International Journal of Biological Macromolecules 50 (2012) 534–539

Contents lists available at SciVerse ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

In vitro antioxidant activities of the polysaccharides from *Tricholoma lobayense*

Cui Wang, Yan Chen*, Meili Hu, Jingna Ding, Cunji Xu, Ruijun Wang

School of Life Sciences, Anhui University, Hefei 230039, China

ARTICLE INFO

Article history:
Received 2 December 2011
Received in revised form
26 December 2011
Accepted 8 January 2012
Available online 25 January 2012

Keywords:
Tricholoma lobayense
Polysaccharide
Antioxidant activity
UV spectrophotometry
Fluorescence spectroscopy

ABSTRACT

The antioxidant activities of three polysaccharide components (TLH-1, TLH-2, TLH-3) extracted from *Tricholoma lobayense* were evaluated by three different in vitro methods, namely superoxide radical ($^{\bullet}O_2^-$) scavenging activity, inhibition of mice erythrocyte hemolysis (MEH) and malondialdehyde (MDA) mediated by hydrogen peroxide (H_2O_2) and investigation of oxidative modification of human serum albumin (HSA) induced by 2,2-azobis(2-amidinopropane)dihydrochloride (AAPH) through fluorescence spectroscopy. The antioxidant experiments showed that the polysaccharides had a notable activity in scavenging $^{\bullet}O_2^-$ in a concentration-dependent manner; H_2O_2-induced MEH and formation of MDA were effectively inhibited; by fluorescence spectroscopy, it was demonstrated that the polysaccharides could obviously inhibit AAPH-induced oxidative modification of HSA. The experimental data obtained from the in vitro models clearly revealed that TLH-3 had stronger antioxidant potency than TLH-1 and TLH-2, which indicated that TLH-3 might be exploited as effective natural antioxidant to alleviate oxidative stress.

1. Introduction

Oxidation is essential to many organisms for the energy production of biological processes. However, the uncontrolled production of oxygen derived free radicals can lead to many diseases such as cancer, rheumatoid arthritis, and atherosclerosis as well as in degenerative processes associated with aging [1]. In order to reduce damage to the human body, many synthetic antioxidants are used widely at the present time. However, the most have been suspected of being responsible for liver damage and carcinogenesis [2–4]. Thus, it is essential to develop and utilize effective natural antioxidants.

Many reports have indicated that fungus polysaccharides in general have strong antioxidant activities and can be explored as novel potential antioxidants [5–7]. Mushrooms are famed for their nutritional and medicinal values. A variety of compounds with important pharmacological properties have been isolated from mushrooms.

Tricholoma lobayense Heim, also known as *Tricholoma giganteum Massee*, belongs to *Tricholoma* species of *Basidiomycotina* [8], is a kind of precious edible fungus in development. French mycologists Heim first discovered it in Africa, and named in 1970. Recently, related experts pointed out that *T. lobayense* Heim will be developed into the leading product in China in recent years. *T. lobayense* Heim is abundant in nutrients. According to the analysis, it contains protein 27.56%, total sugar 38.44%, crude fat 9.58% and crude fiber 8.20% in per kilogram of dry product. Liu et al. [9] suggested that *T. lobayense* polysaccharide–protein complex exhibited immunomodulation and antitumor activity by in vivo and in vitro antitumor assay. Wang et al. [10] described that *T. lobayense* extract could inhibit cell-free translation.

The fluorescence spectroscopy which is a sensitive, non-destructive and highly selective method of detection has been widely used to investigate the interaction of drug and protein in recent years. After the protein is treated by quenchers of different concentrations, quenching of the protein intrinsic fluorescence can be used to infer the binding mechanism and to obtain unique structural and dynamic information [11–13]. The manner in which the fluorescence emission spectra of the bound drug–protein affected by simultaneous presence of ligands which bind specifically on the protein, leads to use the drug as a fluorescent probe to determine the environment at the drug binding site [14,15]. The fluorescence technique has been used to investigate inclusion complex formation of antioxidants [16] and interaction of disaccharide with HSA [17]. In the present study, the binding of *T. lobayense* polysaccharides to HSA and the effect of *T. lobayense* polysaccharides on oxidative modification of HSA were investigated by fluorescence spectroscopy.

