Communications to the Editor

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EFFECT OF PROTEIN-BOUND POLYSACCHARIDE (PS-K) ON MICROTUBULE POLYMERIZATION

Yoshiyuki Kondo, Toshihiro Fujii, Toshimichi Koyama, Michiyo Fujii, and Kosuke Ohki*
Department of Functional Polymer Science
Faculty of Textile Science and Technology
Shinshu University, Ueda, 386, Japan

A protein-bound polysaccharide (PS-K) partially inhibited brain microtubule polymerization, though it had little effect on the reconstituted microtubules. The heat treatment at 100°C and digestion by trypsin, papain, pronase E, proteinase K, and glycosidase mixture did not affect the inhibitory action of PS-K.

KEYWORDS——brain microtubule protein; inhibitory factor; PS-K; microtubule polymerization; stable factor

Polymerization and depolymerization of cytoplasmic microtubules are characteristic events playing important roles in cell motility such as cell division, secretion, and the formation of cell shape.[1] A number of antimitotic reagents including colchicine, podophyllotoxin, griseofulvin, ansamitocins, maytansinoids, and Vinca alkaloids are thought to mediate their antitumor effects through their binding to the tubulin molecule, a main component of microtubule proteins.[2] Moreover, these drugs are known to inhibit microtubule polymerization and to induce depolymerization of microtubules.

A protein-bound polysaccharide, PS-K, isolated from Basidiomycetes, has been reported to possess an antitumor activity against various experimental tumors such as sarcoma-180, hepatoma AH-13, Ehrlich tumor, and 3-methylcholanthrene-induced fibrosarcoma.[3] The antitumor effect of PS-K was supposed to restore the depressed functions of lymphocyte or macrophage in tumor-bearing hosts.[3a,4] However, the precise mechanism of action of PS-K on the cellular components remains unknown. Thus, it is of interest to determine whether microtubule proteins are a target for PS-K.

Microtubule proteins were prepared from porcine brain by a slight modification of the method of Shelanski et al.[5] with three cycles of polymerization and depolymerization.[6] Protein was determined by the method of Lowry et al.[7] using bovine serum albumin as a standard.

Polymerization was determined at 37°C by measuring the turbidity change at 350 nm with a recording spectrophotometer (Japan Spectroscopic Co., Ltd., UVIDEC-410) equipped with a thermostatically regulated sample chamber. The reaction mixture

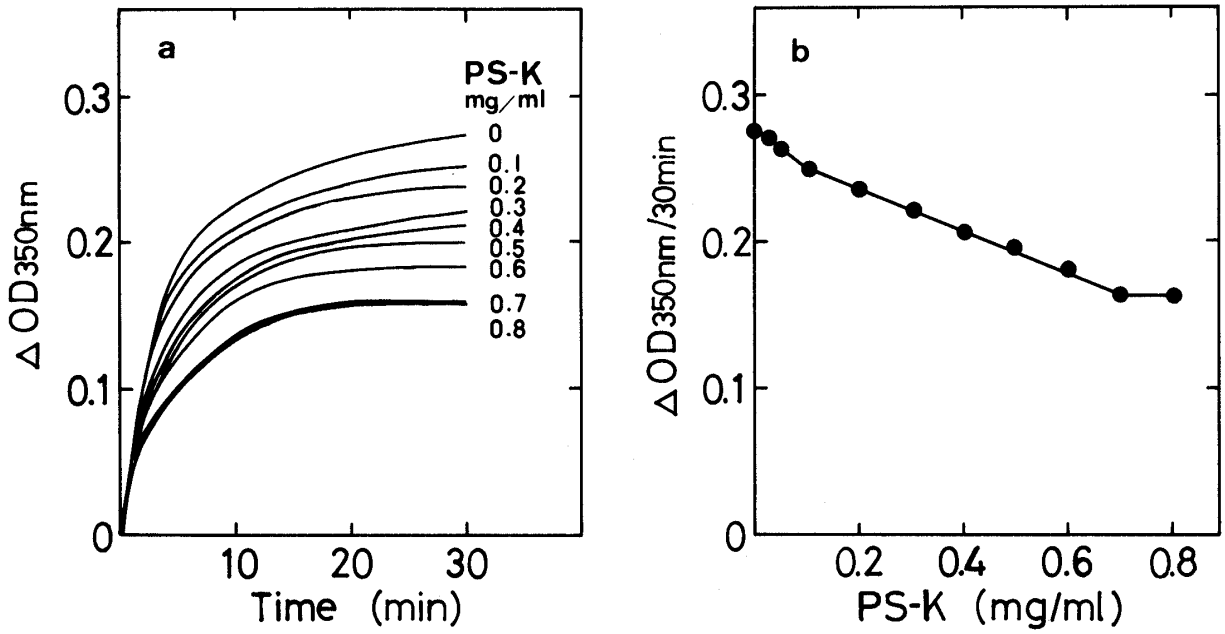

Fig. 1. Inhibition of Microtubule Polymerization by PS-K

contained 2 mg microtubule proteins in 1 ml of a buffer solution composed of 60 mM 2-(N-morpholino)ethanesulfonic acid (MES)-KOH (pH 6.5), 1 mM $Mg(CH_3COO)_2$, 0.5 mM ethyleneglycol-bis(2-aminoethyl ether)-N,N,N',N'-tetraacetic acid, 12.5% glycerol, and 1 mM GTP.

