Journal of Food Engineering xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Journal of Food Engineering

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

Characterization of resveratrol–milk protein interaction

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ARTICLE INFO

Article history:
Received 18 November 2014
Received in revised form 21 May 2015
Accepted 27 May 2015
Available online xxxx

Keywords:
β-casein
β-lactoglobulin
Bovine serum albumin
Resveratrol
Fluorescence
Circular dichroism
Transmission electron microscopy

ABSTRACT

Resveratrol is a natural polyphenolic compound which is poorly soluble in aqueous solutions. Due to its polyphenolic structure, it possesses antioxidant activity and anticancer effects. Two different milk proteins (β-lactoglobulin (BLG), β-casein (BCN) and bovine serum albumin (BSA)) were used to investigate the influence of protein–resveratrol interaction and its influence on the binding sites, structure and the conformational changes of the proteins. Circular dichroism results showed that resveratrol did not caused any significant change to the secondary structure of proteins. However, steady-state fluorescence results indicated that resveratrol was able to quench the intrinsic fluorescence of the proteins. Fluorescence results were used to calculate the affinity constants between resveratrol and the three proteins. BLG was the protein with the highest accessible fluorophore exposure in comparison with BSA and BNC. In addition, transmission electron microscopy experiments were carried out to visualize the complex resveratrol–protein formation.

1. Introduction

3,5,4′-Trihydroxystilbene, commonly known as resveratrol (RES), is a natural polyphenolic compound found in peanut butter, red wine, and grape juice. Resveratrol exists a trans- and cis-isomers (Fig. 1). Most of its biological activities are attributed to the trans-isomer. Trans isomer of resveratrol converts to its cis-isomers under exposure to light. The stability of trans-resveratrol ranges from hours to several days and it depends on the pH of the solution (Jimenez-Garcia et al., 2012; Bertelli et al., 1996).

This compound has aroused widespread interest due to biological effects associated with health benefits (Pace-Asciak et al., 1995; Liang et al., 2007; Stervbo et al., 2007). It displays various pharmacological activities and is widely known for its cardioprotective, chemopreventive, antioxidant, anti-inflammatory, analgesic, neuroprotective, and anti-aging activities (Baur and Sinclair, 2006; Jang et al.,1997). Frankel et al. showed in 1993 that resveratrol can inhibit the oxidization of LDL cholesterol, thus eventually reducing the risk of coronary artery disease (Frankel et al., 1993). In addition, resveratrol was known to inhibit arachidonate metabolism in leukocytes and to inhibit platelet aggregation in plateletrich plasma (Yoshiyuki et al., 1985; Bertelli et al., 1996). The free radical scavenging capacity of resveratrol and other polyphenols might also contribute to the beneficial effects of this compound (Fauconneau et al., 1997). The very short half-life (approximately 8–14 min) and extremely low oral bioavailability of resveratrol have raised concerns regarding its systemic action (Baur and Sinclair, 2006; Das et al., 2008; Baur et al., 2006).

In order to have biological activity, molecules need to be capable of reaching the action site without losing integrity and be able to cross the lipophilic membrane. Plants bioactive compounds have restrict application as pharmaceutical products considering their limited water solubility, poor bioavailability, and the fact that they can be easily modified by environmental factors such as temperature, pH and light (Fang and Bhandari, 2010; Munin and Edwards-Lévy, 2011; Pinho et al., 2013).

Bovine serum albumin (BSA) found in whey, milk, and plasma, is a highly soluble protein without bulky appending carbohydrates, which is stable and available at high purity and low cost. It is constituted by 585 amino acid residues, including 35 cysteines (17 disulfide bond bridges), which confer a relatively strong stability

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http://dx.doi.org/10.1016/j.jfoodeng.2015.05.032
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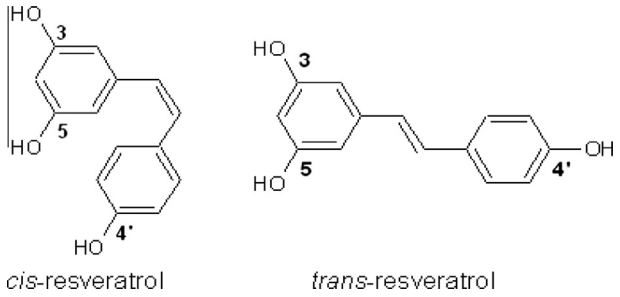

Fig. 1. Cis and trans isomers of resveratrol.

to the protein (Carter and Ho, 1994; Tang and Shen, 2013). BSA can also act as emulsifiers for the reason that it has a strong tendency to adsorb at oil–water interface to lower the surface tension (Han et al., 2010; Suslick and Grinstaff, 1990; Grinstaff and Suslick, 1991). BSA has been widely used in drug delivery studies because of its abundance, low cost, ease of purification, and unusual ligand-binding properties (Elzoghby et al., 2012). Previous studies showed that BSA has the highest binding capacity and considerable surface activity among other individual milk proteins (Singh and Ye, 2009; Kühn and Zhu, 2007; Livney, 2010). Moreover, the interaction between resveratrol and human serum albumin assessed by CD spectroscopy, showed no major alterations at low resveratrol concentrations. However, the results indicate a partial stabilization of protein secondary structure at high resveratrol content (N′Soukpoé-Kossi et al., 2006). Furthermore, it has been found that the driving forces for BSA-Quercetin association were hydrophobic interaction and hydrogen bond, and the latter was involved in the mechanism of Quercetin stabilization, which makes BSA a good carrier to deliver hydrophobic flavonols (Fang et al., 2011).

