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Solution properties of a water-insoluble (1 → 3)-α-D-glucan isolated from *Poria cocos* mycelia

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Abstract

A water-insoluble (1 → 3)-α-D-glucan (wc-PCM3-I) was isolated from the *Poria cocos* mycelia by extracting with 0.5 M NaOH aqueous solution at room temperature. wc-PCM3-I dissolved in dimethylsulfoxide (DMSO) containing 0.25 M LiCl was fractionated by a nonsolvent addition method into 10 fractions, and the solution properties of seven of them were studied by size exclusion chromatography combined with laser light scattering and viscometry at 25 °C. The dependences of intrinsic viscosity $[\eta]$ and radius of gyration $\langle s^2\rangle_z^{1/2}$ on weight-average molecular mass M_w for this glucan were found to be $[\eta]=(9.77\pm0.1)\times10^{-3}M_w^{0.77\pm0.02}$ ($cm^3\ g^{-1}$) and $\langle s^2\rangle_z^{1/2}=(1.83\pm0.4)\times10^{-2}M_w^{0.58\pm0.04}$ (nm) in the range of M_w from 4.16 to 29.0 × 10^4. On the basis of current theories for wormlike chain model, the conformation parameters of wc-PCM3-I were calculated to be 690 (nm^{-1}) for molar mass per unit contour length (M_L), 4.5 (nm) for persistence length (q), and 11.9 for characteristic ratio (C_∞), suggesting that the (1 → 3)-α-D-glucan exists as a relatively extended flexible chain in the 0.25 M LiCl/DMSO solution.

Keywords: *Poria cocos* mycelia; (1 → 3)-α-D-glucan; Conformation; Molecular mass; Intrinsic viscosity; Solution properties

1. Introduction

Poria cocos is an important herb in China and other Asian countries, and polysaccharides (mainly glucan) extracted from it have mitogenic, complement activating (Yamada et al., 1992), antimutagenic, anti-inflammatory effect (Schinella, Tournier, Prieto, Mordujovich de Buschiazzo, & Rios, 2002), and immunoactivities (Wang, Wen, & Hu, 1995). Recently, much work focused on the mechanism of antitumor activity has been done on the polysaccharides from *P. cocos*, indicating that the polysaccharides may potentiate the host defense mechanism through the activation of immune system (Lee & Jeon, 2003; Park, Kim, Kim, Kim, & Kim, 2003; Yu & Tseng, 1996). It has been reported that molecular mass, the degree of branching, chain conformation, and chemical modification of the polysaccharides significantly affect their antitumor and immunomodulatory activities (Adachi, Ohno, Ohsawa, Oikawa, & Yadomac, 1990; Chihara, 1992; Kiho, Yoshida, Nagai, Ukai, & Hara, 1989; Mischnick, 1995; Ohno, Miura, Chiba, Adachi, & Yadomae, 1995). Interestingly, two sulfated derivatives from the (1 → 3)-β-D-glucan and (1 → 3)-α-D-glucan all have significantly higher antitumor activity against Ehrlich ascites carcinoma than the underivatized original glucans, correlating the antitumor activity with the water solubility increase due to the expanded chains of the sulfated glucan in aqueous solution (Zhang, 2000). Therefore, a basic understanding of the secondary structure of the polysaccharides is essential for the successful interpretation of their antitumor action.

Using infrared spectrum, gas chromatography, ^{13}C NMR, and other techniques, we have revealed that the water-insoluble polysaccharide (PCM3-I) extracted from *P. cocos* mycelia is a linear (1 → 3)-α-D-glucan (Jin et al., 2003). It has been reported that *P. cocos* is mainly composed of a polysaccharide known as pachyman, which is a (1 → 3)-β-D-glucan (Chihara, Hamuro, Maeda, Arai, & Fukuoka, 1970). The solution properties of pachyman from *P. cocos* sclerotium (Zhang et al., 1999) and *P. cocos* mycelia (Ding, Zhang, Xu, Zhang, & Wu, 2001) have been investigated in our laboratory. However, the solution