The PS-K used was a generous gift from Dr. C. Yoshikumi, Kureha Chemical Industry Co. Papain and proteinase K were purchased from Sigma Chemical Co., glycosidase mixture from Seikagaku Kogyo Co., trypsin from Worthington, and pronase E from Kakenkagaku Co. Other chemicals used were reagent grade.

It was found in this experiment that PS-K had an inhibitory action on microtubule polymerization as shown in Fig. 1a. There was a concentration dependent inhibition in the extent of the polymerization (Fig. 1b). Saturation of the inhibitory effect was observed when the concentration of PS-K exceeded 0.7 mg/ml in the reaction mixture containing 2 mg/ml microtubule proteins. As compared with the inhibitions of colchicine, podophyllotoxin, and Vinca alkaloids on microtubule polymerization,[2a] PS-K showed a weak inhibitory effect and, further, it did not induce the depolymerization of reconstituted microtubules (data not shown).

Microtubule polymerization in vitro can be suppressed by adding monovalent ions, Ca ions, and GDP.[8] Since the preparation of PS-K was capable of inducing inhibition of microtubule polymerization after its dialysis against 20 mM MES-KOH (pH 6.5) for 48 h (molecular weight cutoff; 6,000-8,000), the inhibitory effect was not considered to be caused by contaminating ions or the low molecular weight components In addition, rapid hydrolysis of GTP did not seem to occur, because the addition of 0.2 mM phosphoenolpyruvate-2 μg/ml pyruvate kinase mixture failed to sequester the inhibitory activity of PS-K.

Some properties of PS-K are summarized in Table I. The inhibitory effect of PS-K was heat-stable, retaining its activity even when treated at 100°C for 2 h.

Table I. Inhibitory Activity of PS-K after Various Treatments

Treatment	Relative activity (%)
None	100
100°C 0.5 h	94
1 h	109
2 h	106
Trypsin (0.1 mg/ml) 37°C 1 h	110
Papain (0.1 mg/ml) 37°C 1 h	102
Pronase E (0.1 mg/ml) 37°C 1 h	97
Proteinase K (0.1 mg/ml) 37°C 1 h	87
Glycosidase mixture (0.1 mg/ml) 37°C 24 h*	107

After incubating of 5 mg/ml PS-K with the indicated treatment, the samples were heated at 100°C for 10 min to inactivate enzyme activities. *PS-K dissolved in 10 mM acetate buffer (pH 4) was treated with glycosidase mixture and then dialyzed against 10 mM MES-KOH (pH 6.5). Relative activity represents the activity ratio of treated to untreated PS-K as a percentage.

Therefore, the inhibitory effect does not seem to be caused by the proteolytic action of PS-K on microtubule proteins. Recently, a polysaccharide, mainly composed of fucose, which inhibits microtubule polymerization and induces depolymerization of microtubules was isolated from sea urchin egg.[9] Active substance of PS-K has been also known to be a protein-bound polysaccharide which was mainly composed of glucose.[10] We examined whether or not the inhibition of microtubule polymerization takes place after treatment of PS-K with various proteases and glycosidases. The inhibitory effect of PS-K was not destroyed by incubating with trypsin, papain, pronase E, proteinase K or a glycosidase mixture containing glucosidases. Furthermore, treatment of pronase E or proteinase K after incubation of PS-K with glycosidase mixture did not reduce the inhibitory activity. Bryan et al.[11] reported that RNA not only inhibited the initial steps of microtubule polymerization but also induced a transient depolymerization of microtubules. Therefore, the mode of the inhibitory effect of RNA was quite different from that of PS-K.

In cell division, microtubules have been considered to play significant roles, because spindle fibers consist mainly of microtubule proteins and, further, chromosome movements depend on the polymerization and depolymerization of microtubules.[12] Therefore, if PS-K is incorporated into cells, it may affect mitosis. The present study provides the first evidence that a factor contained in PS-K inhibits microtubule polymerization. The striking features of this factor are its extreme heat stability and the insensitivity of the various proteases and glycosidases. Purification of this inhibitory factor is necessary to determine if it is the same as that which restores the depression of lymphocyte or macrophage function. An experiment along this line is in progress.

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