

INFLUENCE OF MOLECULAR WEIGHT AND PERIODATE-MODIFICATION OF β -D-GLUCANS FROM *PORIA COCOS SCLEROTIUM* ON ANTITUMOR ACTIVITIES*

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Abstract In this work, influence of molecular weight and periodate modification of β -D-glucans isolated from *Poria cocos sclerotium* on the antitumor activities against Sarcoma 180 and Ehrlich ascites carcinoma (EAC) tumor was studied. The results show that two glucans PC3 (linear β -(1 $\rightarrow$ 3)-D-glucan) and PC4 [β -(1 $\rightarrow$ 3)-D-glucan with a few of branches and glucuronic acid] are devoid of antitumor activity. However, when the glucans were modified by periodate oxidation, borohydride reduction and mild hydrolysis or partially hydrolysis, the derivatives have obvious antitumor activities. The decrease in molecular weight of glucans after periodate modification hardly affects their antitumor actions, but on the other hand, the decrease of molecular weight without periodate modification could lead to an enhancement of the antitumor activities. Moreover, the glucans and these derivatives have much higher enhancement ratios of body weight of mice than that of 5-Fluorouracil (5-Fu), suggesting that they are less toxic than 5-Fu.

Keywords *Poria cocos sclerotium*; Polysaccharide; Periodate modification; Molecular weight; Antitumor activity

INTRODUCTION

Poria cocos, a Heterobasidae belonging to the polyporaceae family, is one of the most important herbs in China and other Asian countries. It is reported that polysaccharides extracted from *Poria cocos* (glucan to be in the majority) have mitogenic, complement activating^[1], antimutagenic^[2], antitumor^[3-4], anticarcinogenic^[5] and immuno activities^[6]. However, correlation of their structures to the bioactivities was not yet fully understood. Chihara et al.^[3] have already reported that pachyman, obtained from *Poria cocos*, considered to be a β -(1 $\rightarrow$ 3) glucan with some β -(1 $\rightarrow$ 6)-linked branches, is completely devoid of antitumor activity. When it

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was modified by periodate to sever the β -(1 $\rightarrow$ 6)-linkages, the derivatives, designated pachymaran, exhibited pronounced antitumor activity. However, Hamuro et al.^[4] suggested that the difference in the behavior between lentinan or pachymaran and schizophyllan denatured with urea may be attributed to the abundance of β -(1 $\rightarrow$ 6)-linked branches existing in the schizophyllan, which has obvious antitumor activity.

In the previous paper^[7-8], we have identified three heteropolysaccharides (PC1, PC2, PC2-A) and two β -D-glucans (PC3 and PC4) from a fresh sclerotium of *Poria cocos* by using a fractionation procedure that involved extraction with 0.9% NaCl aqueous solution, hot water, and separation on a DEAE-Sephadex column, and with 0.5 mol/L NaOH aqueous solution and 88% formic acid, respectively. Among them, a sodium hydrate-extract PC3 (linear β -(1 $\rightarrow$ 3)-D-glucan) and formic acid-extract PC4 [β -(1 $\rightarrow$ 3)-D-glucan with a few of branches and glucuronic acid] are the major fractions of *Poria cocos* polysaccharides. The weight average molecular weights (M_w) of PC3 and PC4 in 20% cadoxen determined by light scattering method were 20.6×10^4 and 39.8×10^4 , respectively. In this work, the influence of periodate-modification and molecular weight of the two glucans on their antitumor activities were studied. Based on the relationship between structures and the antitumor activities of polysaccharides, attempts have been made to exploit new antitumor polysaccharide.

EXPERIMENTAL

Periodate modification

The periodate modification of PC3 and PC4 including periodate oxidation, borohydride reduction and mild hydrolysis were carried out according to Chihara's method^[3]. UV spectrometer (Shimadzu UV-160) was used to monitor the course of reaction. To study the influence of molecular weight after periodate modification on the bioactivity, the derivatives of periodate oxidation and borohydride reduction were divided into two parts. One part was used for mild hydrolysis to sever the β -(1 $\rightarrow$ 6) branches, if existed. The derivatives so prepared were washed with water, then with ethanol, finally dried in vacuum over P_2O_5 and coded as PC3-2 and PC4-2, respectively. And the other part was hydrolyzed with 88% formic acid at room temperature for 12 h to decrease its molecular weight. The partially hydrolyzed samples were rotary evaporated under reduced pressure to remove formic acid, then dried in vacuum over P_2O_5 and coded as PC3-3 and PC4-3, respectively. To study the impact of molecular weight on the antitumor activity, PC3 was partially hydrolyzed by 88% formic acid at room temperature and 100°C for 12 h, and coded as PC3B and PC3C, respectively. All samples (PC3, PC4, PC3-2, PC4-2, PC3-3, PC4-3, PC3B and PC3C) were subjected to viscometry and antitumor assay.

