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Kinetics for the thermal stability of lectin from black turtle bean

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ABSTRACT

The thermal stability of purified lectin from the black turtle bean was studied at temperatures between 70 °C and 90 °C over treatment times from 0 to 30 min. The results of the hemagglutinating activity and the intrinsic fluorescence assays indicated that the thermal inactivation is associated with the unfolding of the protein. Based on the two-way analysis of variance, the heating temperature and treatment time significantly affected the stability of the lectin during thermal treatments. Analysis of kinetic data suggested that the thermal stability of the lectin followed first-order reaction kinetics. The activation energy for the lectin was 78.80 kJ/mol. This higher activation energy suggested hydrophobic stability and thermal sensitivity of the lectin during thermal processing. Additionally, a high temperature and short treatment time might eliminate the anti-nutritional functions of the lectin. The linear relationship observed could be used as a quality indicator for the thermal processing of the black turtle bean.

1. Introduction

Lectins are non-enzymatic proteins of non-immune origin possessing at least one non-catalytic domain, which binds specifically and reversibly to different types of glycoproteins and mono- or oligosaccharides without altering their covalent structures (Rudiger and Gabius, 2001; Wang et al., 2011; Zhang et al., 2009). The lectins are the most predominant natural form of anti-nutritional factors in grain and vegetables. However, plant lectins are currently the subject of intensive studies because of their remarkable biological functions, such as plant protection (Davidson and Stewart, 2004), the recognition of nitrogen-fixing bacteria (Sharon and Lis, 2004) and as their mitogenic, antifungal (Lam and Ng, 2011), antitumor (Abdullaev and Gonzalez de Mejia, 1997) and anti-HIV (Ji et al., 2005) activities. In addition, because of their carbohydrate binding properties, lectins have been widely used for glycoconjugate purification (Cummings, 2008), research monitoring molecular mechanisms (Hirabayashi et al., 2013) and drug delivery/targeting (Jain et al., 2012). Thus, a practical application has been found for the lectins with more specific biological characteristics.

The black turtle bean (*Phaseolus vulgaris*) is an important industrial crop in subtropical and tropical countries (Kumar et al., 2013). However, there are few reports that have characterized the novelty lectin from this bean. The biological functions of lectins depend on their correctly folded structure, and a biphasic pattern of inactivation or a first-order kinetic scheme of lectins might be exhibited above certain temperatures (Ahmad et al., 1998; Biswas and Kayastha, 2004; Shnyrov et al., 1996). However, few detailed reports on the thermal stability of lectin from the black turtle bean based on the inactivation of hemagglutinating activity were provided to the food industry. Studies on the thermal inactivation of the hemagglutinating activity (HA) are becoming increasingly significant for practical application, which could help characterize the stability of the lectins, measure exhibited protein conformational changes, and provide useful information on thermal treatment designs as well as food processing strategies (Dimitrijevic et al., 2010; Zhang et al., 2013).

To determine the influence of thermal parameters on the conformational changes of the lectin, fluorescent spectrometry is the most suitable quantitative method. Changes in lectin conformation induced by thermal denaturation often lead to changes in fluorescence emission (Ahmad et al., 1998; Ali Mohammed Sultan et al., 2006).

The aims of this study will be to investigate and to kinetically analyze the thermal stability of the lectin from the black turtle bean by assessing the hemagglutinating activity. Furthermore, the structural changes will be monitored by intrinsic protein

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fluorescence to better understand the conformational stability of the lectin. The results will provide useful suggestions for food processing and further protein folding studies.

2. Materials and methods

2.1. Materials

Black turtle beans (*P. vulgaris*) were obtained from the local market (Harbin, Heilongjiang, China). The purified lectin was prepared from the crude extract of the black turtle beans using reversed micellar extraction (RME) and cellulose DE-52 (Whatman Chemicals, England) ion exchange chromatography, then exhaustive dialysis and freeze-drying. The purity of the lectin was confirmed by SDS–PAGE and high-performance gel permeation chromatography (HP-GPC) analysis. All chemicals and reagents used were of analytical grade and were obtained from Merck (Darmstadt, Germany).

2.2. Thermal treatment

To make the thermal stability studies more physiologically relevant and realistic, lectin from the black turtle bean was evaluated at a neutral pH (7.2), where it was previously found in a tetramer form (Biswas and Kayastha, 2002; Loris et al., 1998). PBS buffer (4.75 mL, 10 mM, pH 7.2) in a 10 mL flat-bottomed glass tube (16 mm inner diameter, 100 mm depth, and 1.0 mm wall thickness) was heated to the desired temperature using a pre-heated, thermostatically controlled water bath equipped with a shaker. All tubes were completely submerged in the water bath throughout the investigation, and tube plugs were used to prevent water evaporation. The core temperature of the solution was monitored by a digital thermometer. Then, 0.25 mL of lectin solution (20 mg/mL, dissolved in 10 mM PBS buffer, pH 7.2) was added to each glass tube to standardize the test sample to 1 mg/mL as quickly and accurately as possible. A series of thermal treatments were carried out at 70, 75, 80, 85 and 90 °C (±0.1 °C) for 0, 5, 10, 15, 20, 25 and 30 min. At the end of the specified thermal treatment, the test samples were immediately cooled in slurry of crushed ice and water and stored at −30 °C until further hemagglutination assays and fluorescence measurements.

