

## Rheological properties of $\beta$ -D-glucan from the fruiting bodies of *Ganoderma lucidum*



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### ABSTRACT

Rheological properties of a  $\beta$ -(1,3)-D-glucan (GLP20) from the fruiting body of *Ganoderma lucidum* in water at different concentrations were studied with a strain controlled ARES Rheometer at 25 °C. The intrinsic viscosity was measured by Ubbelohde viscometer and estimated to be 1248.05 mL/g from the mean of both intercepts by plotting  $\eta_{sp}/c$  and  $\ln\eta_{rel}/c$  against concentration. Shear-thinning behavior occurred at lower concentrations and very low critical shear rates. The zero shear specific viscosity ( $\eta_{sp,0}$ ) was plotted against the coil overlap parameter ( $c[\eta]$ ) and the slopes of the lines in the dilute and semi-dilute regimes were found to be 1.23 and 4.19, respectively. The critical concentration ( $c^*$ ) was determined to be about 0.79 g/L, which was much lower than that of general disordered polymers. This may be due to the stiff chain conformation of GLP20. The results from dynamic experiments revealed that GLP20 in pure water displayed liquid-like behavior at low concentrations ( $\leq 0.5\%$ ) and could form weak gels with the increase of concentration.

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## 1. Introduction

*Ganoderma lucidum* (*G. lucidum*) has been used as Chinese traditional medicine to cure various human diseases for more than 4000 years (J. Zhang et al., 2010). Many studies demonstrate that polysaccharides are one of the most important components in *G. lucidum* with good biological activities and play an important role in immunostimulation and antitumor activities (Bao, Wang, Dong, Fang, & Li, 2002; Nie, Zhang, Li, & Xie, 2013; Paterson, 2006; Shao, Dai, Xu, Lin, & Gao, 2004). Recently, more and more researches about polysaccharides are focused on their rheological properties and applications because they have many special properties such as high viscosity, stability, gelation properties and so on

(Xu, Xu, Zhang, & Zhang, 2008). Therefore, to investigate the viscous properties of polysaccharide solutions is of practical value for favoring them to the applications in everyday life such as food industry, pharmaceuticals, cosmetics, and so forth (Ahmad, Mustafa, & Man, 2015; Mirhosseini & Amid, 2012; Nishinari, Zhang, & Ikeda, 2000). Now with the development of functional foods containing fungal polysaccharides, the potential commercial importance of these polysaccharides as a functional food and a source for the development of drugs has attracted much attention (Aida, Shuhaimi, Yazid, & Maaruf, 2009). However, comparing with other materials, the corresponding researches on *G. lucidum* polysaccharides are quite limited. Lai and Yang (2007) published the rheological properties of crude polysaccharides extracted with hot-water from the fruiting bodies and mycelia of *G. lucidum*, but the rheology of pure *G. lucidum* polysaccharide is seldom reported.

One  $\beta$ -D-glucan coded as GLP20 was purified from the hot-water extracts of *G. lucidum* fruiting bodies in our lab. Its primary structure has been characterized to be  $\beta$ -(1 → 3)-linked D-glucose as backbone with single  $\beta$ -(1 → 6)-linked D-glucose as branches on every third residue (Y. Liu et al., 2014), which is the same with the repeat units of some other fungal  $\beta$ -D-glucans such as schizophyllan

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and scleroglucan. GLP20 has high average molecular weight with  $4.95 \times 10^6$  Da and shows stiff chain conformation in water solution. Interestingly, it also possesses high viscosity in dilute aqueous solution, and even can form gel at low temperature or in concentrated solution. Although the rheological behaviors of several schizophyllans and scleroglucans were already assessed in some articles (Fariña, Siñeriz, Molina, & Perotti, 2001; Grassi, Lapasin, & Pricl, 1996; Moresi, Lo Presti, & Mancini, 2001; Yanaki, Tabata, & Kojima, 1985), the property information is somewhat discordant probably due to different resources and variability in molecular size. This suggests that GLP20 might possess its own parameters for rheological properties. The main objective of the present study was to investigate the rheological properties of GLP20, such as the steady shear viscosity, dynamic oscillatory behavior and so on.

## 2. Materials and methods

### 2.1. Sample preparation

The  $\beta$ -D-glucan coded as GLP20 was isolated from the fruiting bodies of *G. lucidum* which were cultivated in Zhejiang Province of China by the Institute of Edible Fungi (IEF), Shanghai Academy of Agricultural Sciences (SAAS). This  $\beta$ -D-glucan was extracted with hot-water and precipitated with 20% ethanol according to previously reported method (Y. Liu et al., 2014). The  $M_w$  of GLP20 in water was determined to be  $4.95 \times 10^6$  Da by light scattering combined with high performance size exclusion chromatography.

