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Formation of nanocomplexes comprising whey proteins and fucoxanthin: Characterization, spectroscopic analysis, and molecular docking

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ABSTRACT

Fully elucidating the protein-ligand interaction mechanisms can help us better construct the nanocarrier to delivery fucoxanthin. Thus, in this study, the complexes comprising fucoxanthin and whey proteins were constructed and characterized, using bovine serum albumin (BSA), β-lactoglobulin (β-Lg), and α-lactoalbumin (α-La). The binding processes were also investigated using multi-spectroscopic methods and molecular docking. The results indicated that the skeletons of BSA/β-Lg/α-La were loosened and unfolded, and the protein aggregates were formed with a nano scale (<300 nm) in the presence of fucoxanthin. The number of binding sites was about equal to 1. The binding affinity in decreasing order was BSA, β-Lg, α-La. Based on thermodynamic investigations, all binding processes were spontaneous, and non-covalent interactions were the major driving forces for the formation of whey protein −fucoxanthin nanocomplexes. Docking results also indicated the contribution of van der Waals force, hydrogen bond and hydrophobic interaction when the nanocomplexes were formed, well agreeing with the thermodynamic analysis.

1. Introduction

Fucoxanthin is a major carotenoid present in the chloroplasts of edible brown seaweed (D'Orazio et al., 2012). It is the most abundant of all carotenoids, contributing more than 10% of their estimated total natural production (Peng, Yuan, Wu, & Wang, 2011). Fucoxanthin can provide numerous benefits for human health, lowering the incidence of obesity, cardiovascular disease, diabetes and cancer (Miyashita et al., 2011). Recently, Lashmanova et al. (2015) emphasized the possibility of fucoxanthin as a geroprotective compound to prolong the life and improve the viability of organisms, with a potential to prevent the development of age-related diseases in human. These findings indicate that fucoxanthin can be developed as a nutritional supplement in the diet or functional foods.

Fucoxanthin has a distinct structure, including an unusual allenic bond, epoxy group and conjugated double bond in the polyene chain, leading to its excellent anti-obesity and other activities (Miyashita et al., 2011). However, this structure is unstable and susceptible to some external conditions, e.g., thermal processing, air exposure, illumination, as well as chemical oxidation (Zhao, Kim, Pan, & Chung, 2014; Zhu, Sun, Chen, Wang, & Wang, 2016). The similar results can be observed in some research about the change of carotenoid content in brown seaweed during the drying process (Ling, Yasir, Matanjun, & Bakar, 2015; Moreira, Chenlo, Sineiro, Arufe, & Sexto, 2015; Ryckebosch, Muylaert, Eeckhout, Ruyssen, & Foubert, 2011). Additionally, a previous study on oral administration of fucoxanthin found an area under the curve (AUC) of fucoxanthinol in plasma (0.66 μM h) after a total intake (TI = 47.1 μmol) (Hashimoto et al., 2012). Considering this result, the bioavailability of fucoxanthin is lower than that of other dietary carotenoids, such as β-carotene (AUC = 13.6 μM h, TI = 34 μmol), lutein (AUC = 42.8 μM h, TI = 33 μmol), and astaxanthin (AUC = 2.26 μM h, TI = 67.1 μmol) (Novotny, Kurilich, Britz, & Clevidence, 2005; Odeberg, Lignell, Pettersson, & Höglund, 2003). Therefore, the relatively poor stability and low bioavailability are two major disadvantages of fucoxanthin, possibly restricting its application in food industries.

Many reviews support the viewpoint that designing new delivery systems is an effective approach for improving the stability

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and bioavailability of bioactive compounds (McClements 2015; McClements et al., 2015; Joye, Davidov-Pardo, & McClements, 2014). So far, only a few studies have investigated the fabrication of fucoxanthin carriers, mainly lipid-based carriers, including the solid lipid nanoparticles (Quan, Kim, Pan, & Chung, 2013), microemulsion (Dai et al., 2014), and nanoemulsion (Salvia-Trujillo, Sun, Um, Park, & McClements, 2015). However, these carriers, requiring a relatively large dose of surfactants or cosurfactants for preparation, may bring a huge food safety risk and an undesirable food favor (McClements & Rao, 2011). In recent years, a number of studies on the encapsulation and controlled release methods give more attention to the bovine whey proteins. As the Generally Recognized as Safe (GRAS) ingredients, the whey proteins, mainly including BSA, β-Lg, α-La, lactoferrin and immune globulin, are widely used in food or pharmaceutical industries owing to their important biological, physical and chemical functionalities (Kimpel & Schmitt, 2015; Livney, 2010; Tavares, Croguennec, Carvalho, & Bouhallab, 2014). Besides the high nutritional value, they are also the natural carriers *in vivo*, transporting essential micronutrients and amino acids, as well as immune system components (Livney, 2010). In this regard, the whey proteins are suitable as the alternatives for lipid carriers, with an ability to fabricate more advanced lipid-free vectors loading the bioactive compounds (Livney, 2010; Tavares et al., 2014).

