Food Chemistry 264 (2018) 41–48

Contents lists available at ScienceDirect

Food Chemistry

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

Effect of atmospheric cold plasma on structure, activity, and reversible assembly of the phytoferritin

Rui Yang[a,b], Yuqian Liu[a], Demei Meng[a,b], Desheng Wang[c], Christopher L. Blanchard[d], Zhongkai Zhou[a,b,*]

[a] Tianjin University of Science and Technology, Institute for New Rural Development, 300457 Tianjin, China
[b] State Key Laboratory of Food Nutrition and Safety, School of Food Engineering and Biotechnology, Tianjin University of Science and Technology, Tianjin 300457, China
[c] Tianjin Goubuli Food Co., Ltd., Tianjin 300389, China
[d] ARC Industrial Transformation Training Centre for Functional Grains, Wagga Wagga, NSW 2678, Australia

ARTICLE INFO

Keywords:
Atmospheric cold plasma
Protein cage
Structure
Activity
Reversible assembly

ABSTRACT

Ferritin is characterized by a shell-like structure and a reversible self-assembly property. In this study, atmospheric cold plasma (ACP) was applied to red bean seed ferritin (RBF) to prepare an ACP-treated RBF (ACPF). Results indicated that the ACP treatment retained the shell-like structure of ferritin but reduced the α-helix/β-sheet contents and thermal stability. Iron oxidative deposition and release activities were also markedly changed. The ACPF could be disassembled at pH 4.0 and then assembled into an intact ferritin cage when pH was increased to 7.0, which was a more benign transition condition than that of the traditional method (pH 2.0/7.0 transition). By using this assembly routine, curcumin was successfully encapsulated within the ACPF with a size distribution of 12 nm. Moreover, the encapsulation ratio of curcumin in the ACPF reached 12.7% (w/w). This finding can be used to expand the application of ACP and improve the functionalization of the ferritin.

1. Introduction

Atmospheric cold plasma (ACP), a non-thermal technology, refers to the non-equilibrium plasma generated at near-ambient temperature and pressure (Han et al., 2016; Terpiłowski, Tomczyńska-Mleko, Nishinari, & Mleko, 2017). It is a source of reactive oxygen species, including singlet oxygen and ozone, and can excite molecular nitrogen (Misra, Pankaj, Segat, & Ishikawa, 2016). ACP has been widely applied in food preservation by taking advantage of its inactivation effect on microorganisms, including spoilage organisms and food borne pathogens (Cheng et al., 2014; Han, Patil, Keener, Cullen, & Bourke, 2014; Misra et al., 2016). ACP has shown potential for application in surface hydrophobicity enhancement (Misra et al., 2014), surface modulation (Bahrami et al., 2013), and enzyme inactivation (Mastwijk & Groot, 2010), in addition to food preservation. The applications of ACP have been expanded to include the treatment of biological macromolecules, such as whey protein (Segat, Misra, Cullen, & Innocente, 2015). However, studies are rarely reported on the effect of ACP treatment on the structure and property of ferritin, an iron storage protein that is widely distributed in plants, animals, and bacteria.

Ferritin is a cage-like protein that can store thousands of iron ions in a nanosized cavity (Yang, Zhou, Sun, Gao, & Xu, 2015). Each ferritin consists of 24 similar or different protein subunits. These subunits assemble into a shell-like structure with an internal diameter of 8 nm and an external diameter of 12 nm. Ferritin is principally characterized by iron oxidative deposition and iron release *via* the 3-fold or 4-fold channels (Chasteen & Harrison, 1999). Another distinct feature is reversible self-assembly: ferritin cage can be first disassembled under extremely acidic conditions (pH ≤ 2.0); reassembly of ferritin can then occur when the solution pH is adjusted to a neutral range (e.g., pH 7.0). Given the nanosized inner cavity and the reversible assembly characteristic of the ferritin cage, food nutrient molecules, such as β-carotene, epigallocatechin gallate (EGCG), and rutin, have been successfully encapsulated into the phytoferritin or recombinant ferritin (Chen et al., 2014; Yang et al., 2015, 2016). After encapsulation, these nutrient molecules can be functionalized using the ferritin tool to achieve stabilization, solubilization, and targeted delivery. However, the extremely acidic condition at pH 2.0 during ferritin disassembly may cause the loss of a large fraction of the sample to insoluble aggregates. After pH transition (2.0–7.0), the structural integrity of the reassembled ferritin remains undetermined (Tetter & Hilvert, 2017). Moreover, the extremely acidic condition may abrogate the sensory properties, stability, and bioactivity of certain pH-sensitive molecules. Thus, the successful encapsulation of bioactive molecules in the ferritin cage