Viscometry

To monitor the molecular weight change of the samples during the course of modification and hydrolysis, the viscosities of the glucans and their derivatives were measured at $25 \pm 0.05^\circ\text{C}$ by using a modified capillary viscometer. Cadoxen prepared in our laboratory^[8] was used as solvent. The kinetic energy correction was always negligible. Huggins and Kraemer plots were used to estimate the intrinsic viscosities $[\eta]$.

Assay of antitumor activity

Ehrlich ascites carcinoma (EAC) or Sarcoma 180 tumor cells (5×10^6), provided by Pharmacy college, Tongji Medical University, were transplanted subcutaneously into the right groin of Kunming mice weighting 20 ± 1 g. 5-Fluorouracil (5-Fu) and testing samples were dissolved or suspended in distilled water, respectively, and injected intraperitoneally, daily for 7 days, starting 24 h after tumor implantation. The mice were killed on the next day after the last injection and the tumors were excised. The weights of tumors were compared with those in the control mice. The inhibition ratio of tumor growth was calculated as: inhibition ratio (%) = $[(A - B)/A] \times 100\%$, where A is the average tumor-weight of the control mice, and B is that of the 5-Fu or polysaccharide-treated mice.

RESULTS AND DISCUSSION

The UV absorption for periodate at 223 nm indicated that during the course of reaction the absorption of PC3 reaction solution remains almost constant, while that of PC4 reaction solution decreases slightly. It further confirms the conclusion in the reference [7] that PC3 is a linear glucan, while PC4 has some β -(1 $\rightarrow$ 6)-linked branches and glucuronic acid that can react with periodate, resulting in the decrease of periodate absorption.

The intrinsic viscosities $[\eta]$ of PC3, PC4 and their derivatives are listed in Tables 1 and 2. The change of $[\eta]$ indicates that the molecular weights of the samples PC3-3, PC4-3, PC3B and PC3C are lower than those of glucans PC3 and PC4, respectively. The viscosity of PC3-2 is almost identical with that of PC3, because PC3 has no β -(1 $\rightarrow$ 6)-branches, which can be severed by mild hydrolysis. The slightly decrease of viscosity of PC4-2 compared with PC4 verifies that a few of branches in PC4 are severed by this modification. The reduction of the viscosities of PC3-3 and PC4-3 suggests that the original goal of partially hydrolysis is achieved.

The antitumor activities of samples PC3, PC4, PC3-2, PC4-2, PC3-3, PC4-3 and PC3C against EAC tumor are summarized in Table 1, and PC3 and PC3B against Sarcoma 180 in Table 2. PC3 and PC4 hardly exhibit antitumor activities, similar to the result reported by Chihara^[3]. However, PC3-2 and PC4-2 show much

higher activities than native glucans PC3 and PC4, respectively, but their inhibition ratios are slightly lower than 5-Fu, a well-known chemotherapeutic anticancer agent. The activities of PC3-3 and PC4-3, which have lower molecular weight than PC3-2 and PC4-2, respectively, are nearly the same as those of PC3-2 and PC4-2.

Table 1 The intrinsic viscosities $[\eta]$ and bioactivities against EAC tumor for the glucans and their derivatives

Sample	$[\eta]$ (ml/g)	Dose (mg/kg $\times$ days)	Mice	Enhancement ratio of body weight (%)	Weight of tumors $\bar{X}\pm\text{SD}$	Inhibition ratio (%)
Control			10/10	14.5	1.23 $\pm$ 0.32	
5-Fu		20 $\times$ 7	10/10	2.3	0.61 $\pm$ 0.21	50.4
PC3	38.7	41.25 $\times$ 7	10/10	12.3	1.07 $\pm$ 0.28	13.01
PC3-2	38.2	41.25 $\times$ 7	10/10	12.6	0.74 $\pm$ 0.24	39.84
PC3-3	25.4	41.25 $\times$ 7	10/10	13.5	0.82 $\pm$ 0.18	33.33
Control			10/10	12.0	1.34 $\pm$ 0.28	
5-Fu		20 $\times$ 7	10/10	4.5	0.59 $\pm$ 0.12	55.97
PC4	42.4	41.25 $\times$ 7	10/10	11.5	1.10 $\pm$ 0.25	17.91
PC4-2	40.9	41.25 $\times$ 7	10/10	12.8	0.96 $\pm$ 0.26	28.35
PC4-3	27.9	41.25 $\times$ 7	10/10	11.4	0.75 $\pm$ 0.15	44.03
Control			10/10	27.7	1.20 $\pm$ 0.24	
5-Fu		15 $\times$ 7	10/10	0.08	0.58 $\pm$ 0.23	51.67
PC3C	1.10	40 $\times$ 7	10/10	45.2	0.75 $\pm$ 0.38	37.50

