Chinese Chemical Letters Vol. 12, No. 11, pp 967 – 970, 2001
http://www.imm.ac.cn/journal/ccl.html

Structural Characterization and Chemical Modification of a Glucan from Spores of *Ganoderma lucidum*

Xing Feng BAO, Ji Nian FANG*

Shanghai Institute of Materia Medica, Shanghai Institutes for Biological Sciences,
Chinese Academy of Sciences, Shanghai 200031

Abstract: A linear α-D-(1→3)-glucan, named PSG, was obtained from the spores of *Ganoderma lucidum*. The synthesis of positively and negatively charged polyelectrolytes from PSG was developed. Amine groups and carboxymethyl groups were introduced through nucleophilic substitution with 3-chloropropylamine or chloroacetic acid, respectively. Reaction conditions were varied to obtain insight into the influence of variables on the degree of substitution.

Keywords: α-D-(1→3)-glucan, chemical modification, aminopropylation, carboxymethylation.

The fungus of *Ganoderma lucidum* (Fr.) Karst has been viewed a folk medicine in China and its medical effects on cancer, hypertension, hepatitis and hyperglycemia were demonstrated by pharmacological studies in the last two or three decades[1,2]. On the other hand, interesting biological activities and physico-chemical properties have been reported for the polyelectrolytes derived from neutral polysaccharides, including anti-tumor, anti-HIV and formation of charged hydrogels. In the previous papers, we reported several polysaccharides from the spores of *Ganoderma lucidum*[3-5]. The present paper describes the structural elucidation of a α-D-(1→3)-glucan, isolated from the dilute-alkaline solution extract of the spores of this fungus, and the synthesis of a series of positively and negatively charged polyelectrolytes from this native polysaccharide.

PSG, with a molecular weight of 1.26×10^5, was obtained as water-insoluble pale yellow power (yield: 3.62% from the material). Sugar compositional analysis of PSG indicated that it was only composed of D-glucose. The high positive value of the specific rotation, $[\alpha]_D^{20}$ +243.71 (*c* 0.684, 0.5 mol/L NaOH), and the characteristic absorption at 840 cm^{-1} in the IR spectrum were indicative of the α-D-glucosidic linkages. The ^{13}CNMR spectrum of PSG showed 6 peaks in a close height, among which the signal at δ 102.62 was ascribed to the C-1 of glucosyl residues, and the signal at δ 84.73 was attributed to the O-substituted C-3 of glucosyl residues. Other signals in the spectrum at δ 75.06, δ 73.44, δ 72.95 and δ 63.46 were from the O-substituted C-5, C-2, C-4 and C-6 of glucosyl residues, respectively. All these data suggested that this glucan was a linear α-D-(1→3)-glucan. Methylation analysis of PSG revealed that two components corresponding to 1, 3-linked (98.5%) and terminal (1.4%) glucopyranosyl

---

* E-mail: jnfang@mail.shcnc.ac.cn

residues. This result confirmed the deduction from the ^{13}CNMR data.

Table 1 Influence of the reaction conditions on the aminopropylation of PSG

PSG	[Amine]/[PSG][a]	[NaOH]/[PSG][b]	T (°C)	Reaction time (h)	Yield (g)	N (%)	DS[c]	$[\alpha]_D$ (°)
1	2.0	5.0	50	24	0.91	0.48	0.06	+215.64
2	2.0	5.0	80	24	0.85	0.52	0.06	+208.01
3	3.5	8.0	80	24	0.63	0.99	0.13	+164.56
4	3.5	8.0	80	36	0.52	1.16	0.15	+136.68
5	5.0	11.0	80	24	0.34	2.48	0.35	+120.93
6	5.0	11.0	95	36	0.26	4.48	0.74	+97.44

[a] Ratio of initial concentration of 3-chloropropylamine to PSG monomer units.
[b] Ratio of initial concentration of NaOH to PSG monomer units.
[c] Degree of substitution with respect to the monomer units of PSG.

We used the linear α-D-(1→3)-glucan as the starting material to synthesize a series of positively and negatively charged polyelectrolytes. Substitution reactions with amino-containing electrophilic are commercially applied on, for example, starch[6], however, the degree of substitution is always quite low (of the order of DS 0.15). We tried to obtain material with a higher degree of substitution with 3-chloropropylamine as the etherifying agents (**Table 1**) under similar conditions, as previously described for the aminoethylation of scheroglucan[7]. Since we used the HCl salt of the amine, two equivalents of sodium hydroxide were necessary. In general, a small excess of NaOH was used. From **Table 1**, it is clear that highly substituted product can be obtained by using a molar excess of amine at relatively harsh conditions. However, these products were highly degraded as was indicated by the yield and the [α]. Clearly, under these conditions side reactions became important, which were not obvious under milder conditions. For example, in several cases a conglomeration of the reactors formed and the color became deeper. Possibly, a polymerization of the amine occurred, which was not investigated further. To improve the yield of the products, several experiments were carried out at lower reaction temperatures or shorter reaction time with a lower ratio of amine-PSG. The obtained degree of substitution was lower under these conditions, but yields were much better. On the other hand, the low-substitution products are water-insoluble or poor soluble in water, whereas the high-substitution products are easy to be dissolved in water.

