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The anti-cancer activity of polysaccharide prepared from Libyan dates (*Phoenix dactylifera* L.)

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Abstract

A glucan of cellular origin has been isolated from Libyan dates (*Phoenix dactylifera* L.) and the structure of the purified glucan was characterized using derivatisation methods including methylation, periodate oxidation, and acetolysis. Glucans were found to exhibit potent antitumor activity; this activity could be correlated to their (1→3)-β-D-glucan linkages. This is the first report on studies of antitumor active compounds occurring in dates.

Keywords: Dates; β-D-Glucan; Polysaccharides

1. Introduction

Dates fruits have been an important crop in arid and semiarid regions of the world since ancient times; they have always played an important role in the economic and social lives of the people of these regions. The fruit of the date palm is therefore well known as a staple food. It is composed of a fleshy pericarp and seed (Ahmed & Robinson, 1999; Ishurd, Zahid, Ahmad, & Yuanjiang, 2001; Ishurd, Zahid, Zhou, & Yuanjiang, 2001; Shinwari, 1992). Polysaccharide material from dates has been used as a functional food and a source of active components in the development of drugs (Puri, Sahai, & Kiran, 2000).

In recent years, some (1→3)-β-D-glucans have attracted attention (Akira, Mariko, Takuma, & Motohiro, 1981) because of their inhibitory action on the growth of certain tumors in animals. Structural correlation to antitumor effects of these polysaccharides is not yet fully understood, except for the molecular-weight dependence of activity of some glucans (Sasaki, Takasuka, Chihara, Maeda, & Gann, 1970; Usui, Iwasaki, Tanaka, & Arakawa, 1983). In a preliminary study we showed that the water-soluble glucan exhibits potent antitumor activity against the growth of Sarcoma-180 solid tumors implanted in mice.

We previously reported the isolation of a novel β-D-glucan from dates (Ishurd, Zahid, Zhou, & Yuanjiang, 2002). In our studies we are concerned with the data cell wall glucan introduced by microbial contaminants in ripe dates which contain a high proportion of sugars, thereby creating a suitable environment for microbial growth. We have therefore studied the structure and antitumour activity of two polysaccharides (1→6)-branched, (1→3)-β-D-glucans, isolated from the fruits of dates.

2. Materials and methods

2.1. Plant material

The β-D-glucans was prepared as described previously (Ishurd et al., 2002) from Libyan dates (*Phoenix dactylifera* L.

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2.2. General

^{13}C-Nuclear magnetic resonance (NMR) spectroscopy at 50 °C was used for structural analysis in samples (65 mg/mL) dissolved in D_2O. Spectra were recorded with a Bruker 500 instrument.

2.3. Smith degradations

Samples (25 mg) of dates fruits were cut into small pieces immediately after harvesting and disintegrated in a blender; they were oxidized with 0.05 M $NaIO_4$ (10 mL) at 20 °C in the dark for 48 h. The oxidation was stopped by the addition of 1,2-ethanediol. The solution was then dialyzed against distilled water. The dialyzed material was reduced, by the addition of sodium borohydride (50 mg) in the dark, with stirring for 15 h at room temperature; it was then neutralized with 50% v/v acetic acid, purified by repeated addition and evaporation of water, and the residue (12 mg) was freeze-dried. Second and third sequences of the Smith degradation were performed under the same conditions.

2.4. Selective hydrolysis of the branched glucans

The native glucans were hydrolyzed with 1.0 M trifluoroacetic acid for 1 h at 100 °C. The supernatant was neutralized by evaporation of the excess acid and fractionated by column chromatography on Sephadex G-15.

