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Characterization of milk proteins–lutein complexes and the impact on lutein chemical stability

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ABSTRACT

In this study, the interaction of WPI (whey protein isolate) and SC (sodium caseinate) with hydrophobic lutein was investigated through UV–vis spectroscopy and circular dichroism (CD) as well as fluorescence. The effects on lutein's chemical stability were also examined. The decrease of turbidity of lutein suggested that lutein's aqueous solubility was improved after binding with milk proteins. CD analysis indicated lutein had little impact on the secondary structures of both proteins. Different preparation methods have significant impacts on the binding constant. Fluorescence results indicated that WPI and SC interact with lutein by hydrophobic contacts. Milk proteins have protective effects on lutein against oxidation and decomposition, and SC showed better capability in protecting lutein from oxidation than WPI during 16 days storage. The lutein's chemical stability was increased with increasing of proteins concentration. The results indicated that milk proteins may act as effective carriers for lipophilic nutraceuticals.

1. Introduction

Lutein (Fig. 1) is a kind of oxygenated naturally occurring carotenoids, belonging to the family of the xanthophyll group of carotenoids (Boon, McClements, Weiss, & Decker, 2010). Leafy greens like spinach, collard greens, kale, corn, persimmons, and broccoli are its main sources (Sommerburg, Keunen, Bird, & van Kuijk, 1998). As a natural antioxidant, lutein was found to have a protective effect against oxidative damage of egg yolk lecithin liposomal membranes induced by exposure to UV radiation and incubation (Sujak et al., 1999). The presence in specific eye tissues made lutein unique relative to other carotenoids in humans (Alves-Rodrigues & Shao, 2004). Recently, some epidemiologic studies illustrated that lutein may protect against the development of the two common eye diseases of aging, cataract and macular degeneration (Gale, Hall, Phillips, & Martyn, 2003). Lutein may have impacts on the prevention of cancer, neuronal damage (Li et al., 2012), improving skin health (Wingerath, Sies, & Stahl, 1998) and cardiovascular disease (Mares-Perlman, Millen, Ficek, & Hankinson, 2002). Lutein is used widely in the pharmaceutical, food and cosmetic industries.

However, poor solubility in aqueous phase, low bioavailability, and sensitivity to heat, oxygen and light, which can be easily oxidized, isomerized and degraded, greatly limited the applications of lutein (Boon et al., 2010; Wang et al., 2012). In order to increase lutein's physicochemical stability, bioavailability and solubility in aqueous phase, a lot of efforts have been done to tackle it by food researchers. For example, spray drying formed lutein microcapsules with porous starch and gelatin mixture (Wang et al., 2012), lutein/zein nanoparticles via solution enhanced dispersion by supercritical fluids (Xia, Hu, Jin, Zhao, & Liang, 2012), preparation of lutein microencapsulation by complex coacervation (Qv, Zeng, & Jiang, 2011), and obtained lipid nanocarriers for dermal delivery of lutein by high pressure homogenization (Mitri, Shegokar, Gohla, Anselmi, & Mueller, 2011). Proteins complexes could provide alternative encapsulation carriers for the delivery of functional and nutritional components.

Milk proteins are natural and multifunctional biopolymers, which have already been widely used to deliver bioactive molecules and protect them against oxidation and degradation (Livney, 2010; McClements & Decker, 2000). Sodium caseinates (SCs) and whey protein isolates (WPIs) are two important, widely available, inexpensive, natural, and generally recognized as safe (GRAS) commercial milk protein products (Hasni et al., 2011). SC

Abbreviations: SC, sodium caseinate; WPI, whey protein isolate; SPI, soybean protein isolate; PB, phosphate buffer; CD, circular dichroism; BLG, β-lactoglobulin; ALA, α-lactalbumin; BSA, bovine serum albumin; IG, immunoglobulins; LF, lactoferrin.

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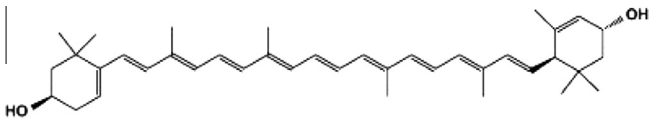

Fig. 1. The chemical structure of all-trans-lutein.

is mainly composed of four fractions named α_{s1}-, α_{s2}-, β- and κ-casein, respectively. And WPI is mainly consisted of β-lactoglobulin (BLG), α-lactalbumin (ALA), bovine serum albumin (BSA), immunoglobulins (IGs), and lactoferrin (LF). Their relative content in milks by weight and the molecular weight were shown in Table S1.

Interactions of carotenoids (β-carotene, β-cryptoxanthin, and α-carotene) with BLG have been reported (Mensi et al., 2013) and the obtained results indicated that carotenoids are bound by BLG with high hydrophobic affinity. The binding between tea catechins with casein was also reported and the results showed that the presence of tea polyphenols resulted in a change of casein's gelation properties (Haratifar & Corredig, 2014). However, to date, no studies were found about the interaction between milk proteins and lutein and the mechanism underlying. But some were published to show that the physicochemical stability can be improved with complex coacervation method or nanoparticle-based delivery system (Dai et al., 2015; Qv et al., 2011). In this work, the interaction between milk proteins (WPI and SC) and lutein was studied by UV–vis spectroscopy, turbidity measurement, fluorescence spectroscopy, and circular dichroism (CD). And the effects of two different proteins (SC and WPI) on lutein' retention under storage were also compared.

