Microtubule assembly from monkey brain microtubule proteins and comparison with porcine brain microtubule assembly system

EISUKE NISHIDA, HIROMICHI KUMAGAI, HIKOICHI SAKAI, SHIN-ICHI HISANAGA and KENJI TAKAHASHI*

Department of Biophysics and Biochemistry, Faculty of Science, University of Tokyo, Bunkyo-ku, Tokyo 113, and *Primate Research Institute, Kyoto University, Inuyama, Aichi 484, Japan

ABSTRACT

Monkey brain microtubule proteins were purified by two cycles of polymerization and depolymerization. Both α and β chains of monkey brain tubulin comigrated with the respective chains of porcine brain tubulin. The microtubule-associated protein fraction of monkey brain contained about thirty components which were mostly identical in electrophoretic pattern to those contained in the microtubule-associated protein fraction of porcine brain. In microtubule assembly, monkey and porcine brain tubulins were indistinguishable from each other. Little difference was detected between the two microtubule assembly systems as regards responses to microtubule poisons, calcium ions, sulfhydryl reagent, and calmodulin. The only difference detected between the two tubulins was the number of reactive sulfhydryl groups, 12 moles for monkey tubulin and 14 moles for porcine tubulin per 110,000 g protein. However, blockade of one mole of the sulfhydryl groups caused complete inhibition of polymerization in both tubulins.

KEY WORDS  monkey brain tubulin / porcine brain tubulin / microtubule-associated proteins / calmodulin / microtubule assembly

Microtubules, which consist of tubulin and microtubule-associated proteins, play roles in a variety of cellular processes including cellular movements (3, 19), mitosis (6, 18, 26), transport of cytoplasmic components (5), organization of cytoskeletons (29), and propagation of membrane excitation (16, 17).

Tubulin seems to show a high degree of evolutionary conservation in physicochemical properties and in some characteristics of its assembly reaction, though its microheterogeneity has frequently been suggested (19). In a comparison of a 25 amino acid sequence in the N-terminal region of tubulin from sea urchin sperm and chick embryo brain, only one residue in the β chains was shown to differ from the other (13). However, recent works have revealed more distinct differences among tubulins purified from different sources, especially among those from protozoa, echinoderms, and mammals. The differences include electrophoretic mobility of tubulin, colchicine binding to tubulin (14, 15), calcium-sensitivity of tubulin in microtubule assembly, and susceptibility of microtubule assembly systems to the calcium-dependent regulatory action of calmodulin (7, 22).

Although some diversity of tubulin molecules in the animal kingdom has recently been suggested, no accurate comparisons have been made on mammalian microtubule proteins obtained from different species in regard to their contents, constituents, and characteristics in the assembly reactions. We have chosen monkey and porcine brains for comparison in this paper, and describe monkey brain microtubule proteins, assembly into microtubules, and some comparisons with the porcine brain microtubule assembly system.

Monkey (Macaca fuscata) brains were dissected freshly at the Primate Research Institute.
of Kyoto University and preparation of monkey brain microtubule proteins was carried out at the Institute. Porcine (Sus scrofa var. domesticus) brains were obtained from Teikoku Zoki Co. immediately after slaughter. Two cycles of temperature-dependent assembly and disassembly were performed as described previously (23) to obtain purified (2-cycled) microtubule proteins (C2S) from both monkey and porcine brains. Purified 6S tubulin was recovered in the flow through fraction by phosphocellulose column chromatography of C2S, and the microtubule-associated proteins adsorbed were eluted with 0.8 M KCl (7). Calmodulin was purified from monkey and porcine brains as described before (23) or by an affinity chromatography using a tubulin-Sepharose 4B column (8).

The yield of microtubule proteins as C2S was 0.61 mg/g brain tissue for monkey brain and 0.45-0.75 mg for porcine brain, indicating nearly the same contents of microtubule proteins in both brains. Phosphocellulose column chromatography separated monkey 6S tubulin from microtubule-associated proteins in a fashion identical to those of porcine brain microtubule proteins. The ratio of monkey brain tubulin to microtubule-associated proteins was 8:2 by weight.

The purified monkey 6S tubulin was incapable of polymerizing into microtubules by itself, as was porcine brain 6S tubulin. Addition of monkey brain microtubule-associated proteins spontaneously initiated microtubule assembly. Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis of monkey and porcine brain C2S and 6S tubulin indicated that the chain weights of tubulin subunits and two major microtubule-associated proteins (two bands near 350 kDa and 250 kDa) are identical. Fig. 1A: SDS-polyacrylamide (10%) slab gel electrophoresis of monkey and porcine brain C2S and 6S tubulin fractions. The proteins were carboxymethylated and subjected to electrophoresis as described previously (22). a, monkey brain C2S (30 µg); b, porcine brain C2S (30 µg); c, monkey 6S tubulin (15 µg); d, porcine 6S tubulin (15 µg); e, monkey 6S tubulin + porcine C2S (15 µg each); f, monkey 6S tubulin + porcine 6S tubulin (15 µg each). B: SDS-polyacrylamide (10%) slab gel electrophoresis of monkey and porcine microtubule-associated proteins (MAPs) prepared by phosphocellulose column chromatography. The proteins were not carboxymethylated. a, monkey brain MAPs (40 µg); b, porcine brain MAPs (40 µg).
MONKEY AND PORCINE BRAIN MICROTUBULE PROTEINS

Assembly of monkey microtubule proteins into microtubules is shown in Fig. 2, in which the sensitivities of the microtubule assembly system to low temperature, colchicine and ansamitocin P3 (a derivative of maytansine) (4) are indicated. Inhibition of the microtubule assembly by a nearly stoichiometric amount of colchicine (11 μM/12 μM tubulin) was 85%, which is similar to the inhibition of porcine brain microtubule assembly (28). Furthermore, ansamitocin P3 at one-sixteenth the stoichiometric amount inhibited the microtubule assembly by 30%. A complete inhibition has been reported at an ansamitocin P3 concentration of 4.4 μM for bovine brain tubulin (24). Maytansine, a similar microtubule poison, is known to inhibit rabbit brain microtubule assembly by 50% at a 0.12:1 molar ratio of the poison to tubulin and 15% at a 0.07:1 molar ratio (25). Fig. 2 also shows the temperature-dependent reversibility of the microtubule assembly.

