Carbohydrate Research, 37 (1974) 245–247

Note

Scleroglucan, an antitumor polysaccharide from *Sclerotium glucanicum**†

PREM P. SINGH, ROY L. WHISTLER,
Department of Biochemistry, Purdue University, Lafayette, Indiana 47907 (U. S. A.)
REIKO TOKUZEN, AND WARO NAKAHARA
National Cancer Center Research Institute, Tsukiji, Tokyo (Japan)
(Received March 16th, 1974; accepted May 6th, 1974)

Antitumor polysaccharides have been obtained from a number of sources, such as yeasts, fungi, lichens, bacteria, and plants. The most active polysaccharides are D-glucans isolated from fungi and lichens[1–10]. Depending upon the source, the glucans vary in structure and in antitumor activity. Lentinan, a (1→3)-β-D-glucan from *Lentinus edodes*, is very effective[2,3] against mouse sarcoma 180, whereas the related pachyman is inactive but gains activity after debranching by partial Smith degradation[1]. Highly branched fungal and yeast glucans containing (1→6)-β-D-glucopyranosyl units on a (1→3)-β-D-glucan backbone are known to be antitumor-active, two examples being schizophyllan from *Schizophyllum commune*[6] and zymosan from yeast cell-walls[11]. We report, here, the antitumor activity of scleroglucan elaborated by *Sclerotium glucanicum*[12]. This glucan has a main chain of (1→3)-β-D-glucopyranosyl units with every third or fourth unit carrying a (1→6)-β-D-glucopyranosyl group[12]. Examination is also made of the antitumor activity of the glucan residue obtained by removal of the (1→6)-β-D-glucopyranosyl side groups by a single Smith degradation.

EXPERIMENTAL

Materials and methods. — Sarcoma 180 tumor cells, 7-day old and in ascites form, were implanted subcutaneously in the right groin of ICR albino mice. This produced a solid tumor at the site of injection in every case. The test samples, dissolved in the minimum amount of saline solution, were injected intraperitoneally daily for 10 days, starting 24 h after tumor implantation. The rate of tumor growth was measured by use of calipers applied to the external surface of the animal and recorded

*Dedicated to the memory of Professor W. Z. Hassid.
†The research performed at Purdue University was supported by a research grant (RO1-CA-10983) from the National Cancer Institute, National Institutes of Health, U.S. Public Health Service, Journal Paper No. 5017 of the Purdue Agricultural Experiment Station.

weekly for 5 weeks. In control mice, the tumor growth was always progressive, while in treated groups some tumors regressed to complete disappearance. At the end of the fifth week, any remaining tumors were dissected out and weighed, and the inhibition ratio determined.

Scleroglucan was obtained from Pillsbury Co., Minneapolis, Minnesota. The polysaccharide was purified by dissolution in 5% sodium hydroxide solution, followed by cold neutralization with dilute acetic acid, and precipitation with ethanol. The precipitated polysaccharide was recovered and dialyzed in water for 24 h. Insoluble material was recovered by centrifugation and was freeze-dried. Ultracentrifugation was conducted in a Spinco analytical centrifuge on a 1% solution of the glucan in 1% aqueous sodium hydroxide, at 44,000 r.p.m. A Smith degradation to remove single (1→6)-β-D-glucopyranosyl branch units from the glucan was performed according to the standard procedure[13]. The debranched glucan had a low solubility in water and was recovered by centrifugation, washed with water, and freeze-dried. In order to give it greater solubility in water, the debranched glucan was further hydrolyzed with 0.1M hydrochloric acid for 1 h at 95°.

RESULTS AND DISCUSSION

Results of bioassay of the glucan and the debranched glucan are given in Table I. Under "complete regression" is given the number of mice in which the tumors completely disappeared after initial active growth, against the total number of mice in the group. It is evident that scleroglucan is effective against sarcoma 180 at a level of 0.5 mg/kg, where it shows 91% inhibition with complete regression in 7 out of 10 mice tested. Pachymaran and lentinan, both linear (1→3)-β-D-glucans, are also antitumor-active; thus, there seemed to be a connection between antitumor activity and linearity of D-glucans of this type. If this were the case, removal of branches from scleroglucan should yield a more antitumor-active glucan. However, removal of branches of scleroglucan gave an inactive glucan. This implies that linearity alone does not account for the activity, as suggested from the findings with pachymaran and lentinan. It is possible that the structure of lentinan and pachymaran may be more complex than first thought.

