Food Chemistry 185 (2015) 261–267

Contents lists available at ScienceDirect

Food Chemistry

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

Fluorescence quenching study of resveratrol binding to zein and gliadin: Towards a more rational approach to resveratrol encapsulation using water-insoluble proteins

Iris J. Joye [a,b], Gabriel Davidov-Pardo [a,*], Richard D. Ludescher [c], David J. McClements [a,d]

[a] Department of Food Science, University of Massachusetts, Amherst, MA 01003, United States
[b] Department of Microbial and Molecular Systems, KU Leuven, 3001 Leuven, Belgium
[c] Department of Food Science, Rutgers, The State University of New Jersey, New Brunswick, NJ 08901, United States
[d] Department of Biochemistry, Faculty of Science, King Abdulaziz University, P. O. Box 80203, Jeddah 21589, Saudi Arabia

ARTICLE INFO

Article history:
Received 6 November 2014
Received in revised form 20 March 2015
Accepted 24 March 2015
Available online 4 April 2015

Keywords:
Zein
Gliadin
Nanoparticles
Microparticles
Resveratrol
Encapsulation
Delivery
Fluorescence

ABSTRACT

Several health benefits have been ascribed to consumption of resveratrol, a polyphenol that can be extracted from grape skins. However, its use as a nutraceutical ingredient is compromised by its low water solubility, chemical stability, and bioavailability. Encapsulation of resveratrol in protein nanoparticles can be used to overcome these issues. Fluorescence quenching experiments were used to study the interaction of resveratrol with gliadin and zein. Resveratrol interacted with both proteins, but the binding constant was higher for zein than for gliadin at 35 °C. Furthermore, binding between resveratrol and gliadin increased at higher temperatures, which was not observed for zein. Analysis of the thermodynamic parameters suggested that resveratrol–gliadin binding mainly occurs through hydrophobic interactions while the binding with zein is predominantly mediated through hydrogen bonds. These results help rationalise ingredient selection and production of protein nanoparticles and microparticles for encapsulation, protection and release of resveratrol and potentially other bioactive compounds.

1. Introduction

Resveratrol (trans-resveratrol; trans-3,5,4′-trihydroxy-stilbene) is a polyphenolic compound mainly found in grape skins, peanuts, and the roots of *Polygonum cuspidatum* (Walle, Hsieh, DeLegge, Oatis, & Walle, 2004). Resveratrol has been shown to have beneficial effects on human health, such as a reduction of the risk of certain types of cancer (Murtaza et al., 2013), anti-inflammatory effects (Catalgol, Batirel, Taga, & Ozer, 2012) and reduction of hypertension, atherosclerosis and thrombosis (Brown et al., 2009). Therefore, there is considerable interest in using resveratrol as a nutraceutical ingredient in food and beverage products. However, the incorporation of this bioactive compound into commercial products is currently limited by its low water-solubility, sensitivity to UV-light (Trela & Waterhouse, 1996) and low oral bioavailability (Patel et al., 2011).

One way to overcome the factors that currently compromise the utilisation of resveratrol in foods is encapsulating it in colloidal delivery systems (Cho, Chun, Kim, & Park, 2014; Davidov-Pardo & McClements, 2014; Isailovic et al., 2013; Matos, Gutierrez, Coca, & Pazos, 2014). One of the most promising encapsulation systems involves the complexation of resveratrol with proteins. Previous studies have shown the potential benefits of using protein-based encapsulation strategies to improve the solubility and stability of the enclosed bioactive components against degradation by UV-light or exposure to oxygen (Liang, Tajmir-Riahi, & Subirade, 2008; Patel, Heussen, Hazekamp, Drost, & Velikov, 2012). Furthermore, gliadin particles have been shown to display mucoadhesive properties and, hence, to significantly increase the bioavailability of enclosed lipophilic drugs by prolonging the residence time of the bioactive in a certain part of the gastrointestinal tract (Arangoa, Campanero, Renedo, Ponchel, & Irache, 2001). The binding of resveratrol to proteins was shown to be a spontaneous process driven by hydrophobic interactions, hydrogen bonding and van der Waals interactions (Acharya, Sanguansri, & Augustin, 2013; Hemar, Gerbeaud, Oliver, & Augustin, 2011).

Quenching of protein fluorescence is a useful technique to gain insight into the complexation of polyphenols and proteins

* Corresponding author at: Department of Food Science, Room 232, University of Massachusetts, Amherst, MA 01003, United States. Tel.: +1 (413) 545 7157; fax: +1 (413) 545 1260.
E-mail address: gdavidov@foodsci.umass.edu (G. Davidov-Pardo).

http://dx.doi.org/10.1016/j.foodchem.2015.03.128
0308-8146/

(Keppler, Stuhldreier, Temps, & Schwarz, 2014; Skrt, Benedik, Podlipnik, & Ulrih, 2012). This method is based on measurements of the decrease in fluorescence intensity that occurs when a fluorophore (such as a protein) interacts with an external quencher molecule (such as a polyphenol) that promotes rapid deexcitation of the excited state. The deexcitation mechanism can also vary but typically involves either acceleration of existing non-radiative decay pathways or through-space resonance energy transfer (FRET) due to overlap of the fluorophore emission and quencher absorption bands. In this study, two fundamentally different quenching mechanisms will be considered, i.e., static (pure association, no molecular motion involved) and dynamic (collisional, molecular motion involved) quenching (Lakowicz, 2010). Static quenching originates from the formation of a complex between the fluorophore and the quencher molecule, while dynamic quenching occurs due to a collision between the two molecules (Lakowicz, 2010). In this manuscript, dynamic and static are, hence, not used in the pure mechanistic meaning of the words. Tryptophan and tyrosine are the most important fluorophores of proteins as phenylalanine typically has a low quantum yield (Lakowicz, 2010). Both tryptophan and tyrosine are typically excited at 280 nm, while their emission maximum is dependent on the properties of the local environment. When the local environment is more polar, the emission maxima for tryptophan and tyrosine typically occur around 350 and 303 nm (Lakowicz, 2010). Conversely, when the local environment is less polar, the emission maxima undergo a blue-shift. Mathematical models have been extensively used to interpret the fluorimetric quenching results so as to explore/quantify the binding of resveratrol and proteins (Acharya et al., 2013; Hemar et al., 2011; Liang & Subirade, 2012; Liang et al., 2008). Quenching results should, however, be analysed with caution as apparent quenching can be caused by high optical densities or sample turbidity.