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properties and conformation of the water-insoluble (1 → 3)-α-D-glucan from *P. cocos* mycelia have been scarcely published. Nowadays, size exclusion chromatography combined with laser light scattering (SEC-LLS) has been demonstrated to be a very powerful method for characterization of polysaccharides (Peng, Zhang, Zeng, & Xu, 2003; Picton, Bataille, & Muller, 2000; Ueda, Itoh, Matsuzaki, Ochiai, & Imamura, 1998). Thus, in the present work, we prepared (1 → 3)-α-D-glucan fractions having different molecular mass, and characterized them by SEC-LLS and viscometry in dimethylsulfoxide (DMSO) containing 0.25 M lithium chloride (0.25 M LiCl/DMSO), hoping to deduce some conformational characteristics of the glucan. Then the data of the intrinsic viscosity ([η]) and weight-average molecular mass (M_w) were analyzed on the basis of the wormlike cylinder model (Yamakawa & Fujii, 1974; Yamakawa & Yoshizaki, 1980) to obtain molecular parameters of the α-glucan in solution.

2. Experimental

2.1. Samples

The (1 → 3)-α-D-glucan sample, wc-PCM3-I, previously extracted from *P. cocos* mycelia with 0.5 M NaOH (Jin et al., 2003) were fractionated as follows. A mixture of acetone and 0.25 M LiCl/DMSO (4:1 by volume) was slowly added to a 0.5% sample in 0.25 M LiCl/DMSO solution at 25 °C until the solution became turbid. The liquid was then heated to 50 °C, and its turbidity was slightly weakened. After cooled to 25 °C and allowed to stand for 12 h, the turbid solution was centrifuged (6000 rpm, 15 min) at about 25 °C to be separated into liquid and gel phase. The gel was removed and the supernatant was subjected to the next fractionation until wc-PCM3-I was divided into 10 fractions. (Some of the fractions were further fractionated by repeating the above procedure once or twice to extend the molecular mass range of the study as wide as possible.) Seven parts of the 10 fractions obtained that had sufficient quantity for molecular mass determination were chosen and coded as F-1, F-2, …, F-7. These fractions were reprecipitated individually from 0.25 M LiCl/DMSO solutions into 80% aqueous acetone, washed with anhydrous acetone four times, and vacuum-dried for seven days to obtain a white powder.

2.2. Viscometry

The viscosity of each fraction in 0.25 M LiCl/DMSO solution was measured at 25 °C using a conventional Ubbelohde viscometer. The kinetic energy correction was negligible. Huggins and Kraemer plots were used to estimate the intrinsic viscosity [η] by extrapolation to infinite dilution as follows

$$\eta_{sp}/c = [\eta] + k'[\eta]^2 c \tag{1}$$

$$(\ln \eta_r)/c = [\eta] + k''[\eta]^2 c \tag{2}$$

where k' and k'' are constants for a given polymer under given conditions in a given solvent; η_{sp}/c, the reduced specific viscosity; $(\ln \eta_r)/c$, inherent viscosity.

2.3. SEC-LLS measurements

SEC-LLS measurements were carried out on a multi-angle laser photometer (DAWN®DSP Wyatt Technology Co., Santa Barbara, CA, USA) at 633 nm in an angular range from 26 to 142° combined with a P100 pump (Thermo Separation Products, San Jose, USA) equipped with a TSK-GEL G4000 H_6 column (7.5 mm × 300 mm) for 0.25 M LiCl/DMSO and a differential refractive index detector (RI-150, Japan) at 25 °C. The eluent was degassed 0.25 M LiCl/DMSO solution purified by a 0.45 μm filter (PTFE, Puradisc 13 mm Syringe Filters, Whatman, England). The samples were dissolved in 0.25 M LiCl/DMSO overnight with stirring. The injection volume was 200 μl with the concentration of 2–3 mg cm^{-3} for each fraction, and the flow rate was 1.0 cm^{-3} min^{-1}. The calibration of the photometer was done with ultra-pure toluene, and the normalization of the RI detector was done with pullulan standards. The refractive index increments (dn/dc) were measured with an optilab refractometer (DAWN®DSP, Wyatt Technology Co., USA) at 633 nm and 25 °C. The value of dn/dc of the samples in 0.25 M LiCl/DMSO was determined to be 0.055 cm^{-3} g^{-1}. Astra software was utilized for the data acquisition and analysis.