Table 2 Influence of the reaction condition on the carboxymethylation of PSG.

PSG	[CA]/[PSG][a]	[NaOH]/[PSG][b]	T (°C)	Reaction time (h)	Yield (g)	DS[c]	$[\alpha]_D$ (°)
1	2.0	3.5	30	3	1.07	0.28	+215.64
2	2.0	3.5	50	3	1.25	0.45	+208.01
3	3.0	4.5	70	3	1.32	0.69	+164.56
4	3.0	4.5	50	3×2[d]	1.45	1.12	+136.68
5	3.0	4.5	70	3×2[d]	1.51	1.44	+120.93

[a] Ratio of initial concentration of chloroacetic acid to PSG monomer units.
[b] Ratio of initial concentration of NaOH to PSG monomer units.
[c] Degree of substitution with respect to the monomer units of PSG.
[d] Experiment was carried out with two successive reactions.

Carboxymethylation of polysaccharide, most notably cellulose, with chloroacetic acid is a well-documented reaction. In the previous reports, this nucleophilic substitution was generally performed in aqueous solution at such a pH (>13) that at least part of the alcohol functions of the polysaccharide were dissociated. However, the polysaccharide would be decomposed and β-elimination reaction and hydrolysis of the etherifying agent were unavoidable under such harsh reaction conditions. So we used the *iso*-propanol as the medium to perform this substitution reaction. In order to determine the influence of variables on the degrees of substitution, several experiments were carried out under different conditions (**Table 2**). Unlike of the aminopropylation, a small excess of one equivalent of NaOH with respect to the molar amount of chloroacetic acid was employed. As shown in **Table 2**, it can be found that increasing the reaction temperature and the ratio of chloroacetic acid–PSG yields products with a higher degree of substitution. By comparison with aminopropylation, the yield of carboxymethylation of PSG is higher and the products are easily dissolved in water.

Experimental

Preparation of aminopropylation of PSG

3-Chloropropylamine was synthesized according to the procedures described by Speziale and Hamm using 3-amino-1-propanol as the starting material (yield: 60.8%)[8]. Glucan PSG (1.0 g) was dissolved in a solution of NaOH in water (40 mL). The amount of NaOH, 3-chloropropylamine and the reaction temperature and time were varied (**Table 1**). After the reaction, the mixture was cooled with ice-bath, diluted and neutralized with 1 mol/L HCl. The product was isolated after extensive dialysis and lyophilization. The degree of substitution was determined by elemental analysis.

Preparation of carboxymethylation of PSG

Glucan PSG (1.0 g) was suspended in a medium of *iso*-propanol (30 mL) at room temperature. After 30 min, a certain amount of 30% solution of NaOH (**Table 2**) was slowly added with stirring over a period of 15 min. Vigorous stirring was continued about 90 min to prevent gel formation. Then a certain amount of chloroacetic acid (**Table 2**) was added and the mixture was stirred for another 3 h at varied reaction temperature (**Table 2**). After the reaction, the mixture was subsequently cooled, diluted and neutralized. The product was isolated as described above and the degree substitution was determined by titration.

References

1. S. Y. Wang, M. L. Hsu, H. H. Hsu, C. H. Tzeng, S. S. Lee, M. S. Shiao, C. K. Ho, *Int. J. Cancer*, **1997**, *70*, 699.
2. G. Franz, *Planta Med.*, **1989**, *55*, 493.
3. X. F. Bao, Q. Dong, J. N. Fang, *Acta Biochim. Biophy. Sin.*, **2000**, *32*, 557.
4. X. F. Bao, J. N. Fang, *Acta Bot. Sin.*, **2001**, *43*, 312.

5. X. F. Bao, C. P. Liu, J. N. Fang, X. Y. Li, *Carbohydr. Res.*, **2001,** *332*, 67.
6. H. L. Han, F. W. Sosulski, *Starch/Stärke*, **1998**, *50*, 487.
7. E. J. Nooy, V. Rori, G. Masci, M. Dentini, V. Crescenzi, *Carbohydr. Res.*, **2000**, *324*, 116.
8. J. Speziale, P. C. Hamm, *J. Am. Chem. Soc.*, **1956**, *78*, 2556.

Received 16 April, 2001