### 2.2. Intrinsic viscosity measurements

GLP20 was separately dissolved in deionized water, and heated in water bath at 80 °C for 30 min under gentle stirring. After being cooled down, 5 mL GLP20 solutions were added into glass Ubbelohde capillary viscometer and equilibrated at  $25 \pm 0.1$  °C for 15 min. The passage time of these solutions with various concentrations flowing through the capillary was measured to calculate the intrinsic viscosity. For rigid polymers in solution, the following equation is used to estimate the intrinsic viscosity  $[\eta]$ :

$$\frac{\eta_{sp}}{c} = [\eta] + k[\eta]\eta_{sp} \quad (1)$$

Where  $\eta_{sp}$  is the specific viscosity,  $c$  is the polymer concentration,  $\eta_{sp}/c$  is the reduced viscosity,  $k$  is the constant for given polymer in given conditions. The equation is valid only when the value of  $\eta_{sp}$  is between 1.2 and 2. If  $\eta_{sp} > 2$ , the interactions between polymer and polymer will become significant, thus higher-order terms will not be negligible. A lower limit of the value (1.2) is also required to eliminate the increasing errors in  $\eta_{sp}$ . The value of intrinsic viscosity  $[\eta]$  was reported as the mean of both intercepts by plotting  $\eta_{sp}/c$  and  $\ln\eta_{rel}/c$  against  $c$  and extrapolating each linear trend line to zero concentration (Qian, Cui, Wu, & Goff, 2012).

### 2.3. Rheological measurements

Both steady shear and dynamic oscillatory tests were conducted on a strain controlled ARES Rheometer (TA Instruments, New Castle, USA) using a parallel plate (40 mm in diameter, 1.0 mm gap). Steady shear viscosity of sample solutions with 8 concentrations (0.06%, 0.08%, 0.1%, 0.2%, 0.4%, 0.6%, 0.8% and 1.0%, w/v) was measured at 25 °C at shear rate ranging from 0.01 to 800  $s^{-1}$ . Dynamic strain sweep measurements were carried out at 1 Hz to determine the linear viscoelastic regime of GLP20 in water with a strain range from 0.3% to 400%. Dynamic frequency sweep measurements were performed to determine the values of storage

modulus ( $G'$ ), loss modulus ( $G''$ ) by varying the frequencies from 0.1 to 50 Hz in a controlled-strain mode. A 5 min delay after sample loading was needed to remove practically all the shear history and to reconfigure the unperturbed state of any sample before any testing.

## 3. Results and discussions

### 3.1. Determination of intrinsic viscosity

Intrinsic viscosity ( $[\eta]$ ) is a parameter which reflects the hydrodynamic volume occupied by polymers. Its value is determined in dilute solution and mainly depends on the molecular size and chain stiffness of the polymer, as well as solvent quality (Moresi et al., 2001). Fig. 1 shows the linear plots of  $\eta_{sp}/c$  and  $\ln\eta_{rel}/c$  against concentration. The  $[\eta]$  of GLP20 in water was easily obtained from the mean of two intercepts as 1248.05 mL/g, which was similar with that (= 1210 mL/g) reported by Moresi et al. (2001) for scleroglucan, but was much smaller than that (= 9560 mL/g) of scleroglucan sample used by Fariña et al. (2001).

For most neutral polysaccharide solutions,  $\eta_{sp}$  usually increase with the increasing of polysaccharide concentration, which can be described by the Huggins equation:

$$\eta_{sp} = C[\eta] + k'[\eta]^2C^2 \quad (2)$$

Huggins' plot has practical advantage of normally giving linear plots (Morris, 1995). However, for polymers with considerable stiff conformation, they will show unique non-linear dependence of viscosity in dilute solutions (Huggins, 1942). Fig. 2 illustrates the dependence of  $\eta_{sp}/c$  for GLP20 on the concentration. This plot is strongly concave upward, similar with the plot of lentinan (Xu et al., 2008), suggesting that GLP20 is considerably rigid. The result is consistent with the previous report that GLP20 exists as stiff chains in aqueous solution (Y. Liu et al., 2014).

### 3.2. Steady shear flow behavior and the critical concentration

The flow curves for GLP20 dispersions in the range of concentration from 0.06% to 0.8% in water at 25 °C are given in Fig. 3. Clearly, the typical flow curve of the sample solutions exhibited shear thinning behavior as the viscosity decreased with the increase of shear rate. This behavior became more obvious at high shear rate and still more pronounced even at lower shear rate as GLP20 concentration increased. However, Newtonian behavior was not apparent in the experiment. It can be expected that the

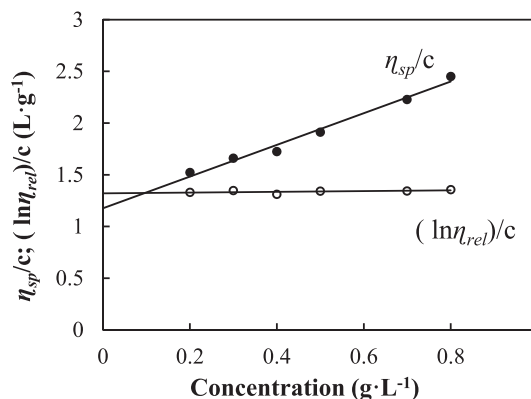


Fig. 1. Dependence of  $\eta_{sp}/c$  (full symbols) and  $(\ln \eta_{rel})/c$  (open symbols) on concentration for GLP20 solutions at 25 °C.

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