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Rapid Communication

Interactions of different polyphenols with bovine serum albumin using fluorescence quenching and molecular docking

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ABSTRACT

Polyphenols are responsible for the major organoleptic characteristics of plant-derived foods and beverages. Here, we investigated the binding of several polyphenols to bovine serum albumin (BSA) at pH 7.5 and 25 °C: catechins [(−)-epigallocatechin-3-gallate, (−)-epigallocatechin, (−)-epicatechin-3-gallate], flavones (kaempferol, kaempferol-3-glucoside, quercetin, naringenin) and hydroxycinnamic acids (rosmarinic acid, caffeic acid, p-coumaric acid). Fluorescence emission spectrometry and molecular docking were applied to compare experimentally determined binding parameters with molecular modelling. Among these polyphenols, (−)-epicatechin-3-gallate showed the highest Stern–Volmer modified quenching constant, followed by (−)-epigallocatechin-3-gallate. Similarly, (−)-epicatechin-3-gallate had the highest effect on the Circular Dichroic spectrum of BSA, while the changes induced by other polyphenols were negligible. Molecular docking predicted high binding energies for (−)-epicatechin-3-gallate and (−)-epigallocatechin-3-gallate for the binding site on BSA near Trp213. Our data reveal that the polyphenol structures significantly affect the binding process: the binding affinity generally decreases with glycosylation and reduced numbers of hydroxyl groups on the second aromatic ring.

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1. Introduction

Polyphenols form a diverse group of aromatic secondary plant metabolites that are widely distributed throughout the plant kingdom (Vermerris & Nicholson, 2008). Due to their almost universal distribution, they are an integral part of the human diet, and they are responsible for the major organoleptic characteristics of plant-derived foods and beverages (e.g. colour and taste) (Cheynier, 2005). Beneficial health effects of polyphenols are believed to be due to their antioxidative properties (Villañoa, Fernández-Pachóna, Moyáb, Troncosoa, & García-Parrilla, 2007). Indeed, they have been shown to induce antitumor, antibacterial, antimutagenic and anticancerogenic effects (Kroll, Rawel, & Rohn, 2003), although many studies have also demonstrated their harmful effects, especially when applied at high concentrations (Arts, Hollman, de Mesquita, Feskens, & Kromhout, 2001; Lambert, Sang, & Yang, 2007). These properties are partially attributed to the interactions between the polyphenols and biomolecules in the body, such as proteins and lipids (Ishii et al., 2009; Nozaki, Kimura, Ito, & Hatano, 2009).

However, a complete understanding of the structural and antioxidative properties of such polyphenols, in their interactions with biomolecules, has not yet been obtained. Polyphenol interactions with different proteins (Dufour & Dangles, 2005; He, Lv, & Yao, 2007; Roy et al., 2010; Soares, Mateus, & de Freitas, 2007; Wang et al., 2008), and bovine serum albumin (BSA) have been the subject of many studies of these interactions (Dufour & Dangles, 2005; Liu, Qi, & Li, 2010; Papadopoulou, Green, & Frazier, 2005; Rawel, Meidtner, & Kroll, 2005; Shi et al., 2010). Serum albumins provide a good insight into our understanding of the interactions of polyphenols under physiological conditions. BSA has been extensively used for such investigations because of its intrinsic structural similarity to human serum albumin.

BSA has a wide range of physiological functions that are associated with binding, transport and distribution of biologically active compounds. The binding of active compounds to serum albumins influences the effectiveness of the biologically active compounds, as well as the activity of the serum albumins. Structurally, BSA is a single-chain polypeptide of 583 amino acids that is folded into three homologous domains, each of which contains two subdomains (A and B) (Carter & Ho, 1994). The BSA polypeptide contains two tryptophan residues (Trp134 and Trp213), and in the folded

Abbreviations: BSA, bovine serum albumin; epicatechin-G, (−)-epicatechin-3-gallate; epigallocatechin, (−)-epigallocatechin; epigallocatechin-G, (−)-epigallocatechin-3-gallate; kaempferol-glu, kaempferol-3-glucoside.

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BSA molecule, Trp134 is located on the surface, and Trp213 is located in a hydrophobic pocket (Kragh-Hansen, 1981).

