Vol. 110, No. 1, 1983 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
January 14, 1983 Pages 287-291

DISASSEMBLY OF MICROTUBULES BY THE ACTION OF CALMODULIN-DEPENDENT PROTEIN KINASE (KINASE II) WHICH OCCURS ONLY IN THE BRAIN TISSUES

Takashi Yamauchi and Hitoshi Fujisawa

Department of Biochemistry, Asahikawa Medical College, Asahikawa 078-11, Japan

Received November 30, 1982

Microtubules assembled by the incubation of GTP at 37 °C were disassembled by the action of calmodulin-dependent protein kinase (Kinase II) which occurs only in the brain tissues. This disassembly required the presence of ATP and physiological concentrations of Ca^{2+} and calmodulin.

It was previously demonstrated that three distinct calmodulin dependent protein kinases were present in rat brain tissues (1). One of them, Kinase II, was found to occur only in the brain tissues (2) and was involved in the activation of tryptophan 5-monooxygenase (1,3) and tyrosine 3-monooxygenase (3), indicating that Kinase II may play a role in the regulation of the biosynthesis of monoamines in the nervous system. We have recently demonstrated that tubulin and MAP 2 were also endogenous protein substrates of Kinase II in rat brain tissues (4,5). In this communication, Kinase II was demonstrated to induce disassembly of the microtubules which had been assembled, suggesting that Kinase II may be involved not only in the regulation of the biosynthesis of monoamine neurotransmitters but also in the

Abbreviations: MAP 2, microtubule-associated protein 2; Mes, 2-(*N*-morphorino)ethanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N'*,*N'*-tetraacetic acid.

0006-291X/83/010287-05$01.50/0

regulation of dynamic cellular processes such as neurotransmitter secretion in the central nervous system.

EXPERIMENTAL PROCEDURES

Rat brain microtubule proteins were purified by two cycles of assembly-disassembly according to a modification of the method of Shelanski *et al.* (6) as described by Karr *et al.* (7). Kinase II was purified about 720-fold with a 36% yield to near homogeneity from the extracts of the rat cerebral cortex. The purification procedures will be published elsewhere. . Calmodulin was prepared from the rat brain by the method of Wang and Desai (8).

Microtubule assembly and disassembly were measured by determining the change in turbidity at 350 nm at 37 °C (9). The incubation medium for microtubule assembly contained 0.1 *M* Mes buffer, pH 6.5, 0.5 m*M* $Mg(CH_3COO)_2$, 0.025 m*M* EGTA, 1 m*M* mercaptoethanol, 0.5 m*M* GTP and microtubule proteins in a total volume of 0.8 ml. Assembly of microtubules was started by elevating the temperature of the mixture from 0 °C to 37 °C. The critical concentration for microtubule assembly under the standard conditions was 0.54 mg of protein/ml.

Protein was determined by the method of Lowry *et al.* (10) with bovine serum albumin as the standard.

RESULTS AND DISCUSSION

Fig. 1 shows the time course of microtubule assembly induced by the incubation with GTP at 37 °C and disassembly induced by the action of Kinase II. When Kinase II, together with the ingredients for necessary for the Ca^{2+}-, and calmodulin-dependent phosphorylation reaction, was added to the microtubules which had been assembled in the presence of GTP at 37 °C for 20 min, microtubule disassembly occurred even at 37 °C and finally reached a level close to the original as judged by the decrement in absorbance at 350 nm. When either Kinase II or Ca^{2+} was omitted, such a relatively rapid disassembly of microtubules was not observed. The requirements of each ingredient added to the mixture for microtubule disassembly were examined by measuring the initial rate of a decrease in absorbance at 350 nm as shown in Table I. The microtubule disassembly required Ca^{2+}, calmodulin, ATP and Kinase II, and it was blocked by the omission of any of these. These results indicate that the phosphorylation of microtubule proteins by Kinase II may induce the microtubule

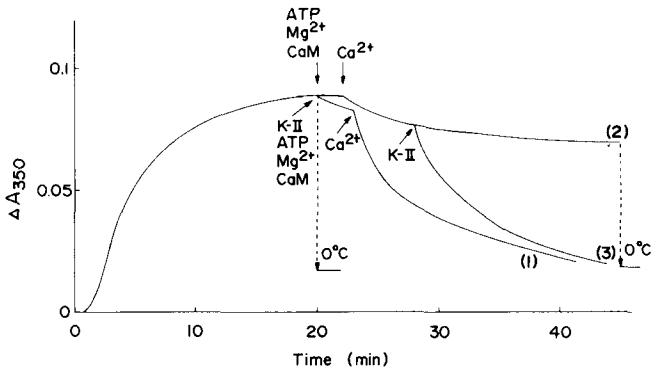

Fig. 1. Disassembly of microtubules by the action of Kinase II. Microtubule proteins were assembled in a mixture containing 0.1 *M* Mes buffer, pH 6.5, 0.5 m*M* $Mg(CH_3COO)_2$, 1 m*M* mercaptoethanol, 0.025 m*M* EGTA, 0.5 m*M* GTP and 0.98 mg of microtubule proteins at 37 °C. After 20 min, 6.2 µg of Kinase II, 0.5 m*M* ATP, 2.5 m*M* $Mg(CH_3COO)_2$ and 5 µg of calmodulin and then 0.1 m*M* $CaCl_2$ were added to the mixture (*line 1*), or 0.5 m*M* ATP, 2.5 m*M* $Mg(CH_3COO)_2$ and 5 µg of calmodulin and then 0.1 m*M* $CaCl_2$ were added to the mixture (*line 2*) and thereafter 6.2 µg of Kinase II were added (*line 3*). After 20 min or 45 min the mixture was cooled in an ice bath for 20 min and then the absorbance was determined in the figure (*dashed lines*). Abbreviations used are: K-II, Kinase II; CaM, calmodulin.

