Food Chemistry 168 (2015) 332–340

Contents lists available at ScienceDirect

Food Chemistry

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

Interaction of chlorogenic acids and quinides from coffee with human serum albumin

Valentina Sinisi [a,*], Cristina Forzato [a], Nicola Cefarin [a], Luciano Navarini [b], Federico Berti [a,*]

[a] Dipartimento di Scienze Chimiche e Farmaceutiche, Università degli Studi di Trieste, via L. Giorgieri 1, 34127 Trieste, Italy
[b] illycaffè S.p.A., via Flavia 110, 34147 Trieste, Italy

ARTICLE INFO

Article history:
Received 10 February 2014
Received in revised form 11 June 2014
Accepted 15 July 2014
Available online 24 July 2014

Keywords:
Human serum albumin
Protein–ligand interaction
Fluorescence spectroscopy
Coffee polyphenols
Chlorogenic acids

ABSTRACT

Chlorogenic acids and their derivatives are abundant in coffee and their composition changes between coffee species. Human serum albumin (HSA) interacts with this family of compounds with high affinity. We have studied by fluorescence spectroscopy the specific binding of HSA with eight compounds that belong to the coffee polyphenols family, four acids (caffeic acid, ferulic acid, 5-*O*-caffeoyl quinic acid, and 3,4-dimethoxycinnamic acid) and four lactones (3,4-*O*-dicaffeoyl-1,5-γ-quinide, 3-*O*-[3,4-(dimethoxy)cinnamoyl]-1,5-γ-quinide, 3,4-*O*-bis[3,4-(dimethoxy)cinnamoyl]-1,5-γ-quinide, and 1,3,4-*O*-tris[3,4-(dimethoxy)cinnamoyl]-1,5-γ-quinide), finding dissociation constants of the albumin–chlorogenic acids and albumin–quinides complexes in the micromolar range, between 2 and 30 μM. Such values are comparable with those of the most powerful binders of albumin, and more favourable than the values obtained for the majority of drugs. Interestingly in the case of 3,4-*O*-dicaffeoyl-1,5-γ-quinide, we have observed the entrance of two ligand molecules in the same binding site, leading up to a first dissociation constant even in the hundred nanomolar range, which is to our knowledge the highest affinity ever observed for HSA and its ligands. The displacement of warfarin, a reference drug binding to HSA, by the quinide has also been demonstrated.

1. Introduction

Phenolic acids are found as secondary metabolites in leaves, roots and especially fruits of many plants. Chlorogenic acids (CGAs) derive from the esterification with D-(−)-quinic acid **1** of certain cinnamic acids, such as caffeic acid **2**, ferulic acid **3** and *p*-coumaric acid **4** (Fig. 1), constituting a large family of different molecules in the form of mono- or multi-esters (Clifford, 2000).

Of all the CGAs present in green coffee beans, which are the best source of CGAs found in plants with an amount of 5–12 g/100 g (Farah, Monteiro, Donangelo, & Lafay, 2008), caffeoylquinic acids (CQAs) represent the main subgroup and 5-*O*-caffeoylquinic acid **5** (5-CQA, Fig. 1) is the most abundant one, indeed it is usually called *chlorogenic acid*. A difference between the two types of coffee was also evidenced since Robusta green coffee turned out to be richer in CGAs than Arabica (Farah, de Paulis, Trugo, & Martin, 2005). The roasting process causes a partial loss of CGAs, due to the occurrence of many reactions including isomerization, degradation, dehydration and lactonization (Fig. 1) (Clifford, 1985; Scholz & Maier, 1990; Schrader, Kiehne, Engelhardt, & Maier, 1996). The latter reaction leads to chlorogenic acid lactones (CGLs) which have also shown potential biological activities (de Paulis et al., 2002).

CGAs and CGLs are extracted during coffee brewing, and their content in the cup depend on the type of roasted coffee used and on the extraction method (Gloess et al., 2013); in a traditional *espresso* coffee beverage (30 ml) the content of monocaffeoyl quinic acids is on average 70 mg (Navarini et al., 2008). The extraction efficiency is higher for CGAs than for CGLs, due to their better water solubility, and in general a *lungo* (about 120 ml) is more rich in CGAs than a regular *espresso* coffee.

