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Non-covalent interaction between dietary stilbenoids and human serum albumin: Structure–affinity relationship, and its influence on the stability, free radical scavenging activity and cell uptake of stilbenoids

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ABSTRACT

Dietary stilbenoids are associated with many benefits for human health, which depend on their bioavailability and bioaccessibility. The stilbenoid–human serum albumin (HSA) interactions are investigated to explore the structure–affinity relationship and influence on the stability, free radical scavenging activity and cell uptake of stilbenoids. The structure–affinity relationship of the stilbenoids–HSA interaction was found as: (1) the methoxylation enhanced the affinity, (2) an additional hydroxyl group increases the affinity and (3) the glycosylation significantly weakened the affinity. HSA obviously masked the free radical scavenging potential of stilbenoids. The stabilities of stilbenoids in different medium were determined as: HSA solution > human plasma > Dulbecco's modified Eagle's medium. It appears that the milk enhanced the cell uptake of stilbenoids with multi-hydroxyl groups and weakened the cell uptake of stilbenoids with methoxyl group on EA.hy 926 endothelial cells. The stilbenoids are hardly absorbed by human umbilical vein endothelial cells in the presence of milk.

1. Introduction

Natural stilbenoids are typically non-flavonoid polyphenols, which mainly exist in red grapes, wines, cranberries, strawberries and peanuts (Mulat, Latva-Maenpaa, Koskela, Saranpaa, & Wahala, 2014; Pineiro, Guerrero, Fernandez-Marin, Cantos-Villar, & Palma, 2013; Xie & Bolling, 2015; Xie, Wei, Lin, Wen, & Qin, 2014; Zraunig, Pacher, Brecker, & Greger, 2014). Resveratrol and its derivatives (Table 1) are the most important dietary stilbenoids associated with many benefits for human health, such as anti-inflammatory activity (Penalva et al., 2015), anti-asthmatic activity (Chen et al., 2015), anti-diabetes activity (Kim, Lee, Eom, & Kim, 2014), hypolipidemic activity (Jo, Kim, & Lim, 2014), anti-oxidant activity (Mulero et al., 2015; Vlachogianni, Fragopoulou, Kostakis, & Antonopoulou, 2015; Wang et al., 2015), anti-cancer activity (Folmer et al., 2014; Fu et al., 2015; Seo et al., 2015) and anti-bacterial activity (Liu et al., 2014). The biological properties of stilbenoids depend on their bioavailability and bioaccessibility. The chemical structure of polyphenols influences their rate and extent of intestinal absorption and the nature of the metabolites circulating in the plasma (Boyer, Brown, & Liu, 2004; Kosinska-Cagnazzo, Diering, Prim, & Andlauer, 2015; Uhlenhut & Högger, 2012; Willenberg et al., 2015; Xiao & Högger, 2014).

Polyphenols are known to non-covalently interact with human serum albumin (HSA) in blood through hydrophobic or hydrophilic interactions (Xiao, 2013; Xiao & Kai, 2012). However, considerable differences are observed due to the stability, the interaction with other food components, the uptake in the intestine, or the binding to plasma proteins. The interactions between resveratrol and serum albumins have attracted great interest among researchers (Nair, 2015; Wu et al., 2009; Xiao, Chen, Jiang, Hilczer, & Tachiya, 2008; Xiao, Zhao, et al., 2011). However, the interactions between other stilbenoids and HSA are rarely reported. Moreover, most of the reports only focused on the binding process, such as the binding forces, binding distance, energy transfer, and molecular modeling. It was found that the effect of polyphenol–protein interaction on the bioavailability of polyphenols is not equivocal. Few reports have focused on the structure–affinity relationship of stilbenoids on binding to HSA. Herein, the structure–affinity relationship of the stilbenoid–HSA interaction, and its influence on the stability,

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free radical scavenging activity and cell uptake of stilbenoids are investigated in detail.

2. Materials and methods

2.1. Chemicals and reagents

Isorhapontigenin (>95%), oxyresveratrol (>95%), piceid (>95%), pterostilbene (>98%), pinostilbene (>97%), piceatannol (>98%), and resveratrol (>98%) were purchased from Tokyo Chemical Industry Co., Ltd (Shanghai, China). 1,1-Diphenyl-2-picrylhydrazyl (DPPH) was purchased from Aladin Co. Ltd. (Shanghai, China). Methanol (HPLC grade) was obtained from Merck (Darmstadt, Germany), and acetonitrile (HPLC grade) was purchased from Fisher Scientific (Schwerte, Germany). Penicillin, streptomycin, L-glutamine, nonessential amino acids, LSGS and fetal bovine serum (FBS) were all purchased from Biochrom AG (Berlin, Germany). Dulbecco's modified Eagle's medium (DMEM) and medium 200 were obtained from Sigma (St Louis, MO, USA). HSA was purchased from Sigma-Aldrich (Shanghai, China). Human plasma was obtained from the blood banks of the University Hospital of Wuerzburg (Germany). The working solutions of stilbenoids (1.0×10^{-2} mol/l) were prepared by dissolving each stilbenoid in methanol. All other reagents and solvents were of analytical grade and ultrapure. Millipore water was used throughout the experiment.

