Vol. 84, No. 3, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
October 16, 1978 Pages 721-726

INHIBITION OF MICROTUBULE FORMATION BY DNA

Wolfram Vater, Hubert Müller and Eberhard Unger

Department of Experimental Cell Research,
Central Institute of Microbiology and Experimental Therapy,
Academy of Science of GDR, GDR 69 Jena

Received July 18, 1978

SUMMARY. In vitro assembly of porcine brain microtubular protein to microtubules is affected by calf thymus DNA. Dependent on mass ratio of DNA/MTP microtubule formation is partly inhibited or blocked. Microtubules formed in presence of DNA are not to be distinguished from those assembled without DNA by electron microscopy. Addition of DNA to microtubules in assembly buffer causes their disassembly.

BRYAN et al (1) have found RNA and other polyanions inhibit in vitro assembly of beef brain microtubular protein. It is proven by turbidity measurements that this action is due in part to elimination of factors favouring microtubule formation. It might be expected that also DNA is able to cause an inhibition of microtubular protein (MTP) assembly. Experimental results reveal this expectation.

MATERIAL AND METHODS

Porcine brain MTP was isolated and purified by two or three cycles of disassembly/assembly using a procedure principally described by SHELANSKI et al (2). The first MTP assembly was carried out in buffer I: 10 mM MES (2(N-morpholino)ethanesulfonic acid), 40 mM KCl, 1 mM $MgSO_4$, 2 mM EGTA (ethylenebis(oxyethylenenitrilo)tetraacetate), 1 mM ATP, 4 M glycerol, pH 6.4 and the second and third one in buffer II: 50 mM MES, 1 mM $MgSO_4$, 2 mM EGTA, 1 mM GTP, 4 M glycerol, pH 6.4. Isolated microtubules were stored at -130 °C.

1. Electron microscope assay of assembly inhibition

Isolated microtubules were resuspended in buffer II without glycerol, for 30 min at 0 °C, and the resulting suspension

0006-291X/78/0843-0721$01.00/0

centrifuged in a VAC 601 centrifuge (VEB Zentrifugenbau Engelsdorf) for 60 min at 0 °C and 100 000g. Supernatant was mixed with calf thymus DNA (Isolation procedure see (3), protein content 0.21 %, DNA was kindly provided by E. SARFERT, Dept. Biochem., ZIMET, Jena) at 0 °C (DNA-concentration: 73 µg/ml, MTP concentration: 0.015-4.2 mg/ml) and incubated 30 min at 37 °C. To observe microtubules the mixtures were placed on Formvar carbon-coated copper grids for 30 sec and blotted with filterpaper. Grids were negatively stained with 2 % uranyl acetate (3 min) and observed with a SIEMENS Elmiskop I electron microscope.

For detection microtubules 6 grids and 10 squares per grid and per mass ratio DNA/MTP were investigated.

2. Turbidity assay of assembly inhibition

MTP solutions were prepared as described in 1, using buffer II without glycerol and GTP. Free GTP and glycerol was separated by gel filtration with Sephadex G-25.

MTP assembly was started by addition of GTP (final concentration 1 mM) at 37 °C immediately after mixing MTP with DNA and monitored using the increase in turbidity at 368 nm (SPECOL with EK 5, Standard Compensation Recorder G1B1, VEB Carl Zeiss Jena) by adapting the method of GASKIN et al (4). After 30 min incubation microtubule formation was proven by electron microscopy.

3. Turbidity assay of microtubule disassembly

MTP assembly without DNA was performed for 30 min as described in 2. Then DNA was added to a final concentration of 73 µg/ml and incubation continued for 10 min. Samples for electron microscopy were taken immediately before DNA addition and 10 min after incubation with DNA and prepared as described in 1.

Protein concentrations were determined by the method of HARTREE (5) using bovine serum albumin as a standard.

(Terminology: Tubulin-main component of microtubules; microtubule associated proteins (MAPs)-proteins copurify with tubulin when microtubules are isolated by in vitro depolymerization/polymerization procedures; microtubular protein-tubulin and MAPs together).