Table 2 The intrinsic viscosities $[\eta]$ and bioactivities against Sarcoma 180 tumor for PC3 and PC3B

Sample	$[\eta]$ (ml/g)	Dose (mg/kg $\times$ days)	Mice	Enhancement ratio of body weight (%)	Weight of tumors $\bar{X}\pm\text{SD}$	Inhibition ratio (%)
Control			10/10	51.8	1.09 $\pm$ 0.33	
5-Fu		20 $\times$ 10	10/10	35.2	0.51 $\pm$ 0.13	53.21
PC3	38.7	40 $\times$ 10	10/10	47.0	0.92 $\pm$ 0.22	15.59
PC3B	23.5	40 $\times$ 10	10/10	33.4	0.69 $\pm$ 0.21	36.60

From the comparison of the antitumor activities of all these six polysaccharides, it is conceivable that the antitumor activity could be enhanced greatly by periodate modification. By comparing PC3-2 and PC3-3 or PC4-2 and PC4-3, the decrease in molecular weight after periodate modification does not significantly affect their antitumor actions. However, as listed in Tables 1 and 2, PC3B and PC3C, which have lower molecular weight than native glucan PC3, show higher antitumor activity against Sarcoma 180 tumor than PC3. It suggests that the decrease of molecular weight without periodate modification could also increase the antitumor activity.

It is worth noting that antitumor activities of the derivatives PC3-2, PC4-2, PC3-3, PC4-3, PC3B and PC3C are slightly lower than that of 5-Fu, but their enhancement ratios of body weight are much higher than that of 5-Fu group. It implies that the polysaccharides do have antitumor activities, yet they do not obstruct the

normal growth of the mice, i.e. they do not have the same toxicity as 5-Fu, which kills normal cells as well as cancer cells. This result is in agreement with the conclusion that antitumor action of β -(1 $\rightarrow$ 3) glucan is derived from the stimulation of the immunoresponse mechanisms of the hosts^[9].

We concluded that periodate modification could result in the enhancement of antitumor activities of *Poria cocos* Sclerotium glucans PC3 and PC4. The decrease in molecular weight after periodate modification do not significantly affect their antitumor activities, while the decrease in molecular weight without periodate modification increases their antitumor activity. The antitumor activities of the polysaccharide derivatives PC3-2, PC4-2, PC3-3, PC4-3, PC3B and PC3C are slightly lower than 5-Fu, but their toxicities are much lower than 5-Fu. This makes it possible to produce the nontoxic or low toxic polysaccharide antitumor drugs in the future.

REFERENCES

- [1] Yamada, H., Kiyohara, H., Takemoto, N., Zhao, J., Kawamura, H., Komatsu, Y., Cyong, J., Aburada, M., Hosoya, E., *Planta Med.*, **1992**, 58(2), 166
- [2] Nunoshiba, T., Ohtsuka, A., Nishioka, H., Suemitsu, R., *Sci. Eng. Rev. Doshisha Univ.*, **1990**, 30(4), 166
- [3] Chihara, G., Hamuro, J., Maeda, T., Hamuro, J., Maeda, Y., Arai, Y., Fukuoka, F., *Nature*, **1970**, 225, 943
- [4] Hamuro, J., Maeda, Y., Arai, Y., Fukuoka, F., Chihara, G., *Chem-Biol. Interactions*, **1971**, 3(1), 69
- [5] Japan Rubber Co. Ltd., Jpn. Kokai Tokkyo Koho, *JP Pat.*, 82 38,794 (Cl C 07G1/00) 03 Mar., **1982**
- [6] Wang, S., Wen, Y., Hu, C., *Phytother Res.*, **1995**, 9(6), 448
- [7] Zhang, L. N., Ding, Q., Zhang, P., Feng, H., *Chem. J. Chinese Univ.*, **1997**, 18(6), 990
- [8] Zhang, L. N., Ding, Q., Zhang, P., Zhu, R., Zhou, Y., *Carbohydr. Res.*, **1997**, 303, 193
- [9] Missaki, A., Kawaguchi, K., Mujaji, H., Nagae, H., Hokkoku, S., Kakuta, M., Sasaki, T., *Carbohydr. Res.*, **1984**, 129, 209