2. Materials and methods

2.1. Materials

Sodium caseinate (SC) powder (protein content > 95% and 1.0% fat on dry basis by manufacturer) was obtained from Fonterra Co-operative Group (Auckland, New Zealand). Whey protein isolate (WPI; Hilmar 9410, 93.0% protein, 0.2% lactose, 1.0% fat, and 2.0% ash, on a dry basis, and 4.5% water) was obtained from Hilmar Food International, Inc., Livingston, CA, US. Lutein (98% pure) was purchased from Zhejiang Medicine Co., Ltd. (Zhejiang, China). All other reagents are analytical grade and used without further purification. Ultrapure water was used in all experiments.

2.2. Sample preparation

0.5 g SC and WPI were dissolved in 10 mM phosphate buffer (PB, pH7.4), stirred for four hours using magnetic stirrer, diluted to 100 mL volumetric flask, and stored at 4 °C for use, respectively.

Stock lutein alcoholic solution was prepared freshly at a concentration of 2 mM by dissolving in ethanol fully and stored at 4 °C for use. Stock 200 μM lutein PB solution were prepared by adding drop-by-drop stock lutein alcoholic solution to 10 mM PB (pH 7.4) under stirring. Lutein–protein complexes were prepared based on the method of (Liang, Tremblay-Hebert, & Subirade, 2011) with slight modification. Add stock lutein PB or lutein alcoholic solution to proteins solution diluted in PB drop-by-drop under magnetic stirring to get different concentration of lutein (0, 5, 10, 20, 40, 60, 80, and 100 μM), while the protein content remains constant (0.5 mg/mL). All samples were blended by magnetic stirring and then incubated for at least 1 h at room temperature before analysis.

2.3. Measurement of lutein and lutein–protein complexes solution turbidity

Turbidity was determined from the transmission at 500 nm according the method by (Liang et al., 2011), using a 2802 UV–vis spectrophotometer (Unico, U.S.A.) and expressed as (100% transmission). All experiments were performed in triplicate at room temperature (25 °C).

2.4. Steady-state fluorescence measurement of protein

The intrinsic fluorescence was measured using a fluorescence spectrometer (Hitachi F-7000, Japan) at a constant protein concentration (0.05%, w/v) in the presence of 0, 5, 10, 20, 40, 60, 80, and 100 μM lutein. Emission spectra were recorded from 290 to 500 nm at an excitation wavelength of 280 nm. The spectral resolution of both excitation and emission was 5 nm. In this study, the fluorescence spectra of controls were subtracted from the respective spectra of samples to offset any contribution that was due to the Raman peak and other scattering artifacts.

2.5. Circular dichroism measurements on bovine milk protein

CD spectra were determined by a model Mos-450 CD spectropolarimeter (Biologic, Claix, France) between 190 and 250 nm with an interval of 1 nm at 25 °C. The concentration of SC and WPI solution were both 0.5 mg/mL. The phosphate buffer solution (10 mM, pH 7.4) was used as the blank for all samples. The scan rate was 5 nm/s. Five scans were averaged to obtain one spectrum. The molar ellipticities of protein samples were calculated as $[\theta]$ (deg cm^2 dmol^{-1}) = $(100 \times X \times M)/(L \times C)$, where X is the signal (millidegrees) obtained by the CD spectrometer, M is the average molecule weight of amino acid residues in the protein, C is the protein concentration (mg/mL) of the sample, and L is the cell path length (cm). A quartz cell with a 1 cm path length was used, and a constant nitrogen flush was used during wavelength scanning. The secondary structure composition was calculated from far-UV CD spectra data using DichroWeb online (http://dichroweb.cryst.bbk.ac.uk/html/home.shtml).

2.6. Lutein's storage stability

To analyze the lutein stability in lutein–protein complexes, samples with different concentration of proteins (SC and WPI) prepared following the method mentioned previously (Section 2.2) were put in a 1.5 mL plastic centrifuge tubes and were stored at 25 °C in a constant temperature incubator. The same content of lutein ethanol dispersed in PB was used as control. *n*-Hexane was used to extract lutein for three times, then organic phase was collected, and dried under nitrogen. The lutein extract was dissolved in ethanol, and diluted exactly in 10 mL brown volumetric flask. The absorbance value was measured in 445 nm, with 2802 UV–vis spectrophotometer (Unico, U.S.A.). All experiments were performed in triplicate at room temperature (25 °C).

2.7. Statistical analysis

All measurements were performed at least three times and were reported as mean ± standard error. The data were analyzed by analysis of variance (ANOVA) using the SPSS 17.0 package (IBM, New York, U.S.). Duncan's multiple range test was used to determine the significant differences of the mean values ($p < 0.05$).