Journal of Traditional and Complementary Medicine 7 (2017) 178–187

Contents lists available at ScienceDirect

Journal of Traditional and Complementary Medicine

journal homepage: http://www.elsevier.com/locate/jtcme

Original article

Boiling-induced nanoparticles and their constitutive proteins from *Isatis indigotica* Fort. root decoction: Purification and identification

Jianwu Zhou, Jie Liu, Dai Lin, Guanzhen Gao, Huiqin Wang, Jingke Guo, Pingfan Rao, Lijing Ke*

SIBS, CAS-ZJGSU Joint Centre for Food and Nutrition Research, Zhejiang Gongshang University, Hangzhou 310012, China

ARTICLE INFO

Article history:
Received 6 April 2016
Received in revised form
27 June 2016
Accepted 15 August 2016
Available online 20 October 2016

Keywords:
Nanoparticles
Boiling-induced assembly
Glycated protein
Herbal decoction
Colloidal aggregates
Chromatographic purification

ABSTRACT

Colloidal particles are essential components of sun-dried *Isatis indigotica* Fort. roots (Ban-Lan-Gen in Chinese, BLG) decoction. Nanoparticles (NPs) were isolated from BLG decoction with size exclusion chromatography and characterized. Their average diameter is ~120 nm, reversibly responding to pH and temperature changes. They promoted the growth of normal cells but suppressed that of cancerogenic cells and macrophages. Two constitutive glycated proteins were identified from the NPs, namely BLGP1 and BLGP2. Their N-terminal amino acid sequences were V-X-R-E-V-V-K-D-I and V-V-R-E-V-V-K-D-I-A-G-A-V-Q-T-N-E-Q-Y. Their full-length cDNA sequences were cloned to obtain the highly homological amino acid sequences of non-glycated proteins, whose theoretical molecular weights are 21831.64 Da and 21841.67 Da. Using pepsin hydrolysis and mass spectrometry, four possible glycation adducts were identified in BLGP1, whereas one in BLGP2. To conclude, bioactive nanoparticles isolated from the herbal decoction are intelligent nanoassemblies composed of a new boiling-stable protein. Glycation plays a critical role in heat-induced formation of these nanoassemblies. The novel, intelligent, safe and stable nano-carriers for drug delivery may be developed using BLG NPs as prototype.

Copyright © 2016, Center for Food and Biomolecules, National Taiwan University. Production and hosting by Elsevier Taiwan LLC. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Like many other Chinese herbs, *Isatis indigotica* Fort. (Chinese woad) is a biennial herbaceous plant species planted widely in China with a few hundred years of medicinal use. To date, the sun-dried roots of *I. indigotica* (Radix Isatidis, Ban-Lan-Gen) and their extracts are popularly used alone or in combination with other herbs to treat a wide variety of infectious and inflammatory diseases, such as influenza, herpes, acute hepatitis, arthritis and encephalitis B.[1–3] Like many other Chinese herbal medicine, decoction is its major administration formula. The granules of Radix Isatidis are also widely used, which is basically the dried powder of its decoction.[4] The decoction can inhibit viral infection via protection of host cells rather than killing virus,[5] increase the spleen weight and number of lymphocytes and antagonize the immunosuppressive actions of hydrocortisone.[6] One of the most notable chemical changes occurred during preparation of Ban-Lan-Gen decoction is the significant reduction in the content of free basic amino acids, i.e. arginine and lysine, and reducing sugars, i.e. glucose, after sun-drying and boiling. The Maillard reaction occurred is the driven force of the above chemical evolution and produces a high content of non-enzymatic glycated proteins[7] with elevated structure stability and solubility at higher temperature,[8,9] e.g. in decocting process. Besides, Ban-Lan-Gen decoction contains a great number of colloidal particles and aggregates.

Boiling extraction (decocting) is the earliest and most popular way of preparing herbal decoction. The intensive boiling process migration a great number of compounds from plant materials to the soup, interaction among extracted coexisting actives and the formation of a multiple-phase dispersion. As reported in a previous study, colloids-like aggregates were observed in all the decoctions of 60 medicinal herbs and 24 Chinese herbal formulae, and were able to survive in the gastro-intestinal environment, pass through the Caco-2 cell monolayer and correlated to the activities of decoction.[10] Aggregates even as the precipitates from a two-herb decoction, containing *Aconitum carmichaelii* Debx. (Fu-Zi) and *Radix Rhizoma Glycyrrhizae* (licorice, Gan-Cao), increased the mean

* Corresponding author. Room 407, No. 1 Laboratory Bld., No. 149 Jiaogong Road, Hangzhou 310012, China. Fax: +86 571 8805 6656.
E-mail address: lijingke@zjgsu.edu.cn (L. Ke).

Peer review under responsibility of The Center for Food and Biomolecules, National Taiwan University.

http://dx.doi.org/10.1016/j.jtcme.2016.08.007

2225-4110/Copyright © 2016, Center for Food and Biomolecules, National Taiwan University. Production and hosting by Elsevier Taiwan LLC. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

residence time and absorbed doses of diester diterpenoid alkaloids in gastrointestinal tract and blood after oral administration.[11] On the other hand, the synergic effects between various herbal ingredients and compositions of TCM decoction have been proven by studies *in vitro* and *in vivo*,[12,13] despite the general mechanism of which has not yet been elucidated.[14] Therefore, in respect to the individual active compositions, the aggregates facilitated by noncovalent bonding among multiple molecules may provide an infrastructure for active compositions to conduct their synergic curative effects. Among these aggregates and precipitates, colloidal particles may be the dominant participates, as they are self-assembled by the extracted compounds carrying principle bioactive phytochemicals, i.g. ephedrine embedded colloidal nanoparticles in Ma-Xing-Shi-Gan decoction are expected to cause much less safety concerns[15] and displayed similar biological function with the decoction itself.[16] Wang et al. reported the cluster size of compositions from water decoction of *Pueraria lobata* var. *thomsonii* Benth (radix, Feng-Ge) is correlated to the amount of herb used and is relevant to the oral drug absorption efficiency and the reduction of the octapeptide angiotensin II.[17] Colloids in TCM decoction is merely the sole case of particulates which integrate various active compounds and therefore change their properties and biological functions. For instance, milk protein forms aggregates with chocolate flavonoids and coffee polyphenols and caused the reduction in both bioavailability and health benefits.[18,19] The opposite effects of molecular assembly or aggregates occurred in the above complex system of natural products indicates the composition, bioactivity and structural characteristics of these colloids ought to be studied to understand their role in the healing decoction.

