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DESTABILIZING EFFECT OF CALCIUM ON MICROTUBULES IN VITRO

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The extent of disassembly of microtubules in duced by calcium has been determined at various temperatures. The effect of calcium increases with decreasing temperature. Length distributions of microtubules were measured before and after partial disassembly under the influence of calcium and in steady state in the presence of calcium. Partial disassembly due to calcium resulted in shorter microtubules indicating endwise disassembly. Calcium in steady state induced an increase of length implying that the influence of microtubule associated proteins (MAPs) is suppressed and that dynamic instability is pronounced.

Key Words: Microtubules, calcium dynamic instability.

INTRODUCTION

The multiple roles* of microtubules in the cell are truly amazing. As one of the major components of the cytoskeleton microtubules are involved in cell movement, intracellular transport and maintaining cell morphology. As spindle fibres in mitosis, microtubules are important for chromosome movement. Rapid assembly and disassembly, depending on the environment, enable microtubules to change their network in the cell. The wals of microtubules (since they are cylinders) are formed of tubulin, a dimer composed of two polypeptides, each having a molecular weight of 50000 (Amos, 1979). Within the microtubule, wall tubulin is arranged in a lattice. A few low and high molecular weight proteins are also associated with the microtubule wall. They are known as microtubule associated proteins (MAPs). Microtubules assemble by adding tubulin bound to guanosine triphosphate (GTP). Following assembly GTP hydrolizes into guanosinediphosphate (GDP). Tubulin GTP forms stable ends, but the tubulin GDP core is unstable (Engelborghs, 1989). As a consequence, when the tubulin GDP core is not protected by tubulin GTP, microtubules disassemble rapidly. This alternation between the growing and shrinking phase is called dynamic instability (Mitchison and Kirshner, 1984a,b). Assembly and disassembly result in rapid microtubule turnover, with a half life of 30s and less in animal cells (Salmon et al., 1984). One of the principal purposes in microtubule research is to understand

the regulation of their organisation in the cell and special attention has been paid to inhibitors and promoters of assembly. Calcium is a strong inhibitor of microtubule assembly. Also, calcium ions change assembly and disassembly kinetics; they decrease the net rate of assembly (Gal et al., 1984) and increase; dramatically the disassembly rate (Gal et al., 1988).

In this work, we investigate further the effect of calcium on microtubule stability. The calcium ion is of special interest since it may regulate microtubule network and turnover in the cell through its effect on the kinetic characteristics of both assembly and disassembly.

MATERIALS AND METHODS

Microtubule protein (tubulin + MAPs) was prepared from bovine brain by cyclization in the presence of glycerol (Shelansky et al., 1973). Protein was determined spectrophotometrically using an extinction coefficient of 1.11 $mlmg^{-1}$ cm^{-1}(278nm). The assembly buffer pH 6.6 contained 0.1 M Mes, 1 mM GTP. Assembly was induced by a temperature jupm from 4^0C to an elevated temperature and it was followed by turbidity measurements at 350 nm. Samples for electron microscopy were negatively stained with uranyl acetate and examined in a Philips 300 electron microscope. Length distributions of microtubules were determined from electron micrographs using a semi automatic picture analyzer (Leitz ASM 68K).

RESULTS AND DISCUSSION

We wished to examine destabilization of microtubules by Ca^{2+}. We studied the decrease of the extent of assembly and properties of microtubules through redistribution of their length.

Decrease of assembly A typical assembly, followed by turbidity measurements is presented in Figure 1. The extent of assembly is the difference between the turbidity of the plateau and the lowest turbidity. When Ca^{2+}, or any other inhibitor, is added to the system in steady state (plateau), microtubules disassemble until a new steady state (new plateau) is reached and the new extent of assembly can be determined (Figure 1). Alternatively, the extent of assembly in the presence of Ca^{2+} can be measured if the assembly proceeds in the buffer containing Ca^{2+}, but since in that case assembly is slow (Figure 1b) the former method is more suitable. We have determined the inhibitory effect of Ca^{2+} (mostly by addition to already formed microtubules) at various temperatures. These results are given in Figure 2. It can be seen that effect is dependent on temperature. Namely, at a lower temperature the destabilization is stronger.

