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Properties of aqueous solutions of lentinan in the absence and presence of zwitterionic surfactants

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ABSTRACT

Morphological and conformational transitions of lentinan (**LT**), a β-glucan extracted from Shiitake mushrooms (*Lentinula edodes*), were investigated at different concentrations of aqueous NaOH, using Small Angle X-ray Scattering (SAXS) technique. At low $NaOH_{(aq)}$ concentration **LT** chains are self-associated and adopt the triple helix form where as at higher NaOH concentrations the polymer chains undergo a transition to random coil chains. Also, the presence of fractal dimensions was observed through analysis of the exponential decay of the scattering intensity as a function of the scattering angle. In addition, the lateral radius of gyration was determined for **LT** in different concentrations of NaOH solution, indicating a rigid triple helix present as a small rod-like structure. Interactions of **LT** with two zwitterionic surfactants were investigated by surface tension, fluorescence, and static light scattering measurements. Experimental data showed that the formation of **LT**–(surfactant) complexes occurred through a cooperative process.

1. Introduction

The study of biopolymers has grown considerably in recent decades because of their wide application in different areas and relatively low cost. In addition, such polymers have low impact on the environment, since they are biodegradable and biocompatible. The polysaccharides produced by algae, bacteria and fungi have been extensively used in technological applications (Silva et al., 2006). β-Glucans, polysaccharides of high molecular weight extracted from the cell walls of edible mushrooms, are used in medicine due to their bioactive, immunomodulatory and antitumor properties (Zhang, Cui, Cheung, & Wang, 2007). Lentinan (**LT**), one such polysaccharide, has a high molecular weight (5×10^5 g mol^{-1}) and contains only D-glucopyranose units in its macromolecular structure, with mostly β-(1→3)-glucose linkages in its regularly branched backbone and β-(1→6)-glucose side-chains (Zong, Cao, & Wang, 2012) (Fig. 1).

In aqueous solution, **LT** is self-associated in the form of triple helix chains (Xu, Wang, Cai, & Zhang, 2010; Xu, Zhang, Zhang, & Wu, 2004). It is known that this triple helix conformation is maintained due to intra- and intermolecular hydrogen bonding in aqueous solution (Zhang, Zhang, & Xu, 2004). This conformation changes when **LT** is dissolved in dimethyl sulfoxide (Wang et al., 2009; Wang, Zhang, Zhang, & Ding, 2009) or if the temperature is increased (Zhang, Xu, & Zhang, 2008). Likewise, it can alter when **LT** is dissolved in aqueous NaOH: a triple helix string formation occurring at concentrations of NaOH below 5×10^{-2} mol L^{-1} and a random conformation being observed at concentrations of NaOH greater than 8×10^{-2} mol L^{-1} (Zhang et al., 2004).

A better understanding of the morphological transitions of β-glucans such as **LT** is an important prerequisite to assessing the relationship between bioactivity and morphology, since a number of biological and immune pharmacological activities described in the literature are dependent on the conformation of these polysaccharides (Surenjav et al., 2005; Wasser & Weis, 1999). Sakurai et al. (2005) have shown that β-glucans with β-(1→3)-glucose linkages, for instance, **LT**, curdlan and schizophyllan, form macromolecular complexes with polynucleotides, such as DNA and RNA, and also interact with other molecules and conjugated polymers forming nanocomposites (Li, Zhang, Xu, & Zhang, 2011; Liu, Xu, Zhang, & Yu, 2012).

Many studies on the relationship between the molecular conformation and antitumor activity of **LT** and other β-glucans have been reported (Tang et al., 2012; Zong et al., 2012), but little is known about the interaction of **LT** with amphiphilic molecules and its behavior in the presence of such molecules. In this context, in this study, we extracted **LT** from the fruiting bodies of Shiitake mushrooms (*Lentinula edodes*). After the characterization of **LT**, its morphological structure was investigated at different concentrations of aqueous NaOH by means of the Small Angle

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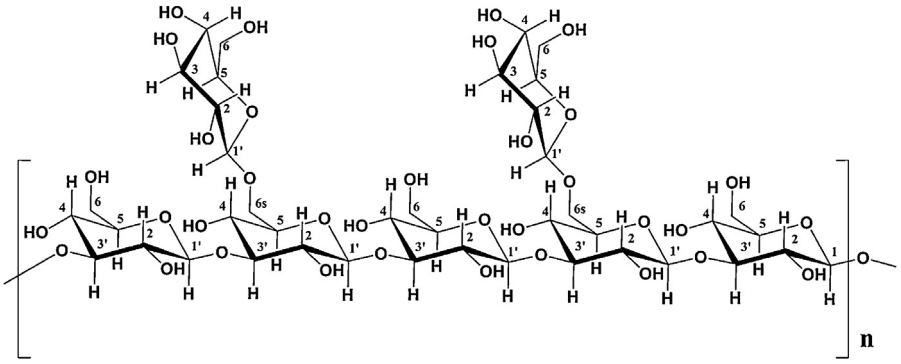

Fig. 1. Representation of the chemical structure of **LT**.