The whey proteins have many significant physicochemical properties beneficial for encapsulating the active ingredients, and the most important one is their excellent binding ability. A variety of molecules can be bound to whey proteins at different degrees of affinity (Livney, 2010), and the binding parameters (binding constants and sites) are dependent on the chemical nature of ligands, physicochemical conditions of media, and conformation of proteins (Tavares et al., 2014). For hydrophobic molecules, the protein-binding mechanisms are varied, mainly hydrophobic interaction, van der Waals attraction and hydrogen bond (Kimpel & Schmitt, 2015). Recent studies indicated that the whey proteins could bind and delivery some dietary carotenoids by the above mechanisms, such as α-carotene (Mensi et al., 2013), β-carotene (Li, Wang, Chen, & Lu, 2015; Mensi et al., 2013, 2014), β-cryptoxanthin (Mensi et al., 2013), astaxanthin (Li et al., 2015), lutein (Yi, Fan, Yokoyama, Zhang, & Zhao, 2016), bixin (Zhang & Zhong, 2012), and norbixin (Zhang & Zhong, 2013). However, more recently, literature with a contradictory conclusion excluded the possibility of complex formation between lycopene and BSA, owing to the domination of dynamic fluorescence quenching process (Rodríguez Galdón, Pinto Corraliza, Cestero Carrillo, & Macías Laso, 2013). Thus, a further investigation of the interaction between whey proteins and fucoxanthin is needed, and more importantly, this aspect have not been studied.

Fully elucidating the protein-ligand binding mechanism can help us better construct the nanovehicles to delivery fucoxanthin or other similar bioactive molecules. Therefore, the objective of this study is to investigate the ability of native whey proteins to bind fucoxanthin, analyze the particle size and morphologic property, as well as probe the molecular interaction mechanism. Besides, three main components of whey proteins, including BSA, β-Lg and α-La, serve as the model proteins to evaluate the potential of fucoxanthin vehiculization.

2. Materials and methods

2.1. Materials

Fucoxanthin was prepared from *Undaria pinnatifida* and stored in the dark at 4 °C according to a previous study (Zhu et al., 2016). The concentration of fucoxanthin dissolved in ethanol was determined using an HPLC system (1260, Agilent Technologies Inc., Santa Clara, CA, USA). Purified BSA, bovine β-Lg, and calcium depleted bovine α-La (apo α-La) were purchased from Sigma Chemical Company (St. Louis, MO, USA). All other chemicals were all of analytical reagent grade and procured from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). BSA and β-Lg dispersions were prepared in a phosphate buffer solution (PBS) of pH 7.4 (20 mM), and α-La solution was prepared by the 20 mM PBS (pH 7.4) containing 35 mM ethylenediamine tetraacetic acid disodium salt (EDTA-2Na) to maintain the apo state. All stock solutions were kept in the dark at 4 °C before diluting to a final concentration, which were determined spectrophotometrically according to the extinction coefficients $\varepsilon_{280} = 44{,}720\ M^{-1}\ cm^{-1}$ for BSA (Li et al., 2015), $\varepsilon_{278} = 17{,}600\ M^{-1}\ cm^{-1}$ for β-Lg (Hadian et al., 2016), and $\varepsilon_{280} = 28{,}540\ M^{-1}\ cm^{-1}$ for α-La (Mohammadi & Moeeni, 2015).

2.2. Fluorescence spectra measurements

All fluorescence spectra measurements were carried out using an F-4600 fluorescence spectrophotometer (Hitachi Ltd., Tokyo, Japan) equipped with a 1.0 cm quartz cell and thermostat bath. Very dilute protein solutions were used in the experiments ([BSA] = [β-Lg] = [α-La] = 5.0 μM), and the fucoxanthin concentration was adjusted from 0.0 to 15.0 μM. Additionally, ethanol added with the fucoxanthin never exceeded 3% (v/v). The mixtures of BSA/β-Lg/α-La with fucoxanthin were vortexed for 5 min before measurement of the fluorescence intensity. The excitation and emission slit widths were fixed at 10 nm. The photomultiplier tube voltage was set as 400 V. The excitation wavelength was set at 280 nm, and the emission spectra were recorded in the range of 300–450 nm at different ambient temperatures (298, 304, 310 K).

For synchronous fluorescence measurements, the excitation spectra were recorded from 250 to 320 nm when the interval between excitation and emission wavelength (Δλ) was fixed at 15 and 60 nm, respectively. Other scanning parameters were identical to the above fluorescence experiment.

For three-dimensional fluorescence measurements, the emission wavelength was collected between 200 and 500 nm with a scanning rate of 12,000 nm min^{-1} and the initial excitation wavelength was set to 200 nm with an increment of 5 nm. Other scanning parameters were also identical to the above fluorescence experiment.

2.3. Absorption spectra measurements

UV–vis absorption measurements were performed using a 2102PC spectrophotometer (Shanghai Unico Instruments Co., Ltd., China) equipped with 1.0 cm quartz cells. The absorption spectra of samples containing fucoxanthin and various proteins were recorded in the range of 200–320 nm. PBS buffer (control) and various samples were placed in the reference and sample cuvettes, respectively.

2.4. Fourier transform infrared spectroscopy

Fourier transform infrared spectroscopy (FT-IR) were performed on a Nicolet iS10 spectrometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). The spectra of whey proteins, in the absence and presence of fucoxanthin, were recorded in the range 400–4000 cm^{-1} with the potassium bromide pellet method. The molar ratio of fucoxanthin to whey proteins was maintained at 3:1. A background spectrum was recorded before each sample measurement. Then a total of 64 scan were recorded and averaged. The secondary structures of free whey proteins and its fucoxanthin complexes were determined from the shape of the amide I band. Fourier self-deconvolution and second derivative resolution