2.2. High performance affinity chromatography (HPAC) analysis

HPAC analysis was performed on a Waters HPLC with a 1525 binary HPLC pump, a 717plus auto sampler, and a model 2487 UV/VIS dual wavelength absorbance detector (MA, USA). Data collection and integration were accomplished using BreezeTM software version 3.30. The chromatography isolation was performed on a CHIRALPAK-HSA column (150 mm × 4 mm, I.D., 5 μm) (Chrom Tech Ltd., Congelton, Cheshire, UK). The mobile phase consisted of 85% ammonium acetate buffer (10 mmol/l, pH 6.9) and 15% (v/v) acetonitrile. The flow rate was 0.9 ml/min and the detection wavelength was 270 nm. A 10 μl sample volume was injected. All sample compounds were dissolved in methanol.

Reference compounds (isorhapontigenin, oxyresveratrol, piceid, pterostibene, pionstilbene, picestannol and resveratrol) were subjected to HPAC to calculate the capacity factor (k') from the retention time using Eq. (1):

$$k' = (t_R - t_M)/t_M \quad (1)$$

t_R, the retention time of samples, t_M, the retention time of the unrestrained compound.

The known binding values of the reference compounds were plotted against the measured protein binding values calculated according to Eq. (2):

$$\%\ \text{Protein binding} = [k'/(k'+1)] \times 100 \quad (2)$$

A calibration curve was generated by linear regression and the % protein binding of the test compounds with unknown protein binding.

2.3. HPLC analysis

The HPLC system consisted of a Waters 1525 Binary HPLC pump (USA), a Waters 2487 Dual λ absorbance detector, and a Waters 717 Autosampler. Separation was carried out on a YMC-Pack Pro C18 column (4.6 × 150 mm, 5 μm, Japan). The mobile phase consisted of 0.1% phosphoric acid (A) and acetonitrile (B), and was degassed prior to use. The gradient elution was carried out as follows: 8–10% (B) for 0–5 min, 10–20% (B) for 6–10 min, 20–25% (B) for 11–22 min, 25% (B) for 23–25 min, 25–50% (B) for 26–33 min, 50% (B) for 34–42 min, and 50–8% (B) for 43–45 min with a flow rate of 1.0 ml/min. The detection wavelength was 280 nm.

2.4. DPPH free radical scavenging assay

The DPPH free radical scavenging activities of stilbenoids in the absence and presence of HSA were measured according to the literature with minor modifications (Cao, Chen, & Yamamoto, 2012). The working solution of HSA (1.0×10^{-5} mol/l) was prepared by directly dissolving HSA with Millipore water. The stilbenoid solutions (5.0×10^{-4} mol/l) were obtained by diluting the working solutions of stilbenoids (1.0×10^{-3} mol/l). The stilbenoids (5.0×10^{-4} mol/l) were mixed with HSA (1.0×10^{-5} mol/l) (1:1, v/v) and incubated at 37 °C for 30 min to prepare the samples for testing. The samples with different volume were added to 500 μl of DPPH solution (0.2 mmol/l in methanol) and was diluted with 50% methanol to 1 ml. Following incubation in the dark for 30 min, the absorbance at 517 nm was measured. Stilbenoids was replaced by methanol in the control group. The control group consisted of 750 μl methanol and 250 μl of Millipore water. The DPPH free radical scavenging potential was calculated using the following Eq. (3):

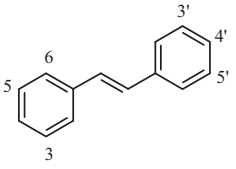
$$\text{DPPH free radical scavenging activity}\ (\%) = (1 - A_1/A_0) \times 100 \quad (3)$$

where A_0 is the absorbance of the control, and A_1 is the absorbance of sample. Each sample was tested three times ($n = 3$). The absorbance was found to be reproducible within experimental errors.

2.5. Stability of stilbenoids

In the current research, the stabilities of stilbenoids in HSA, DMEM and human plasma were investigated, respectively. HSA was dissolved in PBS to obtain a 1.0×10^{-5} mol/l working solution. DMEM was supplemented with 10% FBS, 100 U/ml penicillin, 100 mg/l streptomycin and 2 mmol/l L-glutamine. Fifty microlitres stock solution of stilbenoids (1.0×10^{-3} mol/l) were added to

Table 1
The capacity factor (k') and %protein binding of stilbenoids determined with HPAC.

Stilbenoids	Structure	Substitutions			R_t/min	k'	%Protein binding
		OH	OCH_3	Others			
Resveratrol		3,5,4′			18.34	9.75	90.70
Isorhapontigenin		3,5,4′	5′		12.86	6.54	86.74
Oxyresveratrol		3,5,4′,6′			23.73	12.91	92.81
Pinostilbene		3,4′	5		26.68	14.64	93.61
Pterostilbene		4′	3,5		43.65	24.59	96.09
Piceid		3,4′		5-*O*-glucoside	6.09	2.57	71.99
Piceatannol		3,5,4′,5′			18.66	9.94	90.86