3. Results and discussion

3.1. Mark-Houwink equation

The SEC patterns of the fractions F-1 to F-7 in 0.25 M LiCl/DMSO at 25 °C are shown in Fig. 1. Obviously, each fraction having a different M_w was eluted at a different elution volume, namely elution time. Moreover, SEC-LLS, an absolute method, is basically applicable for the wc-PCM3-I fractions over the M_w range tested in this research. The results from Fig. 1 indicated that the molecular mass distributions of the fractionated samples of wc-PCM3-I are relatively narrow. The single peak of each sample showed that there was no aggregation of the glucan in 0.25 M LiCl/DMSO. Therefore, the seven fractions could be used for the investigation of their solution properties. The values of M_w, $\langle s^2\rangle_z^{1/2}$ and polydispersity index M_w/M_n obtained from SEC-LLS, [η] and Huggins coefficient' values k' of these fractions determined by viscometry are summarized in Table 1.

The molecular mass dependence of [η] for the (1 → 3)-α-D-glucan in 0.25 M LiCl/DMSO at 25 °C is shown in Fig. 2. The Mark-Houwink equation for the α-glucan with M_w range from 4.16 to 29.0 × 10^4 (the solid line in Fig. 2),

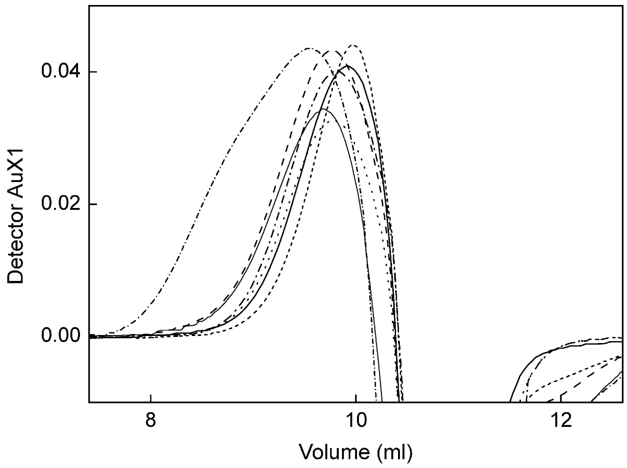

Fig. 1. Size exclusion chromatogram of the fractions from F-1 to F-7 (from left to right) determined by RI-150 detector (detector AUXI) in 0.25 M LiCl/DMSO at 25 °C.

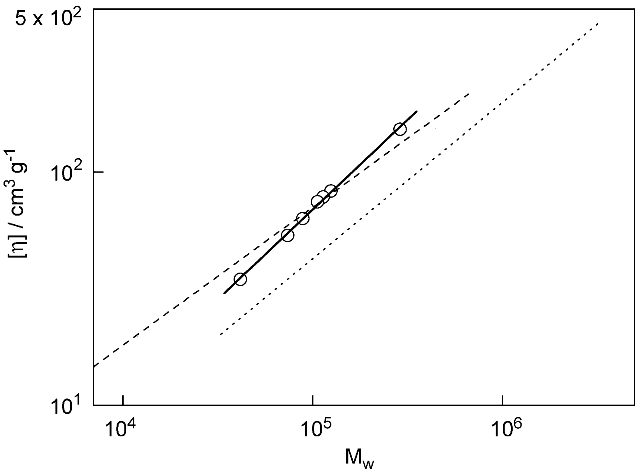

Fig. 2. The double-logarithmic plot of $[\eta]$ against M_w for the fractions of wc-PCM3-I in 0.25 M LiCl/DMSO at 25 °C, in comparison with the linear $(1\rightarrow3)$-α-D-glucan (- - -) from *Ganoderma lucidum* in 0.25 M LiCl/DMSO at 25 °C and pullulan in water at 25 °C (…).

could be represented by:

$$[\eta] = (9.77 \pm 0.1)\times 10^{-3} M_w^{0.77\pm0.02}\ (\mathrm{cm^3\ g^{-1}}) \tag{3}$$

The exponent α value is related to the shape of the macromolecular and nature of the solvent. For flexible linear polymer in a good solvent, the α value is usually in the range from 0.5 to 0.8. Therefore, the α value of 0.77 for the $(1\rightarrow3)$-α-D-glucan in 0.25 M LiCl/DMSO lies in the range of a flexible chain polymer.