Recently, protein nanoparticles have drawn rising attention in the field of nanotechnology. A range of plant-source proteins have been used to fabricate colloidal delivery systems, including zein, whey proteins, soy proteins, caseinate and boiling stable proteins (hydrophilins).[20] Many of these proteins are GRAS (generally recognized as safe) food ingredients.[21] Taking whey proteins as an example, comprehension of the aggregation mechanism of these proteins is warranted for manipulating the properties of proteins colloids and facilitating the possible applications in food and pharmaceutical industry. The changes of protein structures were observed prior to protein aggregation, which include partial unfolding of the tertiary structure and conformational changes of secondary structure. As a consequence, hydrophobic sites or free −SH groups are exposed to molecular surface, therefore enhance intermolecular interaction led to the formation of soluble aggregates.[22,23] The triggers of protein aggregation, including heating[24] and protein glycation,[25] were extensively investigated while the latter has been considered as a promising approach for protein modification.[26] It can alter the hydrophobicity and the secondary structure of the protein, improve the thermo-stability and facilitate protein aggregations.[27]

In this study, the colloidal nanoparticles (NPs) were isolated from Ban-Lan-Gen boiling water extracts, whose constitutive proteins were characterized with SDS-PAGE. To get further insights on the assembly mechanisms of NPs from Ban-Lan-Gen decoction, which may be induced upon glycation and boiling, the characterization of these NPs and structural characterization of their component proteins were performed.

2. Material and methods

2.1. Raw materials, chemicals and cell lines

The sun-dried roots (Ban-Lan-Gen, BLG) and fresh roots of *I. indigotica* Fort. were collected from the same GAP (Good Agricultural Practice) field in Fuyang (Anhui Province, China). All the chemicals used in this study were of reagent/analytical grade from Sinopharm Chemical Reagent Co., Ltd (Shanghai, P. R. China). Protein Molecular Weight Marker (Beyotime Biotechnology), MTT (Sigma–Aldrich). Human normal hepatocytes (L-02), human hepatoblastoma cells (Hep-G2), rat alveolar macrophage cells (NR8383) and human cervical carcinoma cells (HeLa-229) were purchased from the Type Culture Collection of Chinese Academy of Science (Shanghai, China).

2.2. BLG decoction preparation

To prepare the BLG decoction, sliced BLG were soaked in distilled water (1:8, w/v) for 30 min at room temperature with stirring, then boiled for 60 min, cooled to room temperature and filtered through two layers of cotton gauze.

2.3. Separation of nanoparticles from BLG decoction

The nanoparticles from BLG decoction were separated with a size-exclusion gel chromatography with multi-angle laser light scattering (SEC-MALLS) as described in Ref. 15. The fraction with strong signals at both light scattering and UV280 nm were collected and pooled for further analysis.

2.4. Dynamic Light Scattering measurement

Particle size measurements were performed by Dynamic Light Scattering analysis (DLS) on a Zetasizer Nano device (Malvern Instruments, Worcestershire, UK). Sample aqueous suspension (1 mL) was centrifuged at 400 g for 10 min (Model CF16RXII, Hitachi Koki Co., Ltd., Japan). The supernatant, without dilution, was moved to the disposable 10 mm cuvettes for measurement at 25 °C. Deionized water or chromatograph elution buffer was used as background control.

2.5. Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) was performed with a Cold Field Emission S-4800 Scanning Electron Microscope (Hitachi, Tokyo, Japan) operated under an acceleration voltage of 5 kV. The particles were collected with 0.22 μm cellulose acetate membrane and coated with gold with sputter coater (E-1010, Hitachi Instruments, Japan) to render them electrically conductive. The images were taken with 15 k magnification and 500 nm scale bar.

2.6. Denatured gel electrophoresis and periodic acid–Schiff (PAS) staining

Gel electrophoresis (SDS–PAGE) was performed under reducing conditions on a 0.1% SDS–12% polyacrylamide slab-gel according to Laemmli method.[28] PAS staining was performed as below: gel was fixed with 10% trichloroacetic acid for 60 min, washed twice with deionized (DI) water for 1 min, oxidized with 1% periodic acid for 60 min, washed twice with DI water for 15 min, stained with Schiff reagent for 60 min, washed with DI water for 1 min, added 0.25% sodium metabisulphite–3.5% acetic acid solution for overnight at 4 °C and finally washed with DI water again.

2.7. Separation of constituent proteins from BLG aqueous extract

The ground BLG (100 g) was extracted with 300 mL of PBS buffer (0.01 M, pH 7.2, 0.1 M NaCl) overnight at 4 °C. The extract was centrifuged at 12,000 g for 15 min (Model CF16RX, Hitachi Koki Co., Ltd., Japan) and removed debris.

Download English Version:

https://daneshyari.com/en/article/5635362

Download Persian Version:

https://daneshyari.com/article/5635362

Daneshyari.com