disassembly. The concentration of calmodulin required to produce a maximal effect on the microtubule disassembly was found to be about 0.3 µ*M* under the experimental conditions, which corresponded to about 3% of the concentration of tubulin added to

Table I. Requirements for disassembly of microtubules

System	Disassembly	
	$-\Delta A_{350}/min$	%
Complete	0.022	100
- Ca^{2+}	0.002	9
- Calmodulin	0.002	9
- ATP	0.002	9
- Kinase II	0.004	18

The reaction was initiated by the addition of Ca^{2+} or Kinase II after microtubules were assembled as described in the legend for Fig. 1, and the initial rate of a decrease in absorbance at 350 nm was determined.

the mixture. The concentration of Ca^{2+} required for a maximal effect on Kinase II-dependent microtubule disassembly was lower than 80 μ*M*, although ATP- and Kinase II-independent microtubule disassembly occurred in the presence of much higher concentrations of Ca^{2+}.

Weisenberg first reported that microtubule assembly required the removal of Ca^{2+} (11) and many investigators have demonstrated that Ca^{2+} effects on microtubule assembly and disassembly might be mediated by calmodulin (12-17). However, the mechanism of the Ca^{2+} effects reported previously is distinguishable from that described here in the following respects. (1) The Ca^{2+} effects did not require the presence of ATP and Kinase II. (2) The Ca^{2+} effects required the presence of much higher concentrations of calmodulin than those required for Kinase II-dependent microtubule disassembly.

Sloboda *et al.* (18) reported that MAP 2 was phosphorylated by cAMP-dependent protein kinase and Jameson *et al.* (19,20) recently demonstrated that cAMP-stimulated phosphorylation of microtubule-associated proteins inhibited both the rate and extent of microtubule assembly, suggesting that phosphorylation may play a regulatory role in microtubule-mediated functions.

Burke and DeLorenzo (21) have recently reported that brain tubulin was phosphorylated by a calmodulin-dependent protein kinase. We have more recently demonstrated (4,5) that tubulin and MAP 2 were phosphorylated by Kinase II, the calmodulin-dependent protein kinase which was found to occur only in the central nervous system and was involved in the activation of tryptophan 5-monooxygenase and tyrosine 3-monooxygenase, the rate-limiting enzymes in the biosynthesis of serotonin and catecholamines, respectively. Since tubulin is known to occur abundantly in the brain tissues and to function in dynamic

cellular processes such as cell secretion and intracellular transport (22), it may be conceivable that Kinase II participates in the regulation of both biosynthesis and secretion of a monoamine neurotransmitter in the central nervous system.

ACKNOWLEDGEMENTS

The authors would like to thank Mr. Andrew Grenville who kindly read the first draft and offered suggestions for correcting the English phrasing. This work has been supported in part by a grant-in-aid for Scientific Research from the Ministry of Education, Science, and Culture of Japan.

REFERENCES

1. Yamauchi, T. and Fujisawa, H. (1980) FEBS Lett. 116, 141-144
2. Yamauchi, T. and Fujisawa, H. (1981) FEBS Lett. 129, 117-119
3. Yamauchi, T., Nakata, H. and Fujisawa, H. (1981) J. Biol. Chem. 256, 5404-5409
4. Yamauchi, T. and Fujisawa, H. (1982) Abstracts of 25 annual Meetings of the Japanease Neurochemical Society, p.75-77
5. Yamauchi, T. and Fujisawa, H. Biochem. Biophys. Res. Commun. *in press*
6. Shelanski, M. L., Gaskin, F. and Cantor, C. R. (1973) Proc. Natl. Acad. Sci. U.S.A. 70, 765-768
7. Karr, T. L., White, H. D. and Purich, D. L. (1979) J. Biol. Chem. 254, 6107-6111
8. Wang, J. H. and Desai, R. (1977) J. Biol. Chem. 252, 4175-4184
9. Gaskin, F. and Cantor, C. R. (1974) J. Mol. Biol. 89, 737-758
10. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265-275
11. Weisenberg, R. C. (1972) Science 177, 1104-1105
12. Marcum, J. M., Dedman, J. R., Brinkley, B. R. and Means, A. R. (1978) Proc. Natl. Acad. Sci. U.S.A. 75, 3771-3775
13. Nishida, E., Kumagai, H., Ohtsuki, I. and Sakai, H. (1979) J. Biochem. 85, 1257-1266
14. Kakiuchi, S. and Sobue, K. (1981) FEBS Lett. 132, 141-143
15. Job, D., Fischer, E. H. and Margolis, R. L. (1981) Proc. Natl. Acad. Sci. U.S.A. 78, 4679-4682
16. Berkowitz, S. A. and Wolff, J. (1981) J. Biol. Chem. 256, 11216-11223
17. Lee, Y. C. and Wolff, J. (1982) J. Biol. Chem. 257, 6306-6310
18. Sloboda, R. D., Rudolph, S. A., Rosenbaum, J. L. and Greengard, P. (1975) Proc. Natl. Acad. Sci. U.S.A. 72, 177-181
19. Jameson, L., Fery, T., Zeeberg, B., Dallodorf, F. and Caplow, M. (1980) Biochemistry 19, 2472-2479
20. Jameson, L. and Caplow, M. (1981) Proc. Natl. Acad. Sci. U.S.A. 78, 3413-3417
21. Burke, B. E. and DeLorenzo, R. J. (1981) Proc. Natl. Acad. Sci. U.S.A. 78, 991-995
22. Olmsted, J. B., and Borisy, G. G. (1973) Ann. Rev. Biochem. 42, 507-540