VISCOMETRIC ANALYSIS OF EFFECTS OF CYTOCHALASANS ON *IN VITRO* POLYMERIZATION AND DEPOLYMERIZATION OF MICROTUBULES

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Nineteen cytochalasans including 6 natural cytochalasins, 2 natural aspochalasins, 7 natural chaetoglobosins and 4 their acetates were investigated by viscometry to examine their effects on *in vitro* polymerization and depolymerization of microtubules. Cytochalasin A was proved as an only active cytochalasan, confirming the studies of Himes *et al.*, and others showed very weak or no noticeable effects. This results indicate that a wide range of effects of cytochalasans are not related to interactions with microtubules.

Keywords—cytochalasans; cytochalasins; aspochalasins; chaetoglobosins; microtubules; viscometry

The cytochalasans¹⁾ are known as a group of structurally similar mycotoxins which show unique effects on cell growth, movement, secretion and phagocytosis. Moreover, some cytochalasans have been reported as the inhibitors of glucose transport in the plasma membranes. On the other hand, it is proved that a wide range of effects of the cytochalasans might be attributed to the interaction between the same active site of the drug and the common target protein, the actin.²⁾ Löw *et al.*³⁾ have reported that there is no correlation between the ATP hydrolyzing activity of F-actin elicited by various cytochalasans and their influence on the viscosity. On the other hand, Himes and his co-workers⁴⁾ have reported that cytochalasin A (CA) inhibits the selfassembly of beef brain tubulin though cytochalasin B (CB) at identical and higher concentration has no noticeable effect.

We describe below studies which clarify that 19 cytochalasans, including 6 natural cytochalasins, 2 natural aspochalasins, 7 natural chaetoglobosins⁵⁾ and 4 their acetates, except for CA do not show any marked inhibitory effects on the association of porcine brain tubulin and also on

the dissociation of the microtubule. On the other hand, the studies of Yahara *et al.*²⁾ which indicate that the *in vivo* target protein of cytochalasans is actin, are very important in connection with our results.

MATERIALS AND METHODS

Cytochalasans — The names of cytochalasans are used in the abbreviated forms shown in Table I. The structures of some cytochalasans are shown in Fig. 1.⁵⁾ (a) CA,⁶⁾ CC,⁷⁾ and CE⁸⁾, (b) CB⁹⁾, (c) CD¹⁰⁾, (d) CH¹¹⁾, and (e) AsB and AsD¹²⁾ were provided by D.C. Aldridge (Imperial Chemical Industries, Ltd., Macclesfield, England), Ch. Tamm (Institut für Organische Chemie der Universität Basel, Switzerland), H. Minato (Shionogi Co., Osaka, Japan), R. J. Cole (National Peanuts Research Laboratories, Dawson, Ga.), and W. Keller-Schierlein (Eidgenössische Technische Hochschule Zürich, Zürich, Switzerland), respectively. Chaetoglobosins were isolated from *Chaetomium globosum* and the acetates were prepared as described previously⁵⁾ (Fig. 1).

Other Chemicals — Griseofulvin and daunomycin were provided by Y. Nakayama (Nippon

Kayaku Co., Tokyo, Japan) and M. Tanabe (SRI International, Menlo Park, California, U.S.A.), respectively. Colchicine and vinblastine were commercial products from Wako Chemical Industries, Ltd., Osaka, Japan and Sigma Chemical Co., St. Louis, Mo, U.S.A., respectively.

Preparation of Crude Tubulin Fraction — The preparation of crude tubulin was obtained by the method described by Kuriyama *et al.*¹³⁾ Porcine brain was obtained within 1.5 h after slaughter through the courtesy of Tokyo Metropolitan Meat Market. Details are described in the previous paper.¹⁴⁾

Viscosity Measurement — The procedures were the same as described previously.¹⁴⁾ In detail, 1 ml of partially purified tubulin solution (ca. 6.2–7.4 mg/ml) was added in a tube of Ostwald type viscometers attached with Automatic Viscometer, Shibayama Scientific Co. Ltd., Tokyo, and viscosity was measured in a thermostatic bath for measure of the kinematic

viscosity TV-5S, Thomas Scientific Instruments Co., Ltd., Tokyo. The test samples were dissolved in a 1:1 mixture¹⁴⁾ of dimethylsulfoxide and *N,N*-dimethylformamide, and the sample solutions added to the corresponding proteins, respectively. Final concentrations of the drugs shown in Table I were 200 μ M.

Calculation of Activities of Samples on the Inhibition of Microtubule Polymerization and on Depolymerization of Microtubules — Examples of the time course of specific viscosities (η_{sp}) are shown in Fig. 2 and 3. The *a* and *b* represent the η_{sp} values of control at 20 min after the incubation at 37°C and of the microtubule proteins in which the test sample was added at 0 min of the incubation, at 20 min after incubation at 37°C, respectively. The *a'* and *b'* represent the η_{sp} values at 30 min after the incubation at 37°C and at 10 min after the addition of the sample at the time indicated by the arrow, respectively. The values of $(a - b)/a$ and $(a' - b')/a'$ of each sam-

FIG. 1. Structures of Cytochalasins

a) Cytochalasin A and B, b) Chaetoglobosin A and D, c) Aspochalasin B and D.

