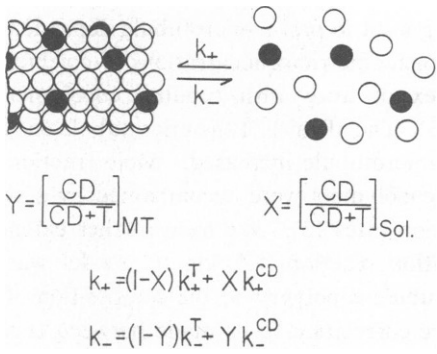

FIGURE 1

$$Y_s = \frac{[CD]_{total}}{[T]_{total} + [\underbrace{(k_-^{CD}/k_+^{CD})}_{affinity_{CD}^{-1}} - \underbrace{(k_-^{T}/k_+^{T})}_{affinity_{T}^{-1}}]}$$

$$Y_s = \frac{[CD]_{total}}{[T]_{total} + \left(\frac{affinity_T}{affinity_{CD}} - 1\right)(affinity_T^{-1})}$$

FIGURE 2

pellet, were determined. The ratio X^s/Y^s was set equal to $affinity_T/affinity_{CD}$, and had values of ~6 ± 2. Furthermore, the assembly reaction was characterized by an abrupt transition from a state of no assembled microtubules to a state of assembled microtubules when total active tubulin concentrations equalled certain minimum concentrations. These minimum, or critical concentrations, depended on CD, and increased with increasing CD_{total}. In accord with the studies of Oosawa (5) and our previous study of CD inhibition (3), we interpreted these critical concentrations as being equal to the tubulin dissociation constants, i.e., $affinity_T^{-1}$. We estimated that $affinity_T^{-1}$ could be approximated well by the expression:

$$affinity_T^{-1} \simeq 1.7 + \frac{4.5\,[CD]_{total}}{1 + 0.5\,[CD]_{total}}\ (\mu M)$$

($CD_{total} \lesssim 5\mu M$). This expression for $affinity_t^{-1}$ and the estimation of ~6 for $affinity_T/affinity_{CD}$ were substituted into our previous expression for Y^s, and the CD mole fraction in the microtubule phase were calculated as a function of CD_{total} and T_{total}. The predicted and measured values were in excellent agreement (Fig. 3). We noted further that, whereas the Y^s values were not very sensitive to total tubulin concentrations the extent to which CD inhibited assembly did depend strongly on tubulin concentration (Fig. 3).

We were also able to model successfully various other aspects of the CD inhibition process

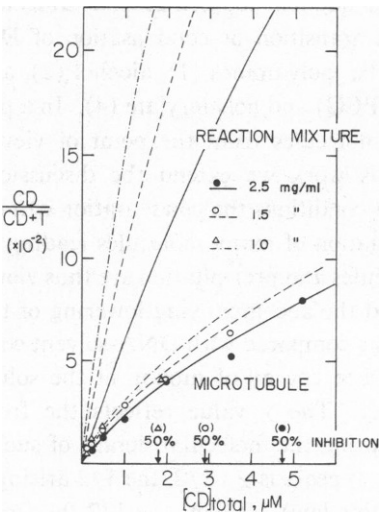

FIGURE 3

using our derived equation for Y^s, and noting that assembly occurs abruptly when total active tubulin concentration equals affinity$_T^{-1}$ (H. Sternlicht et al., unpublished observations). This success lends further support to the validity of our Y^s equation.

CONCLUSION

Our analysis resulted in an expression for the composition of microtubule copolymers assembled in the presence of tubulin and CD. The affinity of the micrrotubule ends for both tubulin and CD correlates with the CD mole fraction in the microtubule phase, and the affinities decrease as CD_{total} increases. The molecular basis for these effects remains to be established.

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REFERENCES

1. Olmsted, J., and G. Borisy. 1973. *Biochemistry*. **12**:4242.
2. Margolis, R., L. Wilson, and B. Kiefer. 1978. *Nature (Lond.)*. **272**:450.
3. Sternlicht, H., and I. Ringel. 1979. *J. Biol. Chem.* **254**:10540.
4. Gaskin, F., C. Cantor, and M. Shelanski. 1974. *J. Mol. Biol.* **89**:737.
5. Oosawa, F., and M. Kasai. 1962. *J. Mol. Biol.* **4**:10.

DNA CONDENSATION AND HOW IT RELATES TO PHASE EQUILIBRIUM IN SOLUTION

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High molecular weight DNA is a randomly coiled polymer usually found to be highly expanded in solution due to its low degree of flexibility. It has been shown, however, that DNA is able to undergo a sudden conformational transition into a highly compacted conformation. The collapse transition or condensation of DNA has been observed to be caused by a number of agents: polyamines (1), alcohol (2), acid (3), and polymer solutions such as polyethylene glycol (PEG) and polyacrylate (4). In a previous paper (5) we discussed the condensation of single molecules from the point of view of Flory's classical polymer solution theory (6). In this work we extend the discussion to take account of higher concentrations. Under these conditions the condensation can occur either as a unimolecular phenomenon or as an aggregation of many molecules leading to precipitation of the DNA. Condensation of single molecules and precipitation are thus viewed as two aspects of the effect of reduced solvent power and the accompanying lowering of the free energy of DNA-DNA and solvent-solvent contacts as compared with DNA-solvent contacts.

In the Flory theory the free energy of mixing of the solute (DNA) and the solvent is governed by a parameter χ. The χ value reflects the free energy of interaction of a solvent-segment contact, ignoring the molecular details of such contacts. In the free energy expressions the quantity that appears is $\chi - 1/2$, the $1/2$ arising from the usual expression for the entropy of mixing (Raoult's law). When $\chi < 1/2$ the free energy of mixing is negative