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RELATIONSHIP BETWEEN THE CHEMICAL STRUCTURE AND ANTITUMOUR ACTIVITY OF GLUCANS PREPARED FROM *Grifora umbellata*

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ABSTRACT

Glucans from *Grifora umbellata* and their enzyme-treated fractions have been tested for antitumour activity against subcutaneously implanted Sarcoma 180 (solid type). The results indicate that the basic common unit of the glucans is of primary importance for the antitumour activity, which is also influenced by the type of sugar linkage, length of branch, branching frequency, molecular size, and molecular conformation.

INTRODUCTION

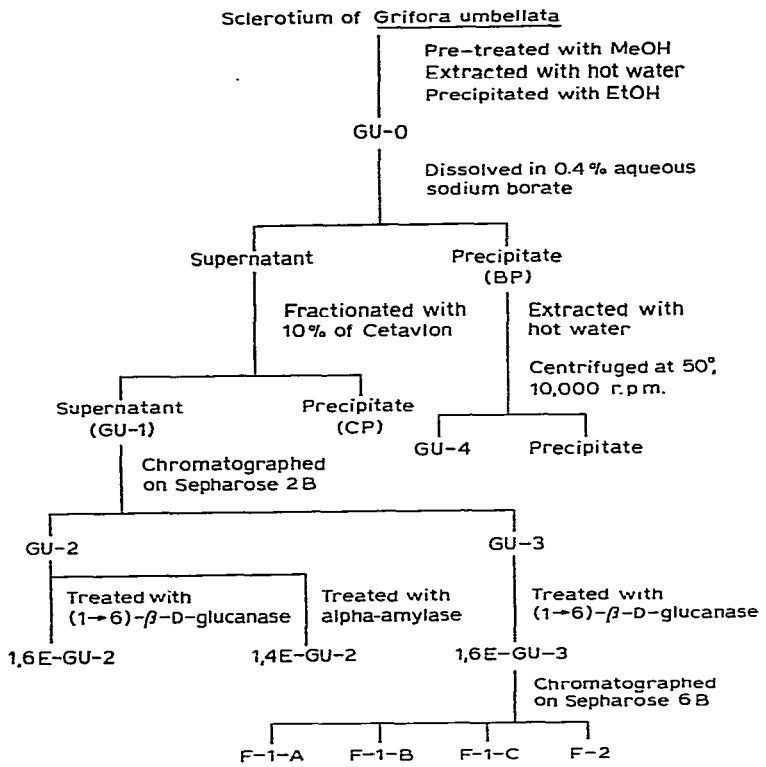
In a previous paper[1], we reported that the water-soluble glucan of *Grifora umbellata* markedly inhibits the growth of subcutaneously implanted Sarcoma 180 in mice. The glucan was further separated into three fractions (GU-2,3,4) by borate–Cetavlon fractionation and gel filtration, as shown in Scheme 1, and the chemical structures of these fractions have been investigated in detail[2]. We now report on the relationship between their structures and antitumour activities.

MATERIALS AND METHODS

Test samples. — Test samples summarized in Scheme 1 were prepared as previously reported[2].

Animals. — Female ICR-JCL mice weighing ~25 g (5 weeks) were obtained from Clea Japan, Tokyo Co. Ltd.

Assay of antitumour activity. — Sarcoma 180 (solid type) was subcutaneously implanted with ~30 mg of tumour tissues. Each test sample of an appropriate concentration in saline was injected at a dose of 1 mg/kg/day or 5 mg/kg/day intra-



Scheme 1. Purification of glucans and preparation of enzyme-treated sample.

peritoneally once daily for 10 days, starting 24 h after the implantation of Sarcoma 180. Assay of antitumour activity of the test sample was carried out as previously described[1].

RESULTS AND DISCUSSION

The results of the antitumour assays are shown in Table I. Of the materials tested, GU-3 and (1→6)-β-D-glucanase-treated GU-2 (abbreviated as 1,6E-GU-2) were the most effective, and complete regression was observed in 9 out of 10 mice at the end of 42 days after the tumour implantation. For GU-2, the antitumour activity (complete regression ratio) was increased on treatment with (1→6)-β-D-glucanase and decreased on treatment with alpha-amylase. (1→6)-β-D-Glucanase-treated GU-3 (1,6E-GU-3) was separated into four fractions (F-1-A, F-1-B, F-1-C, and F-2) by chromatography on a column of Sepharose 6B. Methylation analysis of these fractions by the procedure described in our previous paper[2] gave the following molar ratios of 2,3,4,6-tetra-, 2,4,6-tri-, 2,3,4- + 2,3,6-tri-, and 2,4-di-*O*-methyl-glucitol acetates: 1.0 : 0.7 : 1.9 : 1.0 for F-1-A, 1.0 : 1.0 : 2.1 : 1.0 for F-1-B, 1.0 : 1.3 : 2.5 : 1.0 for F-1-C, and 1.0 : 1.7 : 3.0 : 1.0 for F-2, respectively. These results indicated that the branching ratio decreased with decreasing molecular size, while a significant change was not observed in the ratio of (1→3) and (1→4) + (1→6) linkages of these