2. Materials and methods

2.1. Materials

Kunming mice (SPF grade), average body weight 25 ± 2 g, were supplied by Anhui Medical University (Anhui, China).

* Corresponding author. Tel.: +86 551 5108636.
E-mail address: chenyan91030@yahoo.com (Y. Chen).

0141-8130/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijbiomac.2012.01.005

Malondialdehyde (MDA) test kit was obtained from Nan-jing Biology Engineering Company (Nanjing, China).

2,2-Azobis(2-amidinopropane)dihydrochloride (AAPH), human serum albumin (HSA), pyrogallol and 1.0 M Tris–HCl buffer were purchased from Beijing Solarbio Science & Technology Company (Beijing, China). Water was purified by the Milli-Q system. All other reagents were of analytical grade.

T. lobayense Heim was from Panji Shenshan edible fungus cooperative (Huainan, Anhui Province, China).

2.2. Preparation of T. lobayense polysaccharides

T. lobayense were dried to constant weight, crushed and sifted, the polysaccharide of *T. lobayense* (named TLH) was extracted by hot-water extraction and ethanol precipitation, the extraction rate of the crude polysaccharide was 12.294%.

The TLH contains three components, designated as TLH-1, TLH-2 and TLH-3, they were collected after further purification via DEAE ion-exchange chromatography and Superdex-200 column chromatography; total sugar content of the three components were 82.7%, 89.6% and 92.3%, protein content were 6.1%, 6.5% and 4.5%, uronic acid content were 12.7%, 20.5% and 26.9%, respectively; the molecular weight and monosaccharide components of the three fractions were determined by high-performance liquid chromatography (HPLC). The molecular weight of TLH-1, TLH-2 and TLH-3 were 8.75×10^5 Da, 5.85×10^5 Da and 4.22×10^3 Da, separately. The monosaccharide components of TLH-3 were arabinose, mannose, glucose and galactose, and the molar ratio of arabinose, mannose, glucose and galactose was 15.1:2.3:3.7:1.8. The monosaccharide components of TLH-1 and TLH-2 were the same as TLH-3, and the molar ratios were 8.7:5.9:5.3:10.4 and 2.1:3.3:5.0:9.3, respectively.

2.3. Assay of superoxide radical ($^{\bullet}O_2^-$) scavenging

Superoxide radical was generated in the system of pyrogallol's autoxidation in an alkalescent condition [18]. All experiments were performed at 25 °C. With some modification in this experiment, the reaction was performed in 4.0 mL Tris–HCl buffer (50 mM, pH 8.2) which contained 1.0 mL samples with different concentrations (50–500 μg/mL), 0.3 mL pyrogallol solution (45 mM) was added before testing. The change speed of absorbance (A/min) of the reaction solution was measured at 420 nm. The inhibition of superoxide radical production was calculated according to the following formula (1):

$$\text{Inhibition rate } (\%) = \left[\frac{A-B}{A}\right] \times 100 \quad (1)$$

where A is the change speed of absorbance of the control group in the superoxide radical generation system, and B is the change speed of absorbance of the experiment group.

The antioxidant potential of *T. lobayense* polysaccharides was expressed as IC_{50} (referred to as the amount of the polysaccharides required to scavenge 50% of the superoxide radicals present in the reaction mixture).

2.4. Assay for inhibition of mice erythrocyte hemolysis and formation of MDA

Blood samples were collected from decapitated Kunming mice, and a 0.5% erythrocyte suspension (ES) was obtained according to Malpezzi and Freitas [19].

The in vitro inhibition of mice erythrocyte hemolysis by the polysaccharides was evaluated according to the procedures described by Yuan et al. [20].