* Corresponding author at: Tianjin University of Science and Technology, Institute for New Rural Development, 300457 Tianjin, China.
E-mail address: zkzhou@tust.edu.cn (Z. Zhou).

https://doi.org/10.1016/j.foodchem.2018.04.049
Received 5 January 2018; Received in revised form 13 April 2018; Accepted 14 April 2018
Available online 16 April 2018
0308-8146/ © 2018 Elsevier Ltd. All rights reserved.

while maintaining the integrity of the ferritin structure presents challenges.

The present study aims to evaluate the effect of ACP treatment on the structure and property of ferritin. The morphology, secondary structure, thermal stability, iron oxidative deposition, and iron release properties of ACP-treated apoRBF (ACPF) were evaluated. The disassembly-reassembly feature of the ACPF was emphasized. We also encapsulated the curcumin molecules into the ACPF. This study is designed to expand the application of ACP to include protein modification and to elucidate the effect of ACP on the structure and property of phytoferritin.

2. Materials and methods

2.1. Preparation of apoRBF and analyses of native-PAGE and SDS-PAGE

RBF and apoRBF (RBF deprived of iron ions) were prepared as reported (Li, Yun, Yang, & Zhao, 2013). The molecular weight of apoRBF was determined by native-PAGE using 12% polyacrylamide gradient gels. The running conditions were as follows: 5 mA, 8 h, and 4 °C. SDS-PAGE was performed under reducing conditions in 15% SDS-PAGE. The concentrations of ferritin were determined according to the Lowry method, with bovine serum albumin as a standard sample.

2.2. ACP treatment of apoRBF

ACP was performed using a dielectric barrier discharge plasma reactor (T&C Power Conversion, Inc.). Two electrodes (brass) were covered with dielectric layers made up of quartz to ensure uniform microdischarges. ApoRBF solutions (2 μM, 5 mL) in a quartz reactor were applied to plasma treatment for 120 s by using the aforementioned cold plasma prototype. A plasma discharge was generated by a DC power supply input under the following conditions: operating frequency, 12 kHz; voltage, 60 V; and current, 1.0 A. The treatment was conducted in a 50% ± 2% relative humidity at 20 °C ± 1 °C. The resulting ACP-treated apoRBF was finally refrigerated at 4 °C for further investigation.

2.3. UV/Vis spectra analyses

The UV/Vis spectra analysis of apoRBF and ACPF was performed by an Agilent 8453 spectrophotometer, with scan ranges of 195–300 nm at a speed of 300 nm/min at 20 °C.

2.4. Inherent fluorescence spectrofluorometry analyses

Inherent fluorescence spectrofluorometry (for inherent Tryptophan) was performed using an RF-5301PC spectrofluorophotometer (Shimadzu, Japan). The concentrations of both apoRBF and ACPF were 1.0 μM in 100 mM Mops at pH 7.0. The path lengths for excitation and emission were 1.0 and 0.5 cm, respectively. The excitation and emission wavelengths were set to 290 and 330 nm, respectively. Slits with widths of 10 and 5 nm were set for excitation and emission, respectively.

2.5. Circular dichroism (CD) analyses

CD spectra were recorded by a MOS-450 spectrometer using quartz cuvettes with an optical path length of 1 mm optical path length. The samples were scanned at a wide UV range (190–260 nm) with 3 replicates at 60 nm/min. The band width was 1 nm. The CD data were expressed as mean residual ellipticity, (h) in deg cm^2 $dmol^{-1}$. The contents of secondary structures were calculated based on a previously reported method (Andrade, Chacón, Merelo, & Morán, 1993). The percentage of secondary structure (α-helix, β-sheet, and random coil) content (%) = content of certain secondary structure/total content.