TABLE I

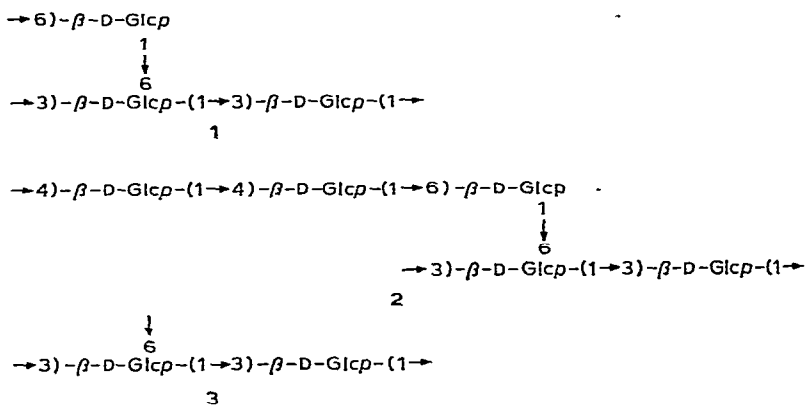
ANTITUMOUR EFFECT OF GLUCANS FROM *Grifora umbellata* AGAINST SARCOMA 180 (SOLID TYPE)

Samples	*No. of mice*	*Average tumour size (mm^3) at intervals*						*Tumour regressed*[a] *at 42 days*	*Tumour not regressed at 42 days*	*Mortality (tumour death) at 42 days*
		7 days	*14 days*	*21 days*	*28 days*	*35 days*	*42 days*			
GU-2	10	667.6	1288.7	607.5	137.6	10.4	0	7/10	—	—
		1557.8	4103.4	16347.5	30476.3	26251.7	29586.6	—	3/10	2/10
1,6E-GU-2	10	920.9	1321.7	1238.7	421.9	0	—	9/10	—	—
		2996.0	3851.3	6128.8	6478.8	6860.0	9784.5	—	1/10	0/10
1,4E-GU-2	10	1033.7	1356.2	1067.9	714.8	0	—	6/10	—	—
		1578.3	2547.1	7946.1	15053.3	26076.1	15933.9	—	4/10	1/10
GU-3	10	503.1	1103.7	843.9	221.4	3.3	0	9/10	—	—
		1355.3	2445.0	2489.6	2547.5	8684.6	9738.4	—	1/10	1/10
1,6E-GU-3	10	1416.9	2213.9	1756.8	421.2	30.3	0	8/10	—	—
F-1-A		643.3	4063.5	8736.3	18200.1	21258.7	24382.3	—	2/10	0/10
1,6E-GU-3	10	1002.5	1965.7	1647.7	190.2	338.2	0	3/10	—	—
F-1-B		1223.8	4111.9	8365.2	19998.4	31028.7	35921.0	—	7/10	3/10
1,6E-GU-3	10	1186.4	1685.3	1690.6	791.7	0	—	5/10	—	—
F-1-C		1287.8	2514.3	6456.8	14270.7	27933.9	31111.2	—	5/10	0/10
1,6E-GU-3	10	1169.6	1919.1	1890.4	511.7	0	—	6/10	—	—
F-2		1079.1	2142.3	5427.1	27421.3	44579.1	49211.8	—	4/10	0/10
GU-4	10	595.9	1302.8	1060.5	719.3	4.5	0	6/10	—	—
		795.4	3817.3	14586.3	18671.4	18098.9	17918.3	—	4/10	2/10
Control (saline, 0.25 ml × 10)	10	1150.7	4034.5	13580.0	26237.2	36413.4	62436.1	—	10/10	9/10

[a]Ratio of number of mice showing complete regression to number of mice tested.

fractions. The highest antitumour activity was observed in GU-3 (complete regression ratio, 9/10) followed by F-1-A (8/10), F-2 (6/10), F-1-C (5/10), and F-1-B which showed the lowest activity (3/10) (Table I). In GU-4, a complete regression ratio (6/10) was observed with a dose of 5 mg/kg/day.