2.3. Determination of protein concentration

The protein concentration was determined using the bicinchoninic acid (BCA) method with an enhanced bicinchoninic acid kit (He et al., 2013).

2.4. Determination of lectin purity

To verify the purity of the purified lectin, a 2 mg/mL protein sample was subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) with a Bio-Rad electrophoresis system (Powerpac Universal, Bio-Rad Laboratories, Inc., USA) using Mini-Protean 4–15% precast gels (Bio-Rad Laboratories, Inc., USA). All analytical HP-GPC profiles were obtained on an Agilent 1100 Series HPLC Value System (Agilent Technologies, Pittsburg, PA, USA) with a TSKgel G3000SW$_{XL}$ (300 × 7.8 mm) column (TOSOH Co., Tokyo, Japan). The column was eluted with the identical solvent gradient as the PBS buffer (10 mM, pH 7.2) containing 0.05% sodium azide at a flow rate of 1.0 mL/min. The UV absorption was measured at 280 nm, and the injection volume was 20 μL.

2.5. Hemagglutination assay

The hemagglutination assay was carried out on the twofold serial dilutions of the lectin solutions. A suspension of rabbit red blood cells (2%) was then added to determine the maximal visible agglutination at 4 °C after 90 min. Because activity was defined as the reciprocal of the highest dilution exhibiting hemagglutination, the hemagglutinating activity (HA, HU/mg) was expressed as the units of activity per mg of protein (Kilpatrick and Yeoman, 1978):

$$HA = (2^n)/(C_{(Pr)} \times V)\ (n \geqslant 1) \quad (1)$$

where n is the highest well number exhibiting hemagglutination in the microtiter V-plate, and $n = 0$ means no visible agglutination. $C_{(Pr)}$ (mg/mL) is the protein concentration of the lectin samples, while V (mL) is the volume of lectin solution added to each well.

2.6. Fluorescence spectroscopy assay

The measurements of the lectin samples after different thermal treatments were performed with a Hitachi fluorescence spectrophotometer (Model: FP-6500, Japan) at room temperature (25 ± 0.5 °C). The intrinsic fluorescence spectra were obtained using a 1 cm × 1 cm cuvette with an excitation wavelength of 280 nm, and the emission spectra were recorded between 315 and 400 nm. The slit widths of the excitation and emission monochromators were set to 3 and 5 nm, respectively. All samples were diluted to 10 ± 2 μg/mL using PBS buffer (10 mM, pH 7.2) and incubated at 25 ± 0.5 °C for 2 h to ensure equilibrium before the measurements. The test samples at the 0 min thermal treatment were used as the native proteins, and readings of the buffer without protein were subtracted from all spectra.

2.7. Kinetic studies

To compare the effects of different thermal treatments on lectin from the black turtle bean, the HA of the lectin during heating was plotted as a function of time (t). Because the reaction rate constant (k) was calculated as the slope of the linear plot, a first-order kinetic model was developed based on the HA to characterize the thermal stability of lectin from the black turtle bean. Then, the kinetic model was expressed according to the following equation:

$$-dHA/dt = kHA \quad (2)$$

The kinetic thermal HA degradation of lectin could be formed by the following integral of Eq. (2) in relation to time:

$$\mathrm{Ln}(HA/HA_0) = -kt \quad (3)$$

where HA_0 is the initial HA of lectin at the thermal treatment of 0 min, HA is the remaining HA of lectin after the specified time (t, min) of heating at the given temperature and k is the reaction rate constant, which can be determined from the slope of the Ln(HA/HA_0) vs. t plot.

The dependence of the reaction rate constant (k) on temperature can be represented by the Arrhenius equation:

$$k = k_0 \exp(-E_a/RT) \quad (4)$$

Then, the first-order kinetic model can be described using the linear relationship represented by Eq. (5):

$$\mathrm{Ln}\,HA - \mathrm{Ln}\,HA_0 = -k_0 \cdot \exp(-E_a/RT) \cdot t \quad (5)$$

The frequency factor (k_0, min^{-1}) and the activation energy (E_a, kJ/mol) can be obtained by evaluating the logarithm of Eq. (4) as:

$$\mathrm{Ln}\,k = \mathrm{Ln}\,k_0 - E_a/RT$$

where R is the universal gas constant (8.314 J/mol/K) and T is the absolute temperature (K).