Step 3: Formation of a Network

Gelation corresponds to the formation of a network of fibers extending throughout the solution. The results of light scattering and clot rigidity measurements support the proposal that gelation of fibrin is a necessary outcome of the (irreversible) assembly of fibers from very long protofibrils. That is, interfiber connections (network branchpoints) will inevitably form in a solution of growing protofibrils or fibers that are forming lateral associations at multiple random points along their length. Fibrin structure at the branchpoints is then proposed to be the same as elsewhere, except that on one side of the branchpoint a portion of the fiber bends away from the remainder (1).

Electron microscope studies of samples obtained at times beyond the light scattering lag time revealed fibers with the cross-striations characteristic of mature fibrin (3). Interfiber connections were found even at early times of assembly.

At times when the light scattering changes are nearly complete, not only are dense fibers observed in the electron microscope, but also long structures that appear to consist of thin fibers loosely arranged in sheets. These loose sheets or fibers also showed the cross-striations characteristic of the dense fibers. Both loose and dense fibers were also observed in samples which included high concentrations of factor XIIIa, which introduces covalent crosslinks into fibrin (rendering the network insoluble even under denaturing conditions). The transformation of loosely to densely packed fibers could contribute to the slow increase in clot rigidity which follows gelation. Alternatively, the continued association of fully formed fibers, which increases the number of interfiber corrections, may be the most important mechanism responsible for the increase in rigidity of the gel.

Received for publication 14 December 1979.

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A PRESSURE RELAXATION STUDY OF TUBULIN OLIGOMER FORMATION

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When microtubules are cooled to 4°C in vitro, ringlike oligomers are formed. These oligomers consist of ~26 tubulin dimers and of the so called microtubule-associated-proteins (MAPs) (1, 2). The role of these rings is often questioned as they are never observed in vivo.

The assembly of microtubules has been studied in terms of a nucleation and a propagation process. The propagation step has been analysed quantitatively (3–5). About the process of nucleation, however, not very much is known. Quantitative information can be deduced from the apparent stoichiometry coefficient of nucleation. This can be obtained from the linear rate, for which we deduced an equation as follows: Integration of equation 1, leads to a rate

equation for the disappearance of the tubulin dimers 2:

$$d^2 \ln c_1/dt^2 = k_+ k_n c_1^n \tag{1}$$

$$dc_1/dt = -c_1\sqrt{2k_+k_n(c_0^n - c_1^n)/n}. \tag{2}$$

This equation clearly shows a maximum, which corresponds to an inflection point in the integrated equation of c_1 vs. t. Due to the gradual change of the slope around the inflection point, a linear region of turbidity increase is observed. Equating $(d^2c_1/dt^2)_{infl} = 0$, allows rate Eq. 2 to be expressed in terms of the total tubulin concentration c_0:

$$(dc_1/dt)_{max} = -\sqrt{(2/(n+2))^{1+2/n} \cdot k_+k_n \cdot c_0^{n+2}}. \tag{3}$$

From this it also follows that the rate constant of propagation $k_{obs} = k_+m$ increases proportionally with $c_0^{n/2}$. For tubulin in equilibrium with MAPs, k_{obs} increases linearly with total concentration ($n = 2$) (4), while PC-tubulin in glycerol shows a very high cooperativity (~10) (6). The major difference between the two solutions is the presence of factors which leads to ring formation in the former. The large difference in cooperativity can be explained if it is assumed that the nucleus can form directly from the rings without complete dissociation.

The fact that rings are never seen when polymerization starts from pure tubulin dimers points to a critical role for intermediates. In previous experiments (4) we could show that completely dissociated rings do not contribute to nucleation but do contribute to the growth of nuclei. We therefore conclude that rings are the product of a side path of MT assembly, but are preceded by common intermediates. This can be understood on the basis of competition between longitudinal and lateral (endothermic) interactions. Here we report preliminary results on the kinetics of ring formation, studied at pH 6.5, I = 0.1, 1 mM GDP. In these conditions only one type of ring is present (2) as confirmed by analytical ultracentrifugation. MT assembly is prevented at all temperatures. Temperature jumps from 3° to 10°, 21°, and 35°C show a decreased turbidity at higher temperatures (Fig. 1). This indicates that ring formation is exothermic. For the study of the kinetics the pressure jump technique proved to be invaluable. It provides a rapid perturbation as well as long term stability which were both essential. The system is also very pressure sensitive (7). When light scattering is followed after a P-jump from 200 atm, three relaxation processes are found. A first step occurs in the millisecond range and is practically concentration independent. Two other steps are found in the seconds range. These are concentration dependent (Fig. 2).

