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Mechanism for improved protection of whey protein isolate against the photodecomposition of folic acid

Xiaojun Fu [a, b], Wusigale [a, b], Hao Cheng [a, b], Zheng Fang [a, b, **], Li Liang [a, b, *]

[a] State Key Lab of Food Science and Technology, Jiangnan University, Wuxi, Jiangsu, China
[b] School of Food Science and Technology, Jiangnan University, Wuxi, Jiangsu, China

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ABSTRACT

Proteins have been widely used to prepare effective carriers for the protection of bioactive components. There were rare studies on proteins' change during protection and its impact on the protection, albeit they are important for the choosing of suitable proteins used as carrier materials. Folic acid is a synthetic form of folate but decomposes under ultraviolet irradiation. In this study, improved protection of whey protein isolate (WPI) against the photodecomposition of folic acid was investigated by using fluorescence and HPLC. During the vitamin decomposition, WPI's change was characterized in term of composition, structure, interaction with the vitamin or its decomposition products. Folic acid decomposition photosensitized indirect oxidation of WPI, leading to unfolding, degradation in concurrence with oligomerization. The increase in the absorbance and antioxidant activity contributed to the protein improved protection for folic acid. WPI could thus be a good carrier material for the encapsulation and protection of folic acid.

1. Introduction

Proteins play a potential role in encapsulation, protection and delivery of bioactive components through functional foods. Whey proteins have been widely used as carrier materials since they possess multiple functional properties including emulsification, gelation and ligand-binding capacity (Betz & Kulozik, 2011; Chen, Remondetto, & Subirade, 2006; De Wolf & Brett, 2000; Oidtmann et al., 2012). Whey protein components, such as beta-lactoglobulin (β-LG), alfa-lactalbumin (α-LA), bovine serum albumin (BSA) and immunoglobulins, are all known as ligand-binding proteins. β-LG constitutes 50% of bovine milk whey proteins and can bind a variety of ligands, such as vitamins, fatty acids and polyphenols (De Wolf & Brett, 2000; Livney, 2010). The affinity of folic acid to β-LG was $2.3 \times 10^5\ M^{-1}$ by using isothermal titration calorimetry, $4.3 \times 10^5\ M^{-1}$ by using 295-nm fluorescence quenching, and $2.0 \times 10^6\ M^{-1}$ by using 280-nm fluorescence quenching (Liang & Subirade, 2010; Perez, David-Birman, Kesselman, Levi-Tal & Lesmes, 2014). The vitamin's affinity to β-LG, α-LA and BSA was similar based on fluorescence quenching (Liang, Zhang, Zhou, & Subirade, 2013). The interaction of whey proteins with bioactive components and protection of formed complex against the latter's loss have been widely studied for the design of protein-based carriers (Liang & Subirade, 2015; Livney, 2010; Yi, Fan, Yokoyama, Zhang, & Zhao, 2016). However, there were rare studies on proteins' change during protection and its impact on the protection, which are important for the choosing of suitable proteins used as carrier materials.

Folate, a natural water-soluble B-vitamin, refers to various tetrahydrofolate derivatives present mainly in leafy green vegetables and legumes. Folate is essential for the biosynthesis of nucleic acids and proteins and its insufficiency is linked to neural tube defect, megaloblastic anemia, pregnancy complications and male infertility problem (Arcot & Shrestha, 2005; Gazzali et al., 2016). Folic acid (FA) is an oxidized and synthetic form of folate and usually used in fortify food and nutritional supplements. Folic acid is composed of pterin, *p*-aminobenzoyl, and L-glutamic acid. However, folic acid is sensitive to UV light, resulting in a splitting of the molecule into inactive 6-formylpterin (FPT) and *p*-aminobenzoylglutamate (PGA), followed by photo-oxidation of FPT to form 6-carboxypterin (PCA) (Gazzali et al., 2016; Off et al., 2005). It

* Corresponding author. State Key Lab of Food Science and Technology, Jiangnan University, Wuxi, Jiangsu 214122, China.
** Corresponding author. State Key Lab of Food Science and Technology, Jiangnan University, Wuxi, Jiangsu 214122, China.
E-mail addresses: fangzheng@jiangnan.edu.cn (Z. Fang), liliang@jiangnan.edu.cn (L. Liang).

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has been reported that addition of low-molecular-weight antioxidants, complexation with proteins, and encapsulation with biopolymer-based coacervate, nanolaminate and fiber could protect folic acid against the photodecomposition (Acevedo-Fani, Soliva-Fortuny, & Martin-Belloso, 2017; Aceituno-Medina, Mendoza, Lagaron, & López-Rubio, 2015; Chapeau et al., 2017; Liang et al., 2013; Wusigale, Fang, Hu, Gao, Li, & Liang, 2017). Folic acid interacted with serum albumins to form complex and human serum albumin delayed the vitamin photodecomposition (Bourassa, Hasni, & Tajmirriahi, 2011; Vorobey, Steindal, Off, Vorobey, & Moan, 2006). The photodecomposition of folic acid was also delayed by proteins ranked in order β-LG > BSA > α-LA (Liang et al., 2013).