X-ray Scattering (SAXS) technique. Subsequently, the interaction of **LT** with the zwitterionic surfactants *N*-dodecyl-*N*,*N*-dimethyl-3-ammonio-1-propanesulfonate (SB3–12) and *N*-tetradecyl-*N*, *N*-dimethyl-3-ammonio-1-propanesulfonate (SB3–14) was investigated in aqueous solutions through surface tension, fluorescence, and static light scattering (SLS) measurements.

2. Experimental

2.1. Materials

SB3–12 and SB3–14 were supplied by Sigma–Aldrich with a purity of 99%, and were used without further purification. Pyrene (Sigma–Aldrich, 99%) was recrystallized three times from methanol and dried before use. The deionized water used in all measurements was obtained through previous distillation, followed by purification employing a Millipore Milli-Q system.

2.2. Characterizations

The Fourier transform infrared (FT-IR) spectra of **LT** were recorded with an ABB FTLA 2000 spectrophotometer. For each sample 100 scans were recorded at between 4000 and 600 cm^{-1}, with a resolution of 4 cm^{-1} using KBr pellet. The ^{13}C NMR spectrum was recorded on a Varian NMR AS 400 spectrometer operating at 100 MHz. This spectrum was obtained in DMSO-d_6 solution with an **LT** concentration of 4×10^{-2} g mL^{-1} at 25.0 °C.

For all solutions used in the SAXS studies the **LT** concentration was kept constant at 1.0 mg mL^{-1}. The concentration of NaOH was varied with each sample. Each sample was prepared 24 h prior to testing and kept under constant stirring for complete dissolution of the polysaccharide. For all aqueous solutions used in the study of the **LT**–surfactant interactions, the concentration of **LT** was maintained constant at 0.5 mg mL^{-1}. All solutions prepared were left to stand for at least two hours before use.

2.3. Extraction of *LT*

The polysaccharide was extracted using the methodology described by Yap (Ann-Teck Yap & Ng, 2001). Fresh shiitake mushroom caps (200.0 g) were mixed with 1.0 L of distilled water and homogenized in a blender. The resultant mixture was placed in a 2.0 L round-bottomed flask and refluxed for 24 h. The mixture was then filtered and the volume of the filtrate was reduced by distillation to 300 mL. The remaining solution was added to an equal volume of absolute ethanol, and the gelatinous precipitate formed was filtered. The precipitate was dissolved in 100.0 mL of water at 80.0 °C under stirring and filtered to remove insoluble matrices. This process was repeated three times. Finally, the sample was dissolved in 50 mL of a solution of NaOH (1.0 mol L^{-1}) and placed in a dialysis membrane (Spectra/Por® 6, *MWCO* = 3500 g mol^{-1}) and dialyzed for seven days. Finally, the resulting aqueous solution was lyophilized, yielding 0.115 g of solid white flakes. IR (KBr, $\bar{\nu}_{max}/cm^{-1}$): 3410 (O–H stretching), 2920 (CH_2 stretching), 1638 (O–H bending), 1370 (CH_2 bending), 1161 (C–O–C stretching), 1078 (C–H β-1→6 glycosidic linkages), 1041 (C–H β-1→3 glycosidic linkages), and 890 (C–H bending). ^{13}C NMR (100 MHz, DMSO-d_6) δ/ppm: 103.9 (C1,C1′), 73.5 (C2), 87.0 (C3), 77.4 (C3′), 69.1 (C4), 76.3 (C5), 71.5 (C6), 70.6 (C6s).

2.4. SAXS measurements

All SAXS studies were carried out on the D11A-SAXS beamline of the Brazilian Synchrotron Light Laboratory (LNLS – Campinas – Brazil). The wavelength (λ) of the incoming beam was set at 0.1488 nm and the samples were injected into a 1 mm-thick sample (Cavalcanti et al., 2004). The collimated beam crossed the samples through an evacuated flight tube ($p < 0.1$ mbar) and scattered to a 2D CCD mar CCD detector with an active area of 16 cm^2. The sample-detector distance was set at 1479.75 mm (silver behenate was used for the calibration of the sample-to-detector distance due to its well-known lamellar structure, $d = 58.48$ Å). The q range covered at this distance was 0.10–2.3 nm^{-1}. 2D-images were found to be isotropic and were corrected by taking into account the dark noise of the detector and normalized by the sample transmission. These images of the samples were subtracted from the corrected and normalized 2D image of the solvent, and the resulting images were then azimuthally integrated, considering the 360° scan to generate the final I as a function of q profiles. This procedure was performed with the use of the FIT2D software developed by Hammersley (Hammersley, 2009).

2.5. Surface tension data

Surface tension measurements were performed using the du Noüy ring method, at 25.0 ± 0.1 °C on a Kruss K8 GMBH interfacial tensiometer equipped with a Pt-Ir-20 ring. The ring was rinsed with a hydrochloric acid solution (4.0 mol L^{-1}) and then with deionized water several times before each measurement. The critical micellar concentration (*cmc*) as well as the critical aggregation concentration (*cac*) and polymer saturation point (*psp*) in aqueous solution were estimated from the intersections between the two linear portions of the surface tension *versus* log *c*(surfactant) plots.

2.6. Steady-state fluorescence

The steady-state fluorescence emission spectra for pyrene were recorded on a Hitachi F4500 spectrofluorimeter equipped with a thermostated cell holder set at 25.0 ± 0.1 °C and the samples were continuously stirred in a quartz cell of 10 mm path length. The