2.5. Assay of antitumor activity

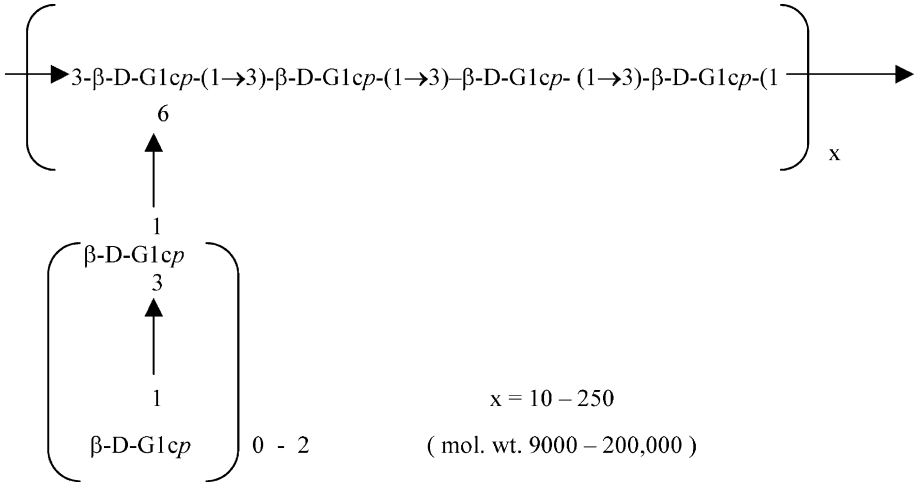
Seven-day-old Sarcoma-180 ascites (0.1 mL, 2×10^6 cells) were transplanted subcutaneously into the right side of female CD1 mice (weighing ~22 g). The test samples, dissolved in saline solution and sterilized for 20 min at 120 °C, and then were injected intramuscularly every day for 10 days, starting 24 h after tumor implantation. At days 10, 20, and 30, the tumor diameter was determined with a caliber square. On day 30, the mice were sacrificed, and the tumors were extirpated and weighed. The inhibition ratio, expressed in a percentage, was calculated by comparing the average weight of the tumors of treated mice with those of untreated controls. This work was done at National Cancer Center Research Institute of China.

3. Results and discussion

We have already described the isolation and characterized the structure of neutral polysaccharides from dates (Ishurd et al., 2002). Briefly, dates were extracted with hot water, and the extract was fractionated by sequential chromatography on columns of DEAE-cellulose, Sephadex 100, and concanavalin A-Sepharose (Pharmacia, Uppsala, Sweden), followed by methylation, Smith degradation, and acetolysis. It was concluded that these polysaccharides have a main chain of (1→3)-linked β-D-glucopyranosyl residues with (1→6)-linked branched saccharide residues (Scheme 1).

3.1. ^{13}C-NMR spectroscopy

The ^{13}C-NMR spectrum of the native date glucans showed multiple resonances (Fig. 1), consistent with their branched (1→3)-β-D-glucan structure. The β-configuration of D-glucosyl residues was clearly evidenced by the presence of two anomeric peaks in the region δ103.5 and δ104, and branchings at C-6 were shown by signals of *O*-substituted C-6 at δ70.8 and of unsubstituted C-6 at δ61.9. The predominance of the latter, together with the typical signal of *O*-substituted C-3 at δ85.6, supported the notion of a high proportion of (1→3) linkages in a linear arrangement



Scheme 1. Structure of D-glucan from Libyan date.

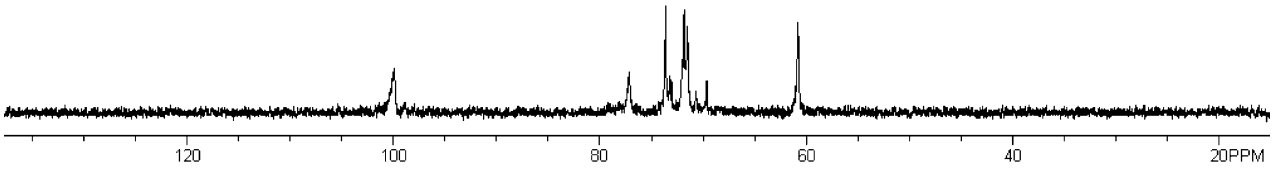


Fig. 1. ^{13}C-NMR spectrum of native D-glucan from Libyan date.