When the critical concentration of monkey brain microtubule proteins for microtubule assembly was measured (Fig. 3) in comparison with that of porcine brain microtubule proteins, both values coincided closely (Fig. 3, inset). The same critical concentration has been reported for microtubule proteins from calf brain (11) and rat brain (2).

Moreover, measurement of the calcium-sensitivity of monkey brain microtubule assembly system gave response curves (Fig. 4) quite similar to those for porcine brain microtubule assembly (21). The inset of Fig. 4 indicates that both tubulin polymerizabilities are identical as a
Fig. 3 Dependence of monkey microtubule assembly on protein concentration. Figures on each curve represent concentration of C2S in mg/ml. Inset shows the critical concentrations of monkey and porcine brain microtubule proteins for assembly by extrapolating to zero turbidity. (○), monkey C2S; (●), porcine C2S. The assembly medium consisted of 0.1 M KCl, 10 mM MES, 0.5 mM MgCl2, 0.5 mM EGTA, and 0.5 mM GTP at pH 6.8.

function of free calcium ion concentration. In bovine brain microtubule assembly, half maximal inhibition was reported to occur at 250 μM Ca²⁺ (12). The calcium-sensitivity of microtubule assembly from porcine brain microtubule proteins has been shown to increase with increasing ionic strength (20). This was the case for monkey brain microtubule proteins as well.

Porcine brain microtubule assembly in a glycerol-free assembly medium is known to be totally inhibited by treatment with the stoichiometric amount of sulphhydril reagent (9, cf. 10). This was also the case for monkey brain microtubule assembly (Fig. 5). Monkey tubulin was found to contain 12 moles of reactive sulphhydril groups per 110,000 g C2S, as measured with Ellman’s reagent (1). When tubulin was dissolved in 0.05 M KCl-containing medium, complete inhibition of microtubule assembly was caused by blocking of two moles of the cysteine residues. In contrast, in 0.1 M KCl-containing medium, blocking of one mole cysteine residue was sufficient for a complete inhibition of microtubule assembly (Fig. 5).

Finally, the effect of calmodulin on the assembly of monkey brain tubulin was examined. Recently, we have demonstrated that the calcium-dependent modulator, calmodulin, suppresses microtubule assembly in a calcium-dependent manner. Several lines of evidence were presented that the inhibition was due to the formation of a tubulin-Ca²⁺-calmodulin complex incapable of polymerizing into microtubules (23, 7). The inhibition also occurred in the case for monkey brain tubulin in the presence of monkey calmodulin and calcium ions. The inhibition was small at low ionic strength and increased with increasing ionic strength. This behavior of monkey brain tubulin was similar to that of porcine brain tubulin (23).

Purified tubulin has been prepared from many animal species, and it has been shown to be conservative in physical and chemical properties (27). However, investigations of microtubule assembly systems using tubulin preparations derived from different animal phyla have shown different assembly characteristics among them.
Fig. 5 Microtubule assembly from monkey and porcine brain microtubule proteins as a function of moles of sulfhydryl groups blocked per 110,000 g of protein. The assembly buffer consisted of 0.1 M KCl, 10 mM MES, 0.5 mM MgCl₂, and 0.5 mM GTP at pH 6.8. Tubulin was preincubated with 0.5, 1.0, and 2.0 equivalent amounts of p-chloromercuriphenyl sulfonate in the assembly buffer for 10 min at 0°C, followed by incubation at 35°C to monitor turbidity increase. (O), monkey C₂S, 2.7 mg/ml; (●), porcine C₂S, 2.7 mg/ml

Sea urchin tubulin, which has an extremely high calcium-sensitivity, does not suffer the modulating action of calmodulin (22). Furthermore, protozoan tubulin is quite different from mammalian tubulin in colchicine binding and even in the apparent chain weights of the subunits (14, 15). In this study, we have detected little difference between monkey and porcine brain microtubule proteins in respect to the amounts contained in both brains, their electrophoretic properties, polymerizability into microtubules, and responses to microtubule poisons, calcium ions, sulfhydryl reagent, and calmodulin. These results, together with the previous ones (2, 11, 12, 24, 25, 28), suggest that tubulin and microtubule-associated proteins are very conservative among the mammal.

We thank Takeda Chemical Industries, Ltd. for the supply of ansamitocin P₃. This study was supported in part by a grant for co-operative research at the Primate Research Institute, Kyoto University and a grant-in-aid for scientific research from the Ministry of Education, Science and Culture, Japan to H. Sakai.

Received for publication 26 May 1980

REFERENCES

15. MAEKAWA S. and SAKAI H. (1978) Characteriza-
tion and in vitro polymerization of *Tetrahymena* tubulin. *J. Biochem.***83**, 1065–1075