Effect on microtubule length distribution A consequence of microtubule dynamic instability is the change in length distribution (Mitchison and Kirschner, 1984a,b). Since an individual microtubule switches from slow growing to fast shortening and vice versa, some microtubules disassemble completely (while

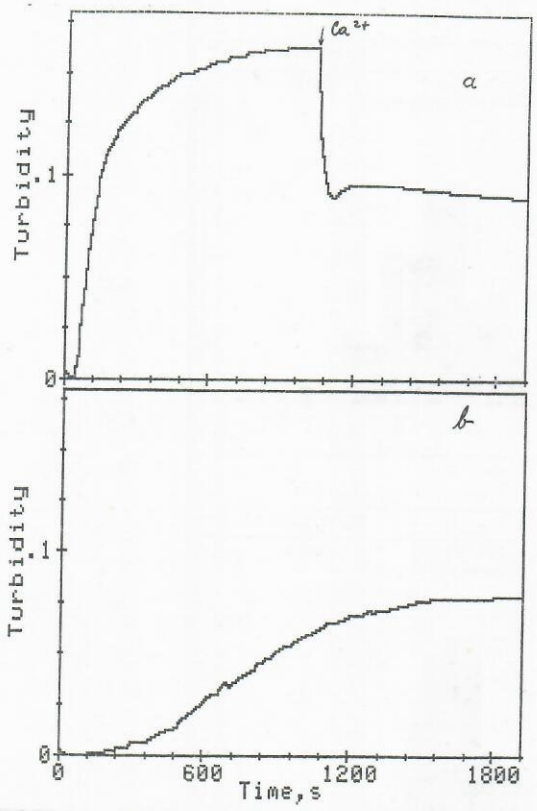

Figure 1. Effect of calcium on microtubule assembly. Microtubule protein, 10 mM, was assembled in 0.1 M Mes, 1 mM GTP, pH 6.6 at 37 °C. a) Calcium (0.7 mM final concentration) was added in steady state. b) Calcium 0.7 mM was added before assembly was induced.

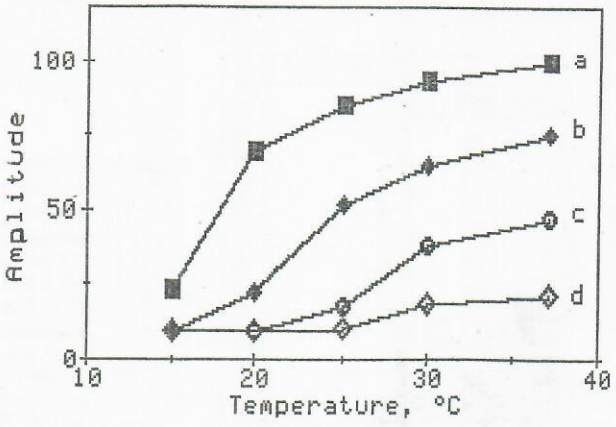

Figure 2. Dependence of microtubule disassembly on calcium concentration and tempereture. Calcium concetration is a) 0, b) 0.65 mM, c) 1 mM and d) 1.45 mM.

