4. Engelborghs, Y., L. De Maeyer, and N. Overbergh. 1977. A kinetic analysis of the assembly of microtubules in vitro. *FEBS Lett.* **80**:81.
5. Johnson, K. A., and G. G. Borisy. 1979. Thermodynamic analysis of microtubule self-assembly in vitro. *J. Mol. Biol.* **133**:199.
6. Carlier, M.-F., and D. Pantaloni. 1978. Kinetic analysis of cooperativity in tubulin polymerisation in the presence of guanosine di-or triphosphate nucleotides. *Biochemistry.* **17**:1908.
7. Marcum, J. M., and G. G. Borisy. 1978. Sedimentation velocity analysis of the effect of hydrostatic pressure on the 30S microtubule protein oligomer. *J. Biol. Chem.* **253**:2852.
8. Weisenberg, R. C. 1974. The role of ring aggregates and other structures in the assembly of microtubules. *J. Supramol. Struct.* **2**:451.

THERMODYNAMICS OF MICROTUBULE ASSEMBLY

Kenneth A. Johnson, *Biochemistry Program, Pennsylvania State University, University Park, Pennsylvania 16802 U.S.A.*

The temperature dependence of microtubule assembly has been the subject of much controversy. Curved van't Hoff plots obtained from birefringence data on the mitotic spindle led Inoué and Sato (1967) to conclude that the condensation mechanism (Oosawa and Kasai, 1962) did not accurately describe the assembly equilibrium in vivo. Subsequent work in vitro has established the validity of the condensation mechanism, but nonlinearity of the van't Hoff plots has been interpreted by a number of different models: (*a*) a contribution of microtubule nucleating species to the apparent critical concentration (Sutherland, 1977), (*b*) a gross conformational change in the microtubule lattice (Gaskin et al., 1974), and (*c*) a large negative heat capacity change (Lee and Timasheff, 1977). In this paper, data will be described which show that the curvature of the van't Hoff plot is solely a function of the microtubule disassembly reaction (see Johnson and Borisy, 1979). The significance of this observation in terms of the mechanisms of assembly and disassembly will be discussed.

The equilibrium for microtubule assembly above 25°C is governed by reactions which can

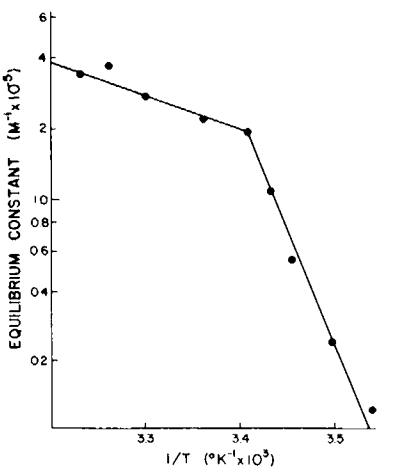

Figure 1 Temperature dependence of the equilibrium constant. Reproduced by permission from Johnson and Borisy (1979).

be described as:

$$M_n + S \underset{k_{-1}}{\overset{k_2}{\rightleftharpoons}} M_{n+1},$$

where M_n is a microtubule polymer, n subunits long and S is a 6S tubulin subunit which adds to the end of a polymer (Johnson and Borisy, 1977). The equilibrium constant is defined by $K_a = k_2/k_{-1}$. In this study, the apparent rate constants k_2 and k_{-1} were measured as a function of temperature by examining the rate of assembly in samples of 6S tubulin nucleated by a fixed concentration of microtubule fragments (Johnson and Borisy, 1977).

The temperature dependence of the equilibrium constant is shown in Fig. 1. The two straight lines define limiting slopes at high and low temperature giving $\Delta H° = 8$ kcal/mol and $\Delta S° = 50$ e.u. above 20°C and $\Delta H° = 46$ kcal/mol and $\Delta S° = 180$ e.u. below 20°C.

Measurements of the apparent rate constants for assembly and disassembly as a function of temperature are shown in Fig. 2. The rate constant for assembly is a simple monotonic function of temperature (Fig. 2 *a*) with $\Delta H°‡ = 8$ kcal/mol. However, the apparent rate constant for disassembly is biphasic with $\Delta H°‡ = 3$ kcal/mol above 20°C and $\Delta H°‡ = -34$ kcal/mol below 20°C (Fig. 2 *b*). Thus, the curvature of the van't Hoff plot is due solely to a reaction in the disassembly pathway which does not affect the rate of assembly.

These data rule out previous proposals to explain the curvature of the van't Hoff plot: (*a*) a shift due to the formation of more oligomers at lower temperature would reduce the effective concentration of 6S tubulin and would principally affect k_2 and not k_{-1}; (*b*) a conformational change in the tubule as proposed (Gaskin et al., 1974) would have affected both k_2 and k_{-1} and in particular is not consistent with the negative activation energy for disassembly (see below); and (*c*) a change in heat capacity could be interpreted in terms of the melting out of water "icebergs" (Tanford, 1968) during assembly and such changes might be expected to affect the assembly reaction at least as much as the disassembly reaction. Moreover, the magnitude of the heat capacity change calculated by Lee and Timasheff is comparable to that observed for

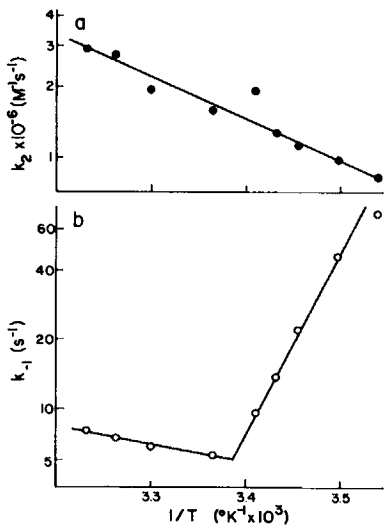

Figure 2 Temperature dependence of the rate constants. Reproduced by permission from Johnson and Borisy (1979).

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.

Received for publication 17 December 1979.

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

H. Sternlicht, I. Ringel, and J. Szasz, *Department of Pharmacology, Case Western Reserve University, Cleveland, Ohio 44106 U.S.A.*

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.