FIG. 2. Calculation Method of Activities of Samples on Polymerization of Microtubule Proteins

FIG. 3. Calculation Method of Activities of Samples on Depolymerization of Microtubules

TABLE I. The Effects of Cytochalasins for the *in Vitro* Microtubule Polymerization and the *in Vitro* Depolymerization of Microtubule determined by Viscometry

Substance	$(a - b)/a$	$(a' - b')/a'$
Cytochalasin A (CA)	0.37 ****a)	0.35 ****a)
Cytochalasin B (CB)	0.05 *	0.00 *
Cytochalasin C (CC)	0.06 *	0.10 **
Cytochalasin D (CD)	-0.03 *	0.00 *
Cytochalasin E (CE)	0.01 *	0.03 *
Cytochalasin H (CH)	-0.08 *	0.05 *
Aspochalasin B (AsB)	0.06 *	0.11 **
Aspochalasin D (AsD)	0.13 **	0.12 **
Chaetoglobosin A (ChA)	0.23 ***	0.16 **
Chaetoglobosin A-Ac (ChA-Ac)	0.19 **	0.06 *
Chaetoglobosin B (ChB)	0.15 **	0.07 *
Chaetoglobosin C (ChC)	-0.03 *	0.01 *
Chaetoglobosin D (ChD)	0.15 **	0.10 **
Chaetoglobosin E (ChE)	-0.05 *	-0.03 *
Chaetoglobosin E-Ac (ChE-Ac)	0.02 *	0.09 *
Chaetoglobosin F (ChF)	0.01 *	0.03 *
Chaetoglobosin F-Ac (ChF-Ac)	0.05 *	0.08 *
Chaetoglobosin J (ChJ)	0.09 *	0.10 **
Chaetoglobosin J-Ac (ChJ-Ac)	0.10 **	0.10 **

a) The values of $(a - b)/a$ and $(a' - b')/a'$ were ranked by the number of the asterisk (*):*, $0.10 > (a - b)/a$ and $(a' - b')/a'$, **, $0.20 > (a - b)/a$ and $(a' - b')/a' \geq 0.10$, ***, $0.30 > (a - b)/a$ and $(a' - b')/a' \geq 0.20$, and ****, $0.40 > (a - b)/a$ and $(a' - b')/a' \geq 0.30$.

ple were calculated and their relative activities were shown by the numbers of asterisk (Table I).

RESULTS AND DISCUSSIONS

Results of 6 cytochalasins, 2 aspochalasins, 7 chaetoglobosins, and their 4 acetates on the inhibitions of *in vitro* polymerization and depolymerization of microtubules were shown in Table I. The structures of cytochalasins are shown in Fig. 1. The dose-response curves of the inhibition of the polymerization of microtubule proteins by CA, CC, and ChA were also shown in Fig. 4.

Firstly, the results given in Table I show that no cytochalasins except for CA have activities in the inhibition of microtubule polymerization *in vitro* and of the depolymerization of polymerized tubulin (microtubules) *in vitro*. The values of $(a - b)/a$ and $(a' - b')/a'$ of CA were 0.37 and 0.35, respectively, and those of ChA follow by 0.23 and 0.16, respectively. All other cytochalasins marked by the asterisk of * or ** have the values of $(a - b)/a$ and $(a' - b')/a'$ less than 0.10 or 0.20, respectively, indicating negligible inhibitory activities on the polymerization of porcine microtubules *in vitro*. Fig. 4 represents the dose-response curves of three cytochalasins: CA,

FIG. 4. Dose-response Curves of the Inhibition of Polymerization of Microtubule Proteins by Vinblastine (Δ), Colchicine ($\blacktriangle$), Griseofulvin ($\circ$), Daunomycin ($\square$), Cytochalasin A ($\bullet$) and C ($\odot$), and Chaetoglobosin A ($\blacksquare$)

Each point is the mean of at least two experiments.

CC, and ChA, with those of vinblastine, colchicine, and griseofulvin which are known as the tentative antimitotic drugs, and daunomycin¹⁵⁾ which is also reported as an active component for representative antimitotic drugs, and daunomycin¹⁵⁾ which is also reported as an active component for perturbing the polymerization of tubulin and microtubule assembly. The results obtained in this experiment indicate that, under the conditions in which vinblastine and colchicine inhibit the polymerization of tubulin into microtubules in low concentration,¹⁶⁾ CA showed a slight but distinct effect. However, its activity was lower than that of griseofulvin,¹⁴⁾ and higher than those of daunomycin and ChA. The dose-response curve of CC represents the activities of all other cytochalasins which showed small $(a - b)/a$ values. On the other hand, the micromolar concentrations of drugs which show the value of 0.2 in reference to $(a - b)/a$ were calculated as follows: vinblastine, 0.04; colchicine, 0.33; griseofulvin, 23; cytochalasin A, 44; daunomycin, 110; chaetoglobosin A, 190.