RESULTS AND DISCUSSION

Twice reassembled MTP in buffer II without glycerol forms microtubules in all investigated concentrations (0.015-4.2 mg/ml). In the lowest concentrations the microtubules are very seldom. Assembly of MTP in MTP/DNA mixtures, however, reveals results partly different (see Table 1).

The number of microtubules diminishes with increasing ratio DNA/MTP. Above a ratio of 0.5 no microtubules can be observed.

Table 1

Electron microscopic assay of microtubule formation, MTP assembly in presence of calf thymus DNA, DNA-concentration: 73 µg/ml, MTP twice polymerized/depolymerized

MTP-concentration mg/ml	DNA/MTP mass ratio	results
0.015 - 0.15	4.9 - 0.5	no microtubules
0.75	0.10	microtubules only in some preparations
1.50 - 4.2	0.05 - 0.02	microtubules in all preparations

The inhibition of MTP assembly by DNA is confirmed by turbidity measurements (see Fig. 1).

Relatively to the control without DNA the half maximal value of turbidity is reached at an DNA-concentration of 8 µg/ml (15 min values of turbidity) corresponding to a mass ratio DNA/MTP of 0.006. A practically complete inhibition of microtubule formation is produced at a DNA/MTP ratio of about 0.02. This value is markedly lower than that received by MTP twice assembled/deassembled (Table 1). This may be due to a higher purity of MTP three times polymerized/depolymerized. Microtubules formed in presence of DNA are electron microscopically not to distinguish from those assembled without DNA.

In many articles it is shown that basic proteins favour the assembly of tubulin to microtubules or that they are necessary factors for the assembly under special given conditions (6)-(13). On the contrary polyanions, e.g. RNA, inhibit microtubule formation (1) via complexation of positively charged assembly promoting compounds and lowering their actual concentrations in

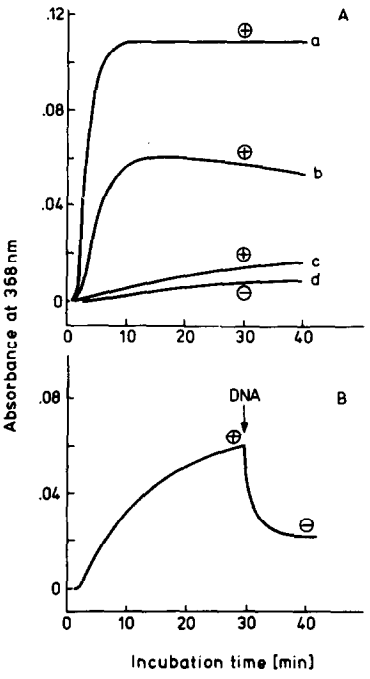

Figure 1. Effect of DNA on MT assembly. (A) Assembly in the presence of DNA, MTP concentration: 1.25 mg/ml, DNA concentration: a, 0 ug/ml; b, 7.3 ug/ml; c, 14.6 ug/ml; d, 21.9 ug/ml. (B) Disassembly of MT by DNA, MTP concentration: 0.57 mg/ml. The arrow indicates the addition of DNA to a final concentration of 73 ug/ml. Electron microscopical control for microtubules: ⊕ MT present, ⊖ MT absent.

this way. It might be possible that such a mechanism is acting also in the case of DNA and is inhibiting partly or completely microtubule formation. The MAPs are positively charged and can be trapped by DNA. On the other hand a binding of tubulin to DNA should be considered. It has been suggested (though disputed (14)) that tubulin is a main component of nonhistone chromatin proteins (15,16). Formation of DNA-tubulin and/or DNA-MAP complexes would lower the actual concentrations of tubulin resp. MAPs. In consequence the conditions for microtubule formation are becoming more unfavourable. The activities of tubulin and MAPs necessary under special conditions for microtubule formation can become too small to fulfill the conditions for

microtubule nucleation and/or elongation. This is in accordance with results recently reported by WICHE et al (17) which say that MAPs and tubulin in presence of MAPs bind to purified DNA and especially to satellite DNA sequences.