Some published data of M_w and $[\eta]$ for linear $(1\rightarrow3)$-α-D-glucan from the fruiting bodies of *Ganoderma lucidum* in 0.25 M LiCl/DMSO (Chen, Zhang, Nakamura, & Norisuye, 1998) and pullulan, a linear α-D-glucan (maltotriose as the repeating unit through the α-$(1\rightarrow6)$-glycosidic linkage) in 0.02% aqueous sodium azide solution (Kato, Okamoto, Tokuya, & Takahashi, 1982), are also compared in Fig. 2. The slope of the line for the fractions of wc-PCM3-I was larger than those for $(1\rightarrow3)$-α-D-glucan from *G. lucidum* and for pullulan, implying a relatively low flexibility of the chains. Compared with other α-glucans (Chen et al., 1998; Kato et al., 1982) shown in Fig. 2, the values of q (4.5 nm) and C_∞ (11.9) of the wc-PCM3-I were larger than those of $(1\rightarrow3)$-α-D-glucan from *G. lucidum* ($q = 1.5$ nm, $C_\infty = 7.2$) and for pullulan ($C_\infty = 4.3$), suggesting that the $(1\rightarrow3)$-α-D-glucan in wc-PCM3-I was more extended in its conformation than the other two polymers. It can be believed that the intra- and inter-molecular interactions of these α-D-glucans in solution are different, strongly affecting the chain flexibility.

Table 1
Results from SEC-LLS and viscometry for the wc-PCM3-I fractions

Sample	$M_w \times 10^{-4}$	M_w/M_n	$\langle S^2\rangle_z^{1/2}$ (nm)	$[\eta]$ (ml g^{-1})	k'
F-1	29.0	1.20	29.2	152	0.36
F-2	12.5	1.35	21.2	82.9	0.32
F-3	11.4	1.87	16.7	78.1	0.32
F-4	10.6	1.92	20.9	74.5	0.41
F-5	8.90	1.35	15.8	63.2[a]	–
F-6	7.41	1.20	16	53.5	0.38
F-7	4.16	1.38	14.6	34.6	0.35

[a] Data obtained by one point method.

3.2. Chain conformation

Based on the data of M_w, $\langle s^2\rangle_z^{1/2}$ and $[\eta]$ of the fractions of wc-PCM3-I, a wormlike cylinder model can be used for conformational characterization of the $(1\rightarrow3)$-α-D-glucan. Bohdanecky (1983) and Bushin, Tsvetkov, Lysenko, Ye, and Yemelyanov (1981) had independently indicated that the Yamakawa–Fujii–Yoshizaki (Y–F–Y) theory for $[\eta]$ (Yamakawa & Fujii, 1974; Yamakawa & Yoshizaki, 1980) of an unperturbed wormlike cylinder could be represented approximately by

$$(M^2/[\eta])^{1/3} = A_\eta + B_\eta M^{1/2} \tag{4}$$

$$A_\eta = \phi_{0,\infty}^{-1/3} A_0 M_L\ (\mathrm{g^{1/3}\ cm^{-1}}) \tag{5}$$

$$B_\eta = \phi_{0,\infty}^{-1/3} B_0 (2q/M_L)^{-1/2}\ (\mathrm{g^{1/3}\ cm^{-1}}) \tag{6}$$

where q and M_L are the persistence length and the molar mass per unit contour length, respectively. The value of A_0 and B_0 had been tabulated (Bohdanecky, 1983), and $\phi_{0,\infty}$ was found to be 2.87×10^{23}. The plot of $(M_w^2/[\eta])^{1/3}$ vs. $M_w^{1/2}$ is shown in Fig. 3. Substituting the intercept and slope of this plot into Eqs. (4)–(6) yielded 690 nm^{-1} for M_L and 4.5 nm for q, indicating a flexible chain characteristic.