Zein and gliadin are the two most abundant storage proteins in cereals, both are prolamine-type of proteins found in corn and wheat, respectively. Zein and gliadin are insoluble in water, but soluble in concentrated ethanol:water solutions [85% and 70% (v/v), respectively], due to the high amount of non-polar amino acids in their primary structure (Rombouts et al., 2009; Shukla & Cheryan, 2001). The average molecular weight for zein is around 24,000 Da (Shukla & Cheryan, 2001), while molecular weights reported for gliadin vary from 30,000 to 80,000 Da (Delcour et al., 2012). Both proteins have already been used to encapsulate lipophilic functional compounds such as curcumin (Patel, Hu, Tiwari, & Velikov, 2010), quercetin (Patel et al., 2012), essential oils (Parris, Cooke, & Hicks, 2005), vitamin E (Duclairoir, Orecchioni, Depraetere, Osterstock, & Nakache, 2003; Luo, Zhang, Whent, Yu, & Wang, 2011), grape seed extract (Davidov-Pardo, Arozarena, & Marín-Arroyo, 2013) and all-trans-retinoic acid (Ezpeleta et al., 1996).

One of the major factors influencing the ability of hydrophobic proteins to encapsulate other compounds is their binding affinity. The aim of this work was therefore to explore the magnitude and nature of the interaction of resveratrol mixed with zein or gliadin using fluorescence spectroscopy.

2. Materials and methods

2.1. Materials

All chemicals, reagents, and solvents were purchased from Fisher Scientific (Waltham, MA, USA) and were of analytical grade unless stated otherwise. Resveratrol (99%) from grape skin extract was purchased from Changsha Organic Herb Inc. (Changsha, China). Gluten was kindly provided by Cargill (Minneapolis, MN, USA). Food grade zein (F4000) was purchased from Flo Chemicals (Ashburnham, MA, USA).

2.2. Sample preparation

100 g of gluten was extracted in 70% (v/v) ethanol (1.00 L) by stirring for 2 h followed by centrifugation at 9900*g* for 12 min. The supernatant was collected and after a night's rest at 4 °C, centrifuged for a second time (9900*g* for 12 min) to remove any precipitated material. The final gliadin extract contained 3.07% (w/v) protein material [86.5% (w/w) protein on dry matter basis]. Assuming an average molecular weight of the protein of 60,000 Da, the protein concentration of the extract would be 500 μM. The same procedure was followed for zein. 30 g of zein was dissolved in 85% (v/v) ethanol (1.00 L). The average molecular weight of zein used in the concentration calculations was 24,000 Da (Shukla & Cheryan, 2001). A stock solution of 1.0 mM of resveratrol was prepared in either ethanol 70% or 85% ethanol (v/v). Resveratrol and protein were mixed and diluted in their respective water–ethanol solutions to reach the desired concentrations. The samples were subsequently incubated in a water bath set at the desired temperature for at least 30 min prior to fluorescence measurements.

2.3. Fluorescence measurements

The fluorescence measurements were performed with a steady state spectrofluorometer (Photon Technology International TCM-1000, Edison, NJ, USA) using a triangular quartz cuvette to exclude the effect of optical properties of the sample (such as optical density and sample turbidity) interfering with the real quenching phenomena. The excitation wavelength was set at 280 nm and the emission signal was collected from 300 to 540 nm. The lamp power was set at 75 Watt, with a step size of 2 nm and integration every 1 s.

3. Results and discussion

3.1. Protein fluorescence

The fluorescence emission spectra of both gliadin and zein were recorded. Both proteins were extracted in ethanol–water mixtures containing 70% or 85% (v/v) ethanol, respectively. As ethanol is a less polar solvent than water, a blue-shift of the emission maximum is expected for both proteins. However, gliadin exhibited an emission maximum around 336 nm (Fig. 1), which is very similar to the emission maxima reported for lactoferrin and whey protein isolate (dissolved in an aqueous buffer) (Hemar et al., 2011). Tryptophan fluorescence is very sensitive to the properties of the local environment of the fluorescent probe. This, rather than the overall polarity of the solvent, explains the blue-shift of the fluorescence which is typically observed for tryptophan in a protein structure. The substantially shorter wavelength of the zein emission maximum, i.e., 304 nm, is attributable to the amino acid composition of this protein. Zein contains a high level of tyrosine residues (approx. 5.0% w/w) which have a typical emission maximum around 304 nm, while the level of tryptophan residues is insignificant (Shukla & Cheryan, 2001).

Monitoring the fluorescence emission spectra as a function of increasing protein concentration showed that the fluorescence steadily increased (Figs. 1A and 2A) (Note that the plateaus in the spectra at high protein concentration in Figs. 1A and 2A are an instrumental artefact, related to the maximum detection limit of the detector). This experiment highlights the necessity of excluding apparent quenching phenomena, which was achieved