The observation that DNA causes microtubule disassembly does not need a new inhibition mechanism for interpretation beside the two ones mentioned. In vitro under steady-state conditions microtubules are in an assembly/disassembly "equilibrium" (18).

At present it is not obvious whether DNA (and also RNA (1)) inhibits MTP assembly also in vivo. It must be taken into account, however, that microtubular organizing centers (MTOC) like kinetochores, centriolar satellites or nucleus associated organelles/spindle pole bodies in fungi contain RNA (19)-(21). DNA is found in/or in near association with kinetochores and spindle pole bodies (22,23). For centrioles the results are controverse (discussion see (24)). These nucleic acids seem to be substantial components of MTOC and may play together or alone an important role in the function of MTOC structures e.g. for initiation of microtubule formation and processes that are related to this and/or anchoring of microtubules.

REFERENCES

1. Bryan, J., Nagle, B. W., and Doenges, K. H. (1975) Proc. Nat. Acad. Sci. U.S.A. 72, 3570-3574.
2. Shelanski, M. L., Gaskin, F., and Cantor, C. R. (1973) Proc. Nat. Acad. Sci. U.S.A. 70, 765-768.
3. Sarfert, E.,and Venner, H. (1962) Naturwiss. 49, 423.
4. Gaskin, F., Cantor, C. R., and Shelanski, M. L. (1974) J. Mol. Biol. 89, 737-758.
5. Hartree, E. F. (1972) Analyt. Biochem. 48, 422-427.
6. Weingarten, M. D., Lockwood, A. H., Hwo, S.-Y., and Kirschner, M. W. (1975) Proc. Nat. Acad. Sci. U.S.A. 72, 1858-1862.

7. Lee, J. C., and Timasheff, S. N. (1975) Biochemistry 14, 5183-5187.
8. Murphy, D. B., and Borisy, G. G. (1975) Proc. Nat. Acad. Sci. U.S.A. 72, 2696-2700.
9. Erickson, H. P., and Voter, W. A. (1976) Proc. Nat. Acad. Sci. U.S.A. 73, 2813-2817.
10. Herzog, W., and Weber, K. (1977) Proc. Nat. Acad. Sci. U.S.A. 74, 1860-1864.
11. Cleveland, D. W., Hwo, S.-Y., and Kirschner, M. W. (1977) J. Mol. Biol. 116, 207-225.
12. Cleveland, D. W., Hwo, S.-Y., and Kirschner, M. W. (1977) J. Mol. Biol. 116, 227-247.
13. Amos, L. A. (1977) Nature 270, 98-99.
14. Comings, D. E., and Harris, D. C. (1976) J. Cell Biol. 70, 440-452.
15. Douvas, A. S., Harrington, C. A., and Bonner, J. (1975) Proc. Nat. Acad. Sci. U.S.A. 72, 3902-3906.
16. Douvas, A. S., and Bonner, J. (1977) in Mechanism and Control of Cell Division (Rost, T. L., and Gifford, E. M. eds.) pp. 44-61, Dowden, Hutchison & Ross, Stroudsburg, Penn.
17. Wiche, G., Corces, V. G., and Avila, J. (1978) Nature 273, 403-405.
18. Margolis, R. L., Wilson, L., and Kiefer, B. J. (1978) Nature 272, 450-452.
19. Bielek, E. (1978) Cytobiologie 16, 480-484.
20. Berns, M. W., Rattner, J. B., Brenner, S., and Meredith, S. (1977) J. Cell Biol. 72, 351-367.
21. Unger, E. (1977) Z. Allg. Mikrobiol. 17, 487-490.
22. Jokelainen, P. T. (1967) J. Ultrastruct. Res. 19, 19-44.
23. Zickler, D. (1973) Histochem. 34, 227-238.
24. Went, H. A. (1977) J. theor. Biol. 68, 95-100.