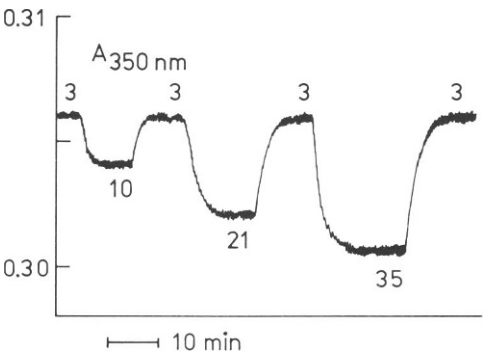

Figure 1 The effect of temperature on the turbidity (350 nm) of depolymerised microtubules. The numbers indicate the temperature (°C) at the plateau levels.

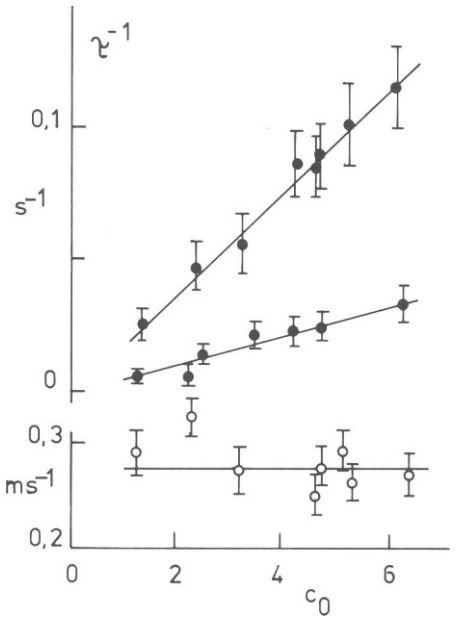

Figure 2 Reciprocal relaxation time as a function of total tubulin concentration (c_0 in mg/ml). The reaction is initiated by a pressure jump from 200 to 1 atm. The dead time of the instrument is 0.2 ms. Reassociation is followed at 0°C by measuring light scattering at 90° using light of 365 nm (Hg-Xe lamp 200 W-Hanovia).

We assume that the concentration independent step is due to an isomerisation equilibrium characterized by a change in light scattering intensity. This assumption is based on considerations about the particle size factor, which reveal that the dissociation of a double ring into a long rod is accompanied by a substantial decrease of the scattering intensity. The subsequent slower steps are probably due to the formation of new oligomers. Assuming that warming up has the same effect as increasing pressure, these results point in the same direction as Weisenberg's observations concerning the decreasing sedimentation coefficient of the rings upon warming up in the analytical centrifugation (8).

The concentration dependence of the overall equilibrium is much less pronounced than predicted by an all or none equilibrium. This means that a substantial number of intermediates are present and/or considerable microheterogeneity of association constants exists. The latter is not surprising in view of the mixture of MAPs present. Therefore volume changes cannot be calculated from the relaxation amplitudes.

Y. Engelborghs is Bevoegdverklaard Navorser of the N.F.W.O., G. Ide is bursar of the I.W.O.N.L.. The authors thank the university for financial support (project OT/VII/14 and OT/IV/17).

Received for publication 19 December 1979.

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THERMODYNAMICS OF MICROTUBULE ASSEMBLY

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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

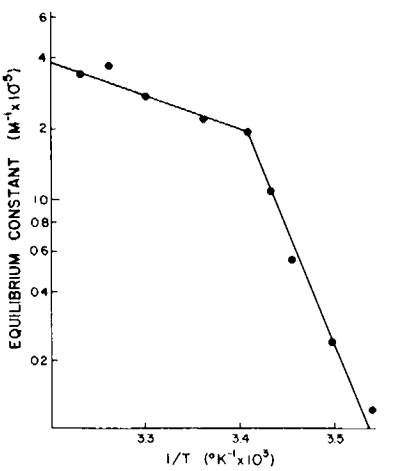

Figure 1 Temperature dependence of the equilibrium constant. Reproduced by permission from Johnson and Borisy (1979).