Scleroglucan is one of the most effective antitumor glucans reported. The structure of scleroglucan is known[12] and it is homogeneous, as indicated by ultracentrifugation. Scleroglucan did not show toxic effects in the test animals, which remained in excellent physical condition throughout the testing period. Scleroglucan is readily available and may prove useful for evaluation of the antitumor action of glucans. Antitumor activity of these glucans is dose-dependent and tends to decrease with an increase in dose (Table I). A similar, dose-dependent, decrease of the antitumor activity has been noted in the case of lentinan[2,3]. It is probable that there are optimum doses for elicitation of a specific immunological response in the host.

It is interesting that scleroglucan and certain other glucans, although having very similar structures, have different levels of activity. The difference in activity

cannot be explained on the basis of present structural knowledge. The inactivity of pachyman may be due to the presence of some (1→2) linkages in the chain, as recently shown by Hoffman *et al.*[14].

TABLE I

ANTITUMOR ACTIVITY OF SCLEROGLUCAN AND DERIVATIVES

Polysaccharide	*Daily dose (mg/kg)*	*Tumor wt. (g)*[a]	*Inhibition (%)*	*Complete regression*[b]
Scleroglucan	0.5	1.0 (0–8.5)	91.6	7/10
	5.0	1.4 (0–6.9)	88.2	5/10
	50.0	7.0 (0–16.0)	41.2	2/10
Debranched scleroglucan	0.5	9.5 (0–21.2)	46.6	2/10
Debranched and hydrolyzed scleroglucan	0.5	14.5 (7.1–19.9)	18.5	0/9
Control	0	11.9 (3.8–15.6)		0/10

[a]Average; in parentheses, minimum and maximum. [b]Ratio of number of mice showing complete regression to number of mice tested.

REFERENCES

1 G. CHIHARA, J. HAMURO, Y. MAEDA, Y. ARAI, AND F. FUKUOKA, *Nature*, 225 (1970) 943–944.
2 G. CHIHARA, J. HAMURO, Y. MAEDA, Y. ARAI, AND F. FUKUOKA, *Cancer Res.*, 30 (1970) 2776–2781.
3 G. CHIHARA, Y. MAEDA, G. HAMURO, T. SASAKI, AND F. FUKUOKA, *Nature*, 222 (1969) 687–688.
4 F. FUKUOKA, M. NAKANISHI, S. SHIBATA, Y. NISHIKAWA, T. TAKEDA, AND M. TANAKA, *Gann*, 59 (1968) 421–432.
5 T. KAMASUKA, Y. MOMOKI, AND S. SAKAI, *Gann*, 59 (1968) 443–445.
6 N. KOMATSU, S. OKUBO, S. KIKUMOTO, K. KIMURA, G. SAITO, AND S. SAKAI, *Gann*, 60 (1969) 137–144.
7 Y. NISHIKAWA, T. TANAKA, S. SHIBATA AND F. FUKUOKA, *Chem. Pharm. Bull.* (Tokyo), 17 (1969) 1910–1916.
8 S. SAKAI, S. TAKADA, T. KAMASUKA, Y. MOMOKI, AND J. SUGAYAMA, *Gann*, 59 (1968) 507–512.
9 S. SHIBATA, Y. NISHIKAWA, M. TANAKA, F. FUKUOKA, AND M. NAKANISHI, *Z. Krebsforsch.*, 71 (1968) 102–104.
10 S. SHIBATA, Y. NISHIKAWA, T. TAKEDA, AND M. TANAKA, *Chem. Pharm. Bull.* (Tokyo), 16 (1968) 2362–2369.
11 W. T. BRADNER, D. A. CLARKE, AND C. C. STOCK, *Cancer Res.*, 18 (1958) 347–351.
12 J. JOHNSON, JR., S. KIRKWOOD, A. MISAKI, T. E. NELSON J. V. SCALETTI, AND F. SMITH, *Chem. Ind.* (London), (1963) 820–822.
13 I. J. GOLDSTEIN G. W. HAY, B. A. LEWIS, AND F. SMITH, *Methods Carbohyd. Chem.*, 5 (1965) 361–370.
14 G. C. HOFFMAN, B. W. SIMSON, AND T. E. TIMELL, *Carbohyd. Res.*, 20 (1971) 185–188.