The polysaccharides with different concentrations (50–500 μg/mL) were added to the 0.5% suspension of erythrocytes according to volume ratio of 1:3. The mice erythrocyte hemolysis was initiated by the addition of 1 mol/L H_2O_2. The samples were incubated at 37 °C for 1 h. After incubation, the samples were centrifuged at 3000 rpm. for 10 min and the absorbance of the supernatant (A) was measured at 540 nm by

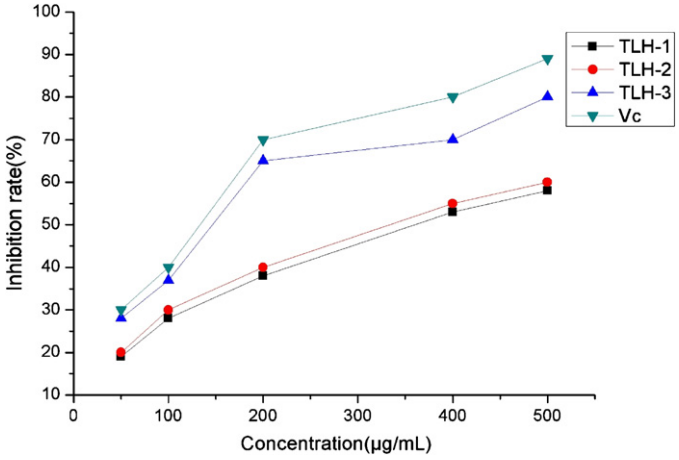

Fig. 1. Superoxide radical scavenging activities of *Tricholoma. lobayense* polysaccharides (TLH-1, TLH-2, TLH-3).

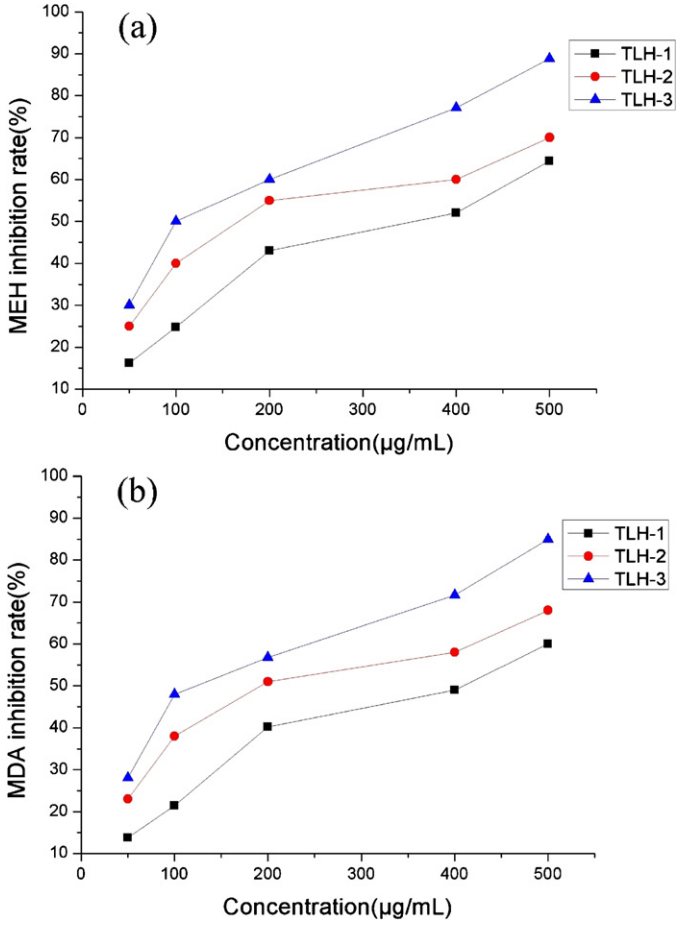

Fig. 2. Effects of *Tricholoma lobayense* polysaccharides (TLH-1, TLH-2, TLH-3) on MEH (a) and MDA (b).