β-Lactoglobulin (BLG) is a 162-residue globular protein, makes up approximately 60% of bovine whey protein (Matalanis et al., 2011; Teng et al., 2013). Because of its flexibility and amphiphilic nature, BLG serves as a natural carrier for various nutrients (Lefèvre and Subirade, 2003; Papiz et al., 1986; Kontopidis et al., 2004). It has an isoelectric point (pI) close to 5, which means that it is negatively charged at neutral pH (Ye and Singh, 2007). By far, various encapsulating systems have been developed with BLG, including emulsions, nanoparticles, and nanocomplexes. These techniques have demonstrated satisfactory protection to the entangled compounds (Given, 2009; Zhang and Zhong, 2010; Ko and Gunasekaran, 2006; Zimet et al., 2009). The studies on the interaction between BLG and resveratrol showed that BLG has the binding constant between 10^4 and 10^6 M^{-1}, as determined by protein or polyphenol fluorescence. However, it had no apparent influence on BLG secondary structure, as resveratrol is bound to the surface of the protein, because BLG-bound polyphenol is in a weaker hydrophobic environment (not in the internal cavity). It does not prevent the isomerization of trans- resveratrol, but can partially improve its photostability (Liang et al., 2007). However, the study of the interaction between BLG and retinol indicates that there are binding sites in the interior cavity and the surface clef (Cho et al. 1994). BLG was shown to bind many hydrophobic molecules by several studies, particularly vitamin D and cholesterol (Wang et al., 1997; Forrest et al., 2005).

β-Casein (BCN) is one of the four major constituents of the milk caseins, a protein widely used in fundamental and applied food emulsion studies (Dickinson, 1992; Dickinson and Stainsby, 1982; McMahon and Brown, 1984; Visser, 1992; McHugh and Krochta, 1994). It could be expected that the hydrophobic part of the molecule adsorbs at the oil–water interface in a "train" configuration, while the hydrophilic chain extends farther away as a "tail" or "loop" (Graham and Phillips, 1979a,b,c). Esmaili et al. showed that BCN increased the solubility of curcumin and the interaction was predominantly an hydrophobic interactions (Esmaili et al., 2011).

According to the several internal and external binding sites these proteins have, diverse interactions, like hydrophobic interactions, hydrogen bonding and electrostatic interactions that may occur between these proteins and ligands. In the current study, we aim to gain a deeper understanding of the interaction (binding) between resveratrol and BSA, BLG and BCN using fluorescence, zeta potential, circular dichroism measurements and transmission electron microscopy. Such results might be of help in the design of future carriers for the stabilization and delivery of resveratrol.

2. Materials and methods

2.1. Materials

Trans-resveratrol (fine crystalline powder with 98% purity) was obtained from Guangzhou Honsea Sunshine (Lougang, Guangzhou, China). β-lactoglobulin, bovine serum albumin and β-casein were purchased from Sigma (Germany).

2.2. Preparation of samples

Each protein stock solution was made by dissolving in 10 mM phosphate buffer at pH 7.4 (physiological condition). Resveratrol stock solution was prepared daily by dissolving at a concentration of 1.2 mM in 75% ethanol and then diluting with 10 mM phosphate buffer at pH 7.4 to a concentration of 120 μM (Bourassa and Kanakis, 2010; Teskac and Kristl, 2010; N′Soukpoé-Kossi et al., 2006). Samples were prepared by mixing protein and resveratrol stock solutions in varying proportions using Ultra-Turrax with the speed of 13,000 rpm/5 min. The highest resulting ethanol concentration was about 7%, which had no appreciable effect on protein structure. The measurements were performed immediately after the sample preparation.

2.3. Circular dichroism (CD) measurement

Far-UV CD spectra of each protein were recorded at bioactive concentrations of 0, 10, 20 and 40 μM using a Chirascan CD Spectrophotometer (Applied Photophysics, Leatherhead, UK). The concentration of proteins was 10 μM in the wavelength range from 180 to 260 nm. The instrument was flushed with nitrogen, the path length was 1 mm, spectral band-width was set to 3 nm and the scan time per point to 10 s. Buffer background was subtracted from the raw spectra.

2.4. Zeta potential

Electrophoretic mobility of samples was determined using a Malvern Zetasizer NS 2000 apparatus (Malvern Instruments, Germany) fitted with a DTS1070 sample cell. The mobilities at room temperature were measured at a certain constant protein concentration of 20 μM and different concentrations of resveratrol (0, 5, 10, 15, 20, 40 and 80 μM). The constant concentration of 20 μM for protein solutions were chosen so that the ability to measure samples at or close to their neat concentration would be desirable as it would minimize any changes in the zeta potential of the sample owing to dilution and to avoid possible self-association of proteins in high concentrations. Results were expressed as the mean of three measurements ± standard deviation.

Please cite this article in press as: Ghorbani Gorji, E., et al. Characterization of resveratrol–milk protein interaction. Journal of Food Engineering (2015), http://dx.doi.org/10.1016/j.jfoodeng.2015.05.032