as was previously demonstrated by chemical analysis. The multiplicity of the signals and the broad C-3 signal at δ85.6 could be ascribed to the presence, in the glucans, of linear (1→3,1→6) branch point and terminal β-D-glucopyranosyl residues. Since the number of terminal residues equals the number of branched points, it was not possible to differentiate their signals on the basis of their relative intensity. However, the signals of the (1→3)-linked linear β-D-glucan could be assigned in the spectrum of the Smith-degraded glucan. After three sequential periodate oxidations, only six well-defined signals were left in the spectrum of the degraded polysaccharide. The assignment of the carbon resonances is shown in Table 1. This confirmed that the linear-extended (1→3) side chains do not exceed three β-D-glycopyranosyl residues. By comparing differences between the spectra of the native and Smith-degraded polymer, it was possible to assign a few other signals in the spectrum of the branched glucan (Table 1). In particular, the second anomeric signal could be ascribed to branching on the linear glucan, either to the (1→3,1→6) branch points or to the terminal residues.

Linear (1→3)-β-D-glucans with various (1→6) linkages could also be obtained by direct mild hydrolysis of the parent material with 0.5 M trifluoroacetic acid for 15 h at 20 °C, which is selective for (1→6)-linkages. The spectrum of this non-Smith-degraded, partially hydrolyzed polysaccharide resembled the spectrum of the Smith-degraded glucans (see Table 1), but still contained numerous extraneous peaks, which indicated that the hydrolysis of the side chains was not complete.

The ^{13}C-NMR-Peak positions are found to be exactly the same as those of linear (1→3)-β-D-glucans and because of the extent of occurrence of (1→6)-linked β-D-glucopyranosyl residues is very small, and the peak separation between (1→3)- and (1→6)-linked β-D-glucopyranosyl residues is not so large (see Table 1), compared with other line-widths, the peaks of the (1→6)-linkages might be buried under the intense signals of (1→3)-linkages. Naturally, the peaks due to (1→3)-linked side chains are collapsed into the peaks that are due to the backbone. The higher peak of low intensity at 61.9 is clearly ascribable to C-6 of (1→3)-linked β-D-glucopyranosyl residues. The other signals of this linked residue (C-1–C-5) seem to be almost completely suppressed.

The incomplete hydrolysis of the appending chains may explain why the hydrolyzed glucan did not form a gel, as does the (1→3)-β-D-glucan scleroglucan (Rinaudo & Vincendon, 1982) and the antitumor β-D-glucan isolated from the fruiting body of *Volvariella volvacea* (Misaki, Nasu, Sone, & Kishida, 1986). However, liquid chromatography analysis of the dialyzable material obtained during partial hydrolysis of our material showed glucose

Table 1
^{13}C-NMR data for D-glucans from Libyan dates

D-Glucan	Residues and linkages	Chemical shifts (δ)					
		C-1	C-2	C-3	C-5		C-6
Native	3)-β-D-G1c*p*-(1→ 6 ↑ 1 β-D-G1c*p* 3 ↑ 1	103.5[a] 103.4	74.6[a] 74.1	85.9 85.6	69.3 69.3	76.7 75.6	70.8 61.9
	$(\beta\text{-D-G1c}p)_{0\text{-}2}$	104.2	73.7	76.6	70.8	77.1[b]	61.9
Smith degraded	→3)-β-D-Glc*p*-(1→	103.4	74.1	85.7	69.3	76.7	61.9
Partially hydrolysed	→6)-β-D-Glc*p*-(1→	103.4	74.0	85.3	70.4[a]	76.7	61.9
	→3)-β-D-Glc*p*-(1→	–[c]	–[c]	–[c]	69.3	76.6	61.9

[a] Assignment made by Colson et al.
[b] Assignment based on the spectrum of gentiobiose.
[c] Not observable due to overlapping peaks.