Folic acid is a photosensitizer with many similarities to riboflavin in the dairy supplements (Martin, Walker, & Soniat, 2009). Riboflavin is also a water-soluble B-vitamin and its sensitized photochemical reaction induced aggregation and degradation of whey proteins under fluorescent light (Gilmore & Dimick, 1979; Jung, Lee, & Kim, 2000). Photodecomposition of folic acid caused unfolding of both β-LG and α-LA and degradation of BSA (Liang et al., 2013). FPT and PCA photosensitized to human serum albumin rather than folic acid itself (Vorobey et al., 2006). Pterin, a constituent of folic acid, and its derivatives are photochemically reactive in aqueous solution and could undergo oxidation to generate reactive oxygen species and to photosensitize the oxidation of biomolecules (Castaño, Dántola, Oliveros, Thomas, & Lorente, 2013; Thomas, Lorente, Roitman, Morales, & Dántola, 2013). Protein oxidation modified a wide range of amino acids, resulting in fragmentation and aggregation of polypeptide chain and change of physicochemical property (Pattison, Rahmanto, & Davies, 2011).

In this study, WPI was used to protect folic acid against decomposition under UV radiation. The susceptibility of WPI to photo-oxidation during the vitamin decomposition was investigated by characterization of amino acid composition and structural change. Furthermore, WPI separated from its folic acid mixture during irradiation was characterized in term of absorption, interaction with FA or decomposition products, and antioxidant activity. It was note that the protection of WPI against the decomposition of folic acid gradually increased during UV irradiation. WPI could thus be a good material to design effective carriers for the protection of folic acid and possibly of other B-vitamins.

2. Materials and methods

2.1. Materials

WPI (Biopro, purity ~92%) was obtained from Davisco International Inc (Le Sueur, MN, USA). FA (~98%), PCA (>98%) and 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB, ≥98%) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). FPT was purchased from Schircks Laboratories (Jona, Switzerland). PGA was obtained from Aladdin Bio-Chem Technology Co. LTD, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid ammonium salt) (ABTS) and β-mercaptoethanol were obtained from Sango Biotech Co., phosphoric acid and methanol of HPLC grade were purchased from Fisher Scientific Co., 2,4-dinitrophenylhydrazine (DNPH, ≥98%) and other reagents of analytical grade were obtained from SinoPharm CNCM Ltd. in Shanghai of China.

2.2. Sample preparation

Exactly 2 mg/mL WPI in ultrapure water was mixed with 2 mM folic acid in 10 mM phosphate buffer at pH 7.4, adjusted to pH 7.0 and incubated for 30 min. WPI had a final concentration of 1 mg/mL, equal to the final molar concentration of folic acid (~55.6 μM), which was calculated based on the molecular weight of β-LG. After irradiation for 0, 60, 120 and 180 min, WPI was separated by ultrafiltration with a molecular weight cut-off of 3000 Da (Amicon® Ultra-15, Millipore, Ireland) under centrifugation at 7500 g for 3 times until folic acid and its decomposition products were not detected in the permeate compartment. The cut-off WPI solution was also used to prepare protein-FA mixture as described above. WPI samples separated from its FA mixture during irradiation for 0, 60, 120 and 180 min were expressed as WPI-0, WPI-60, WPI-120, WPI-180, respectively. All samples were prepared and tested at least in duplicate.

2.3. UV irradiation

Samples were exposed to UV light by using a UVA lamp (peak $\lambda = 365$ nm, Guang Hao Analytical Instruments, Shanghai, China) with a power of 8 W and fluence rate of 1 mW cm^{-2}. Samples were placed at a distance of 9 cm from the light source and analyzed every 20 min.

2.4. Fluorescence measurement

Fluorescence was measured by using a 10-mm quartz cell on a Cary Eclipse fluorescence spectrophotometer (Agilent Co. Ltd, New York, USA). Fluorescence emission spectra of folic acid in the absence and presence of WPI, WPI-0, WPI-60, WPI-120 and WPI-180 were measured at an excitation wavelength of 350 nm to record the intensity at the emission maximum (λ_{max}) around 450 nm. Fluorescence emission spectra of WPI in the absence and presence of folic acid, FPT, PGA or PCA were measured by excitation at 280 nm to record the intensity at λ_{max} around 333 nm. Quenching of protein fluorescence was calculated by using the expression

$$\text{Quenching}\ (\%) = (F_0 - F_L)/F_0 \times 100 \quad (1)$$

where F_0 and F_L are the protein fluorescence in the absence and presence of the ligands, respectively. Fluorescence spectra of WPI and WPI separated from its FA mixture during irradiation were scanned from 300 to 550 nm. The spectral resolution of excitation and emission was 2.5 and 5 nm for folic acid and WPI, respectively.

2.5. High performance liquid chromatography

Content of folic acid, FPT, PGA and PCA was determined by using a Waters HPLC system (Waters, Milford, MA, USA) equipped with a 2695 separation module, a 2998 PDA detector and a Waters Symmetry C18 column (4 μm, 250 × 4.6 mm). The temperature of the column was set to 30 °C. A gradient of mobile phase consisting of methanol (solvent A) and 0.1% phosphoric acid (solvent B) was developed as following: starting with 30% A and 70% B until 12 min to 50% A and 50% B until 27 min and then return to the initial condition within the next 3 min. Absorbance at 280 nm was measured (Wusigale et al., 2017).

2.6. Absorbance measurement

Absorption spectra of WPI, WPI-60, WPI-120 and WPI-180 were measured with a path length of 1 cm on a Shimadzu UV-1800 spectrophotometer (Shimadzu Corp, Kyoto, Japan). Turbidity (100 - %transmittance) of folic acid, WPI and both mixture under irradiation was determined from the absorbance at 600 nm.