An increase in the antitumour activity of 1,6E-GU-2 was associated with a decrease in (1→6)-β-linkage content and a corresponding increase in content of



(1→4)-α and (1→3)-β linkages. The decrease in content of (1→4)-α linkages and the resulting increase of (1→6)-β and (1→3)-β linkages by amylase treatment caused a decrease in the antitumour activity. In view of these results, long or dense (1→6)-β-linked branches in GU-2 make little contribution to the antitumour activity, but the presence of (1→4)-α-linked branches seems to play a role in the antitumour activity to some extent. Polysaccharide A_3 from *Pleurotus ostreatus*[3] is similar to a part of structure in 1,6E-GU-2.

For GU-3, which is a glucan consisting mainly of (1→6)-β and (1→3)-β linkages, a high activity (complete regression ratio, 9/10) was observed. The structure of 1,6E-GU-3, which is devoid of (1→6)-β-linked branches after the enzyme treatment, resembles that of coriolan[4], which is the extracellular glucan from *Coriolus versicolor*. Coriolan is (1→3)-β-linked and branched at position 6, and shows high activity (25/29) against Sarcoma 180 at a dose of 5 mg/kg/day. The activity, however, was low[5] when the dose was 1 mg/kg/day or 10 mg/kg/day.

GU-4 is a glucan consisting mainly of (1→3)-β-D linkages with branches at position 6. Thus, GU-4 is similar to coriolan, but contains a small proportion of (1→6)-β linkages. The antitumour activity of GU-4 (6/10) at a dose of 5 mg/kg/day was weaker than that of coriolan.

During column chromatography on Sepharose 2B, 1,6E-GU-2 was eluted near the void volume, indicating that removal of (1→6)-β-D-linked branches caused some conformational change or molecular association. It is possible that these molecular changes contribute to an increase in the antitumour activity. On the other hand, alpha-amylase-treated GU-2 (abbreviated as 1,4E-GU-2) was similar to GU-2 in the elution pattern, but its activity decreased.

In the column chromatography of 1,6E-GU-3 on Sepharose 6B, F-1-A was obtained in the void volume. F-1-B and F-1-C were similar to GU-3 in the elution volume, and F-2 was eluted in the maximum elution volume. For these four fractions, the highest activity was observed with F-1-A.

GU-4 was viscid, and was eluted in the void volume during column chromatography on Sepharose 2B.

Concerning the relationship between polysaccharide structure and antitumour activity, Chihara *et al.*[6,7] noted that lentinan, an antitumour glucan, is a (1→3)-β-D-glucan branched at position 6, but that pachyman and laminaran which have linkages similar to those of lentinan, were not active[8]. Their findings showed that for glucans having mainly (1→3)-β linkages some, but not others, show activity. The (1→6)-β-D-glucans (GE-3) from *Gyrophora esculanta* showed the activity[9], but lutean from *Penicillium luteum* was inactive. Moreover, pachymaran and CM-pachymaran, derived from pachyman by removing small proportions of (1→6)-β linkages, showed high antitumour activity[10,11]. Furthermore, U-pachyman, obtained by treating pachyman with 8M urea at 70° for 4 h, possesses a high antitumour activity comparable to that of lentinan[12]. Hence, steric factors, rather than the primary structures of the polysaccharides, seem to play a major role in the antitumour activity. In addition, there exists an optimal dosage for the antitumour polysaccharide to show the highest activity[12]. The α-helix structure of bovine serum albumin may be changed[13] by these polysaccharides, and some components of serum protein may increase specifically and temporarily.

The above discussion clearly suggests that the relationship between the structure and antitumour activity of glucans is a complicated matter. From our experimental results, it became obvious that antitumour glucans from *G. umbellata* have a basic common-unit **1**.

As described above, antitumour activity was increased in 1,6E-GU-2 and decreased in 1,6E-GU-3, except for fraction F-1-A. Thus, the same enzymic treatment resulted in an increase in the activity for GU-2, but a decrease in the activity for GU-3. From these results, it is concluded that the presence of a trisaccharide unit (**1**) containing a (1→3)-β linkage with a branch at position 6 is of primary importance for the antitumour activity of the glucans from *G. umbellata*. Furthermore, the degree of antitumour activity is influenced by a number of factors such as the type of sugar linkage, length of branch, branching frequency, molecular size, and molecular conformation. For example, molecular units **2** and **3** are also considered to be associated with antitumour activity. Moreover, species of animals, dosage, and route of administration seem to affect greatly the appearance of the activity[5]. Further correlation of structure and activity will require a more detailed study of the physicochemical properties of antitumour polysaccharides.

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