The double-logarithmic plot of $\langle s^2\rangle_z^{1/2}$ against M_w for the $(1\rightarrow3)$-α-D-glucan fractions is shown in Fig. 4. For the fractions with M_w ranged from 4.16 to 29.0×10^4, the plot can be represented by the following equation:

$$\langle s^2\rangle_z^{1/2} = (1.83 \pm 0.4)\times10^{-2} M_w^{0.58\pm0.04}\ (\mathrm{nm}) \tag{7}$$

The exponent of 0.58 was in the range of a normal flexible polymer (0.5–0.6). Based on Kratky–Porod

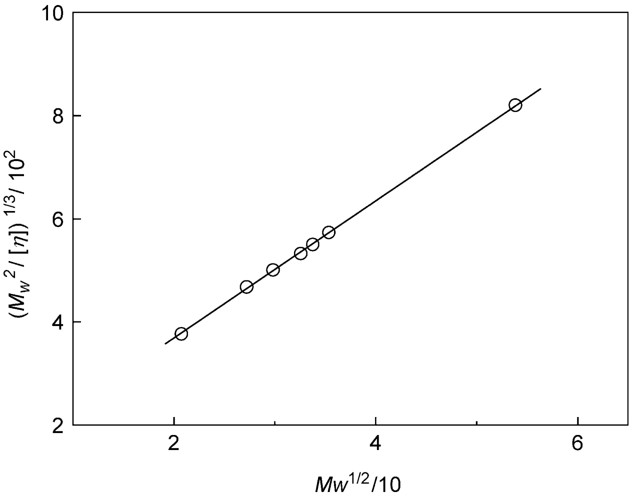

Fig. 3. The plot of $(M_w^2/[\eta])^{1/3}$ against $M_w^{1/2}$ for the fractions of wc-PCM3-I in 0.25 M LiCl/DMSO at 25 °C.

wormlike chain model (Kratky & Porad, 1949), an equation suitable for semi-flexible polymers can be represented by:

$$(M_w/\langle s^2\rangle_z)^{1/2} = (3M_L/q)^{1/2} + 3M_L(3qM_L)^{1/2}/2M_w \tag{8}$$

From a plot of $(M_w/\langle s^2\rangle_z)^{1/2}$ against $1/M_w$, the values of M_L and q were estimated to be 597 nm^{-1} and 5.6 nm, respectively (figure not shown). The value derived from $\langle s^2\rangle_z^{1/2}$ and Eq. (8) of q (5.6 nm) was consistent with the calculated value of 4.5 nm obtained from $[\eta]$ and Eq. (6), suggesting that the wormlike cylinder model could be applied in this system. In addition, the difference between the M_L values obtained from $[\eta]$ and from $\langle S^2\rangle_z^{1/2}$ (690 and 597 nm^{-1}, respectively) might be related to the scatter of the points obtained from $\langle s^2\rangle_z^{1/2}$.

The characteristic ratio (C_∞) reflects the flexibility and conformation of polymer in solution, and is defined as the following (Chen, 1998)

$$C_\infty = M_0/(\lambda M_L l^2) \tag{9}$$

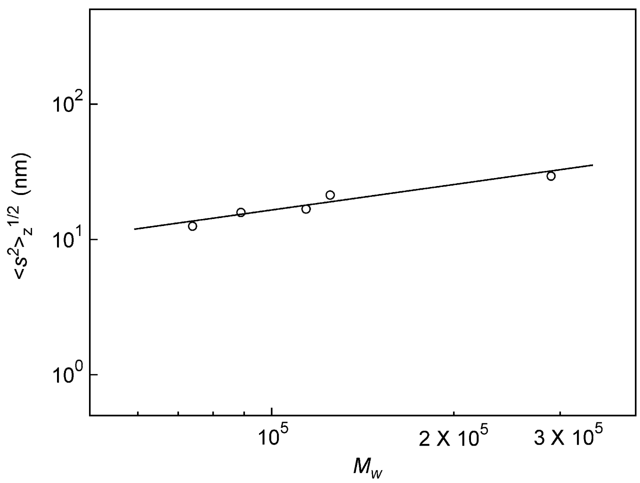

Fig. 4. The double-logarithmic plots of $\langle s^2\rangle_z^{1/2}$ against M_w for the fractions of wc-PCM3-I in 0.25 M LiCl/DMSO at 25 °C.

where M_0 is the average molar mass of a glucose residue repeating unit, λ^{-1} is the Kuhn's segment length ($\lambda^{-1} = 2q$), and l is the virtual bond length which equals to the distance between two successive glycosidic oxygens O(3) and O(3′) in the present case. If we assumed that the value of l for wc-PCM3-I was 0.42 nm, similar to that of (1 → 3)-α-D-glucan (Chen et al., 1998), together with $M_0 = 162$, $\lambda^{-1} = 9.0$ nm, $M_L = 690$ nm^{-1}, C_∞ of the α-D-glucan in 0.25 M LiCl/DMSO was calculated to be 11.9.