2.6. Surface hydrophobicity analyses

The surface hydrophobicities of the ACPF and the untreated apoRBF (2 μM, 100 mM Mops buffer, pH 7.0) were detected using 1-anilinonaphthalene-8-sulfonic acid (ANS) as a fluorescence probe as described in a previous method (Kato & Nakai, 1980). Fluorescence spectrofluorometry was performed using the RF-5301PC spectrofluorophotometer (Shimadzu, Japan). The path lengths for excitation and emission were 1.0 and 0.5 cm, respectively. The excitation and emission wavelengths were set to 337 and 501 nm, respectively. Slits with widths of 10 and 5 nm were set for excitation and emission, respectively. Surface hydrophobicity index (*So*) was obtained by calculating the initial slope of the plot of fluorescence intensity *vs.* protein concentration (Dong et al., 2017).

2.7. Iron oxidative deposition and iron release analyses

Iron oxidation deposition was analyzed in accordance with our reported method (Yang, Yang, Liao, Deng, & Zhao, 2014). Using the Agilent 8453 spectrophotometer, measurement was conducted by recording the absorbance at 300 nm at 25 °C. The final concentration of $FeSO_4$ was 48 μM (in acid water, pH 2.0). Both the apoRBF and ACPF (in 100 mM Mops, pH 7.0) had a final concentration of 0.5 μM each. The kinetics of iron oxidation was recorded in 90 s, and the spectrophotometer was operated in the single-beam mode. The initial rates of iron oxidation were measured by detecting the formation of the μ-oxo diFe(III) complex in accordance with the reported method (Yang, Yang, et al., 2014).

Iron-loaded ferritins (holoferritin) were prepared by adding 600 irons ($FeSO_4$, in acid water, pH 2.0) to each apoRBF or ACPF solution (1.2 mL, 50 mM NaCl, pH 7.0) by 6 increments with 20 min intervals. Iron release from holoferritin was investigated as follows: Each reaction system (1 mL) contained 1.0 μM holoACPF or holoRBF, 500 μM ferrozine, and 50 mM NaCl in 100 mM Mops buffer (pH 7.0). Reactions were initiated by adding of ascorbic acid (1 mM). The formation of $[Fe(ferrozine)_3]^{2+}$ was measured by recording the increase in absorbance at 562 nm, and the iron release was measured using $\varepsilon_{562} = 27.9\ mM^{-1}\ cm^{-1}$ (Hynes & Coinceanainn, 2002). The initial rate of iron release (v_o) was calculated as described in a previous study (Hynes & Coinceanainn, 2002).

2.8. Reversible assembly analyses of ACP-treated apoRBF

The pH of the ACPF (1.0 μM, 3.0 mL) was initially adjusted to pH 4.0 with HCl (1 M) for 50 min to disassemble ferritin into subunits. The pH of the resulting mixtures was then adjusted to 7.0 with NaOH (1.0 M) and then incubated at 4 °C for 60 min to induce the reassembly of the ferritin cage. The effect of pH (2.0, 3.0, 4.0, and 5.0) on ACPF disassembly was investigated to evaluate the effect of ACP treatment on ferritin self-assembly. To prepare the curcumin-loaded ACPF, a curcumin stock solution (1.0 mM) was added into the acid protein solution (pH 4.0) at a mole ratio of 80:1 (curcumin/ferritin = 80:1, molar ratio). The curcumin was encapsulated into the ACPF, and the ferritin cage was reassembled when pH was adjusted to 7.0. The products were then dialyzed (MW 10 kDa cutoff) against the buffer MOPS (20 mM, pH 7.0) with 5 buffer changes (every 1.0 h intervals). Finally, the suspension was further filtered through a 0.45 μm cellulose membrane filter to clarify the sample, resulting in a curcumin-loaded ACPF nanoparticle. A curcumin-loaded apoRBF nanoparticle was also prepared by reversible disassembly/reassembly. The amount of curcumin added into the apoRBF solution was the same as that added into the ACPF. The denatured pH was adjusted to pH 2.0, and the same procedure as that used in the preparation of a curcumin-loaded ACPF nanoparticle was conducted. The curcumin-apoRBF mixtures were also prepared by directly mixing curcumin and apoRBF (curcumin/ferritin ratio = 80:1) at pH 4.0 without disassembly of the apoRBF cage.

Download English Version:

https://daneshyari.com/en/article/7584608

Download Persian Version:

https://daneshyari.com/article/7584608

Daneshyari.com