In the present study, the interactions with BSA were investigated for selected catechins [(−)-epigallocatechin-3-gallate (epigallocatechin-G), (−)-epigallocatechin (epigallocatechin), and (−)-epicatechin-3-gallate (epicatechin-G)], flavones [kaempferol-3-glucoside (kaempferol-glu), kaempferol, quercetin, and naringenin], and hydroxycinnamic acids (rosmarinic acid, caffeic acid, and p-coumaric acid) (Table 1), using fluorescence emission spectrophotometry and molecular docking. The molecular docking was performed to directly compare these predicted data with the experimental data, and to determine the potential modes of action of these polyphenols. The combined data will thus help us to understand the structure–activity relationships between these selected polyphenols and their binding site on the BSA molecule.

2. Materials and methods

2.1. Reagents

Essentially globulin-free bovine serum albumin (BSA; ⩾99%, lyophilized powder) was from Sigma–Aldrich (Munich, Germany). Epigallocatechin-G, epigallocatechin, epicatechin-G, kaempferol-glu, and kaempferol were from Extrasynthese (Lyon, France), quercetin, naringenin, and caffeic acid from Sigma–Aldrich (Munich, Germany), rosmarinic acid from Vitiva (Markovci, Slovenia), and p-coumaric acid from Merck (Frankfurt, Germany). All of the chemicals used were of analytical grade.

The BSA was dissolved in 20 mM HEPES, pH 7.5, and further purified by dialysis against 20 mM HEPES, pH 7.5, for 36 h at 4 °C. The molar concentration of BSA in aqueous HEPES solution was determined spectrophotometrically (Hewlett–Packard UV–VIS spectrophotometer, model 8453) using ε_{280} (BSA) = 42925 M^{-1} cm^{-1} (calculated with ProtParam from ExPASy) and M_W = 66433 g mol^{-1} at 25 °C. The 1 mM stock solutions of each of the polyphenols were prepared in 96% ethanol, except for quercetin, which was prepared as 1 mM in pure methanol. The working solutions of the polyphenols (c = 0.1 mM) were prepared by dilution of these stock solutions in 20 mM HEPES, pH 7.5.

2.2. Fluorescence emission spectrophotometry

2.2.1. General

Fluorescence spectra were recorded with a Cary Eclipse spectrofluorometer (Varian, Australia) equipped with an electro-thermal temperature controller, using a 1 cm path length quartz cuvette. Slit widths with a nominal band-pass of 5 nm were used for both excitation and emission. The intrinsic fluorescence emission spectra of BSA (c = 0.082 ± 0.001 mg ml^{-1}) were recorded from 290 nm to 450 nm, as a function of increasing concentrations of the polyphenols (titration experiment). The maximum excitation wavelengths λ_{ex} and λ_{em} for BSA were observed at 280 nm and 339 nm, respectively. Based on these results the excitation wavelength of 280 nm was used to follow the BSA fluorescence. The emission spectra of BSA in the absence and presence of the polyphenols, as corrected for the solvent blank, were normalized for the dilution and corrected for the photomultiplier-tube response. The wavelengths at maximum emission intensity, λ_{max}, and the fluorescence intensity at 339 nm were determined. All of the experiments were conducted at 25 °C in 20 mM HEPES, pH 7.5,

Table 1
Structures of the polyphenols used in this study.

Catechins	Flavones	Hydroxycinnamic acids
Epigallocatechin-3-gallate R_6, R_8, R'_2 = H R_5, R_7, R'_3, R'_4, R'_5 = OH R_3 =	*Kaempferol-3-glucoside* R_1, R_6, R_8, R'_2, R'_3, R'_5, R'_6 = H R_5, R_7, R'_4 = OH R_3 =	*Rosmarinic acid* R_1 = R_2 = OH
Epigallocatechin R_6, R_8, R'_2 = H R_3, R_5, R_7, R'_3, R'_4, R'_5 = OH *Epicatechin-3-gallate* R_6, R_8, R'_2, R'_5 = H R_5, R_7, R'_3, R'_4 = OH R_3 =	*Kaempferol* R_1, R_6, R_8, R'_2, R'_3, R'_5, R'_6 = H R_3, R_5, R_7, R'_4 = OH *Quercetin* R_1, R_6, R_8, R'_2, R'_3, R'_5, R'_6 = H R_3, R_5, R_7, R'_3, R'_4 = OH	*Caffeic acid* R_1 = H; R_2 = OH *p-Coumaric acid* R_1 = H; R_2 = H
	Naringenin R_1, R_6, R_8, R'_2, R'_3, R'_5, R'_6 = H R_5, R_7, R'_4 = OH	

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