Although data of the present studies were evaluated only on the basis of the results of the viscometric analyses, the results indicate that the *in vitro* target protein of the cytochalasins is not the tubulins or microtubules. In conclusion, our results could be evaluated in connection with the studies of Yahara *et al.*²⁾ which suggested the interaction of cytochalasins with actin.

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REFERENCES

- 1) M.Binder and Ch.Tamm: The cytochalasins: A new class of biologically active microbial metabolites, *Angew. Chem., Intern. Ed.*, **12**, 370–380 (1973); S.W.Tanenbaum (Eds), "Cytochalasins," North-Holland Pub. Company, Amsterdam, 1978.
- 2) I.Yahara, F.Harada, S.Sekita, K.Yoshihira, and S. Natori: Correlation between effects of 24 different cytochalasins on cellular structures and cellular events and those on actin *in vitro*, *J. Cell Biol.*, **92**, 69–78 (1982).

- 3) I.Löw, W.Jahn, Th.Wieland, S.Sekita, K.Yoshihira, and S.Natori: Interaction between rabbit muscle actin and several chaetoglobosins or cytochalasins, *Anal. Biochem.*, **95**, 14–18 (1979).
- 4) R.H.Himes, R.N.Kersey, M.Ruscha, and L.L.Houston: Cytochalasin A inhibits the *in vitro* polymerization of brain tubulin and muscle actin, *Biochem. Biophys. Res. Commun.*, **68**, 1362–1370 (1970); R.H.Himes and L.L.Houston: The action of cytochalasin A on the *in vitro* polymerization of brain tubulin and muscle G-actin, *J. Supramol. Struct.*, **5**, 81–90 (1976).
- 5) S.Natori: Toxic cytochalasins, "Mycotoxins in human and animal health", J.V.Rodricks, C.W.Hesseltine, and M.A.Mehlman (Eds.), Pathotox Publishers, Inc., 1977, pp. 559–581; S.Sekita, K.Yoshihira, S.Natori, and H.Kuwano: Chaetoglobosins, Cytotoxic 10-(Indol-3-yl)-[13]cytochalasins from *Chaetomium* spp. III. Structure of Chaetoglobosins C, E, F, G, and J, *Chem. Pharm. Bull.*, **30**, 1629–1638 (1982), and the references cited therein.
- 6) D.C.Aldridge, and W.B.Turner: Structures of cytochalasins C and D, *J. Chem. Soc. C.*, **1969**, 923–928.
- 7) D.C.Aldridge, J.J.Armstrong, R.N.Speake, and W.B.Turner: The structures of cytochalasins A and B, *J. Chem. Soc. C.*, **1967**, 1667–1676.
- 8) D.C.Aldridge, B.F.Burrows, and W.B.Turner: The structures of the fungal metabolites cytochalasins E and F, *J. Chem. Soc., Chem. Commun.*, **1972**, 148–149.
- 9) M.Binder and Ch.Tamm: Desoxaphomin, das erste [13]cytochalasin, ein möglicher biogenetischer Vorläufer der 24-oxa-[14]cytochalasane, *Helv. Chim. Acta*, **56**, 966–977 (1973).
- 10) S.Hayakawa, T.Matsushima, T.Kimura, T.Minato, and K.Katagiri: Zygosporin A. A new antibiotic from *Z. mosonii*, *J. Antibiot.* (Tokyo), **21**, 523–524 (1969).
- 11) M.A.Beno, R.H.Cox, J.M.Wells, R.J.Cole, J.W.Kinksey, and G.G.Gristoph: Structure of a new [11]cytochalasin, cytochalasin H or kodo-cytochalasin-I, *J. Am. Chem. Soc.*, **99**, 4123–4130 (1977).
- 12) W.Keller-Schierlein and E.Kupfer: Stoffwechselprodukte von Microorganismen. 186 Mitteilung. Über die Aspochalasine A, B, C, und D, *Helv. Chim. Acta*, **62**, 1501–1524 (1979).
- 13) R.Kuriyama and H.Sakai: Viscometric Demonstration of Tubulin Polymerization, *J. Biochem.*, **75**, 463–471 (1974).
- 14) Y.Sato, Y.Saito, Y.Shiratori, S.Shoda, and J.Hosoi: Structure-activity study of griseofulvin and its derivatives for the *in vitro* inhibition of microtubule polymerization and the *in vitro* depolymerization of microtubule, *Nippon Kagaku Kaishi*, **5**, 746–754 (1981).
- 15) C.Na and S.N.Timasheff: Physical-Chemical Study of Daunomycin-Tubulin Interactions, *Arch. Biochem. Biophys.*, **182**, 147–154 (1977).
- 16) J.Howbeke and G.V.Nijen: Quantitative turbidimetric assay for potency evaluation of colchicine-like drugs, *Life Sci.*, **17**, 591–596 (1975).