Table 2
Antitumor effect of mixed glucans from Libyan dates on solid Sarcoma-180

Factor, sample	Dose[a] (mg/kg)	Average tumor weight (g)	Inhibition (%)[b]	Complete[c] regression	Significance[d] ($P<$)
Dose dependence					
Control		4.50		0/10	
D-Glucans	0.2	0.35	92	8/9	0.02
	1	0.002	99	9/10	0.1
	5	0.01	98	7/8	0.01
Effect of pretreatment[c]					
Control		5.4		0/9	
D-Glucans	1[e]	0.31	95	8/10	0.001

[a] D-Glucans were administered by intraperitoneal injections of the glucans, dissolved in saline solution daily for 10 days, starting 24 h after tumor inoculation.
[b] $[(C-T)/C]\times 100$, where T is the average tumor weight of treated group and C is the average tumor weight of control group.
[c] Number of tumor-free mice/number of treated mice, where treated mice were given 1 mg/kg daily for 10 consecutive days, starting 11 days prior to tumor inoculation.
[d] Evaluated according to Student's t test with $P<0.05$ as a criterion of a significant difference.
[e] Treatment with 1 mg/kg daily for 10 consecutive days, starting 11 days prior to tumor inoculation.

to be the predominant sugar, with only traces of dimers and trimers, thus confirming that the side chains consist essentially of single terminal D-glucopyranosyl groups (Ishurd et al., 2002).

3.2. Antitumor activity

The antitumor activity of date glucan was tested on the allogenic solid Sarcoma-180 in mice. This tumor model is known to be very useful for testing immunomodulating substances. In all experiments $\sim 2\times 10^6$ Sarcoma-180 tumor cells (ascites form) were subcutaneously transplanted into the right side of female CD1 mice. The evaluation of the antitumor activity was performed by measuring the tumor diameter at 10-day intervals, and by determining the weight of the excised tumors at day 30 after tumor inoculation. The antitumor effect of the date glucans was dose dependent, with an optimum activity at 1 mg/kg (Table 2).

In all experiments, during the first 10 days after tumor inoculation the tumors of the glucan-treated mice increased at the same rate as the tumors of the control group. After about 15 days, the tumor diameter in the treated group decreased, and in many cases, the tumors showed complete regression by day 30. This delayed antitumor effect suggests an indirect mode of action of the date glucans.

To confirm an immunomodulating action, we examined the antitumor effect after pretreatment of the mice with the glucans; this pretreatment started 11 days before tumor inoculation and was continued for 10 consecutive days, by daily intraperitoneal injections of the polysaccharides. The results clearly indicated that pretreatment has almost the same effectiveness as treatment after tumor inoculation (Table 2). This indirect mode of antitumor action suggests an involvement of the immune system.

Our data are consistent with previous reports concerning a possible correlation between antitumor activity and (1→3)-β-D-glucan structure (Yoshioka, Tabeta, Satro, & Uehare, 1985; Testsuro, Lin, Mase, & Tomio, 1967). The glucans of dates contain two major fractions of different molecular masses which have been separated by Sephadex column chromatography fractions I: $M_t\sim 200{,}000$ and fraction II: $M_t\sim 10{,}000$. These fractions differ by the presence of (1→6)-branched chains consisting of D-glucose and D-Glc*p* -(Glcp-(1→3)-D-Glc*p* groups for the lowest molecular mass polysaccharide or D-Glc*p* -(1→3)-D-Glc*p* for the highest-molecular-mass polysaccharide. These polysaccharides were characterized as a mixture of linear (1→3)-β-D-glucan with various (1→6)-linked mono-, di- and tri-saccharide branches having 0, 1, or 2 (1→3)-β-D-glucopyranosyl residues. The mechanisms of the influence of molecular mass and of the nature of branching chains on the antitumor activity are under further investigation.

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