To verify the q and M_L values calculated from the Y–F–Y theory, a trial-and-error method (Xu, Zhang, Nakamura, & Norisuye, 2002) was used to find the best fit q value, which led to the closest agreement between our M_w and $[\eta]$ data with M_L fixed to 690 nm^{-1}. The unperturbed mean-square radius of gyration $\langle s^2\rangle_0$ of the wormlike chain can be expressed by Benoit and Doty (1953)

$$\langle s^2\rangle_0 = qL/3 - q^2 + 2q^3/L - 2q^4/L^2[1 - \exp(-L/q)] \tag{10}$$

where L and q denote the contour length and persistence length of the chain, respectively. Since L equals the ratio of M (molecular mass) to M_L, the molecular mass dependence of $\langle s^2\rangle_0$ can be determined by substituting the values for q and M_L. The theory for $[\eta]_0$ (the unperturbed intrinsic viscosity) of the wormlike chain contains one additional parameter, namely the chain diameter d (Yamakawa & Yoshizaki, 1980). It is shown in Fig. 5 that $q = 4.7$ nm gave the best fit to the data points. The solid curve in Fig. 5 represents the theoretical values computed with $q = 4.7$ nm, $M_L = 690$ nm^{-1}, and $d = 0.8$ nm. They fit the experimental data points (circles) quite well. Thus the resulting parameters ($q = 4.7$ nm, $M_L = 690$ nm^{-1}, and $d = 0.8$ nm) and the values mentioned above ($q = 4.5$ nm, $M_L = 690$ nm^{-1}) all indicated that the α-D-glucan in wc-PCM3-I exists as a flexible chain having relatively low flexibility in 0.25 M LiCl/DMSO.

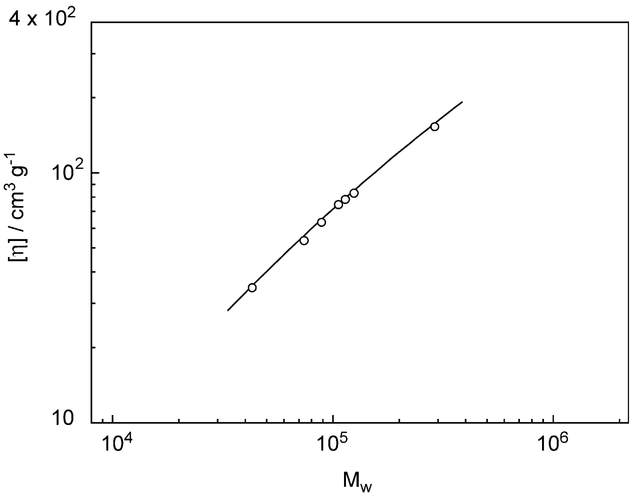

Fig. 5. Intrinsic viscosities (○) for the fractions of wc-PCM3-I in 0.25 M LiCl/DMSO, computed with the theoretical curves for the unperturbed wormlike chain (the solid line) with $q = 4.7$ nm, $M_L = 690$ nm^{-1}, and $d = 0.8$ nm.

4. Conclusions

A water-insoluble (1 → 3)-α-D-glucan (wc-PCM3-I) isolated from the *P. cocos* mycelia by extracting with a 0.5 M NaOH aqueous solution was fractionated successfully into 10 fractions. The Mark–Houwink equation for the fractions in the range of M_w from 4.16 to 29.0×10^4 in 0.25 M LiCl/DMSO at 25 °C were established to be $[\eta] = (9.77 \pm 0.1) \times 10^{-3} M_w^{0.77 \pm 0.02}$ ($cm^3\ g^{-1}$). The conformational parameters of the (1 → 3)-α-D-glucan were 690 nm^{-1} for M_L, 4.5 nm for q and 11.9 for C_∞, indicating a relatively extended flexible chain in 0.25 M LiCl/DMSO.

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