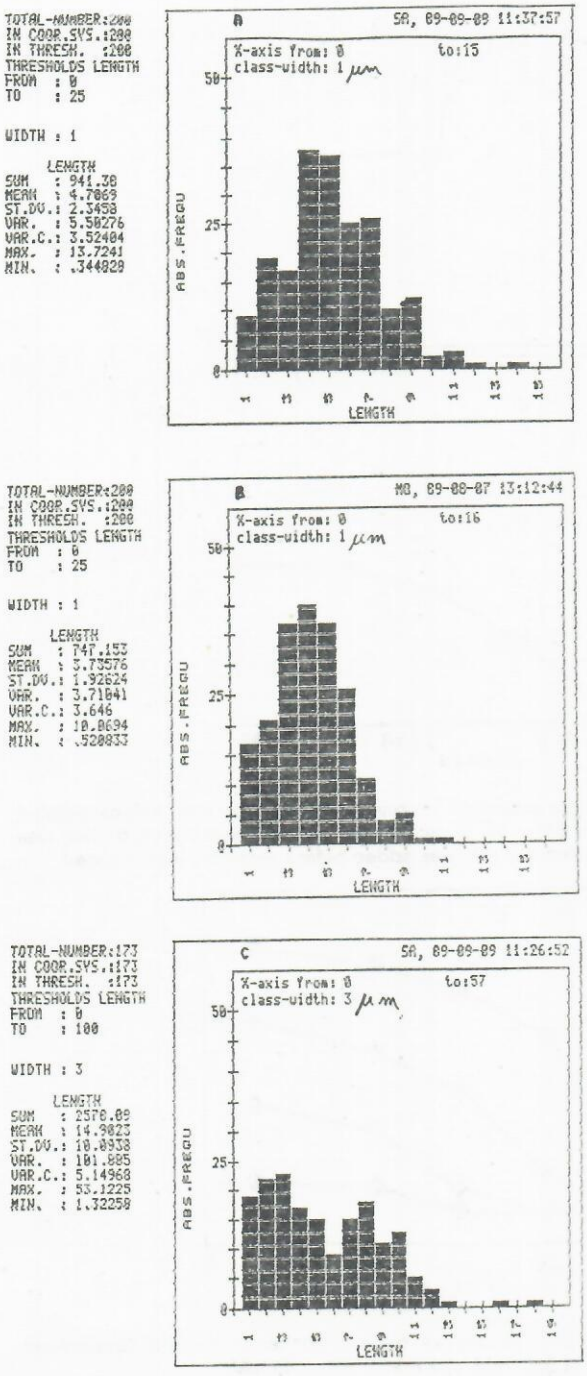

Figure 3. Effect of calcium on microtubule length distribution. Microtubule protein was assembled in 0.1 M Mes, 1 mM GTP, pH 6.6 at 37 °C and when the stady state was reached calcium (0.9 mM final concentration) was added. Histograms of microtubule lengths A) before, B) 3 min and c) 30 min after addition of calcium.

shortening). The liberated tubulin is incorporated into long microtubules for which the probability of complete disassembly is low. Therefore, with time, short microtubules disappear and long ones become longer, with an increase of mean length and a decrease of microtubule number. It should be noted that during these changes in length the concentration of tubulin incorporated into microtubules remains constant, maintaining the system in a steady state, and therefore the turbidity does not change.

In steady state dynamic instability is suppressed by MAPs in the system without Ca^{2+} (Horio and Hotani, 1986) which means that the length distribution does not change with time. The disassembly by Ca^{2+} is endwise (Gal et al., 1988) i. e.partial disassembly should result in a shortening of the microtubules. Therefore, in an experiment where Ca^{+} is added to microtubules in steady state leading to partial disassembly, a change of length distribution toward shorter ones can be expected. In a new steady state in the presence Ca^{2+}, if there is dynamic instability (if the influence of MAPs is suppressed by Ca^{2+}) microtubules will be longer with time, otherwise their lengths will remain constant.

We examined length distributions before, and immediately after partial disassembly by Ca^{2+} as well as in the steady state in the presence of Ca^{2+}. As an example length distributions in steady state without Ca^{2+}, 3 min and 30 min after addition of Ca^{2+} (final concentration 0.9 mM), are presented in Figure 3. The partial disassembly resulted in a change of mean length from 4.73 μm to 3.14 μm, as expected. The dramatic increase of lengths, 14.17μm mean length, 30 min after addition of Ca^{2+} is strong evidence of a very pronounced dynamic instability. This type of experiment was repeated, and the lengths of microtubules always increased in steady state in the presence of Ca^{2+} (data to be published).

In this work we found that the inhibitory effect of Ca^{2+} on microtubules is stronger at lower temperatures and that Ca^{2+} induces dynamic instability in the presence of MAPs. We suggest that the inhibitory effect and enhanced dynamic instability are both consequences of destabilization of the tubulin lattice. As previously suggested by Gal et al. (1988) binding of Ca^{2+} to weak binding sites on tubulin might affect interactions within the microtubule wall resulting in enhanced disassembly rates. The enhanced disassembly rates, with unchanged assembly rates, might be sufficient to decrease the extent of assembly (in steady state) and to suppress the stabilizing influence of MAPs resulting in a high dynamic instability. In is challenging to contemplate the possible biological implications (Bayley, 1993). Does the cell use the enhanced disassembly rates, induced by Ca^{2+} and other metal ions, for rapid reorganization of microtubules arrays? Further investigations on the involvement of metal ions in microtubule activities in the cell should answer this question.

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DESTABILIZACIONI EFEKAT KALCIJUMA NA MIKROTUBULE IN VITRO

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SADRŽAJ

Stepen depolimerizacije mikrotubula, izazvane kalcijumom, je određen na različitim temperaturama. Efekat kalcijuma se povećava sa smanjenjem temperature. Merene su raspodele dužina mikrotubula pre i posle njihove parcijalne depolimerizacije izazvane kalcijumom, kao i u stacionarnom stanju u prisustvu kalcijuma. Parcijalna depolimerizacija kalcijumom izaziva skraćenje mikrotubula što je u saglasnosti sa depolimerizacijom sa krajeva. Kalcijum u stacionarnom stanju izaziva povećanje dužina mikrotubula što ukazuje da je efekat MAPa potisnut i da je dinamička nestabilnost izražena.