Many studies reported that polyphenols are capable to permeate the gastrointestinal barrier and are absorbed in humans, being found in plasma as both intact molecules and as their hydrolysis metabolites, in particular as caffeic acid (Farah et al., 2008; Monteiro, Farah, Perrone, Trugo, & Donangelo, 2007; Nardini, Cirillo, Natella, & Scaccini, 2002; Olthof, Hollman, & Katan, 2001; Renouf et al., 2010); CQAs have been detected in plasma even 4 h after the ingestion.

Abbreviations: CGAs, chlorogenic acids; CQAs, caffeoylquinic acids; 5-CQA, 5-*O*-caffeoylquinic acid; CQLs, chlorogenic acid lactones; DMSO, dimethyl sulfoxide; HSA, human serum albumin; Trp, tryptophan.

* Corresponding authors. Tel.: +39 040 5583920; fax: +39 0405583903.
E-mail addresses: valentina.sinisi@phd.units.it (V. Sinisi), fberti@units.it (F. Berti).

http://dx.doi.org/10.1016/j.foodchem.2014.07.080
0308-8146/

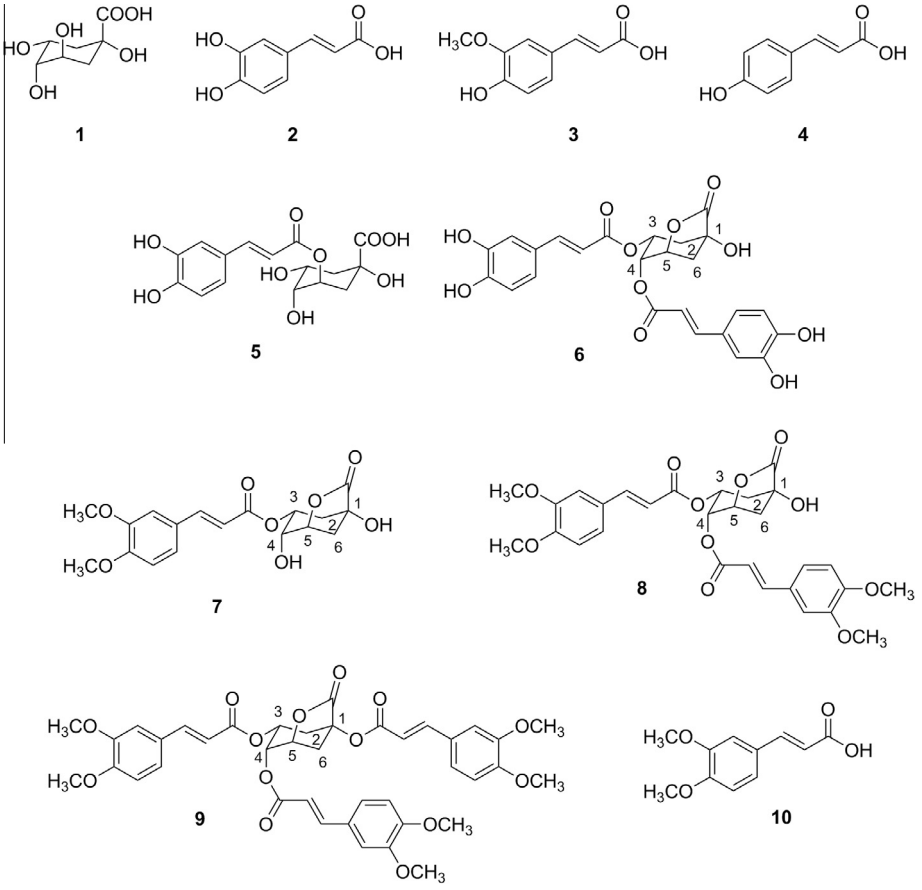

Fig. 1. Molecular structure of (−)-quinic acid (**1**), caffeic acid (**2**), ferulic acid (**3**), *p*-coumaric acid (**4**), 5-*O*-caffeoylquinic acid (**5**) (the IUPAC numbering system for chlorogenic acid (IUPAC, 1976) is adopted and, to avoid confusion, the same numbering system of the carbon atoms both for lactones and for the acid precursors is used), 3,4-*O*-dicaffeoyl-1,5-γ-quinide (**6**), 3-*O*-[3,4-(dimethoxy)cinnamoyl]-1,5-γ-quinide (**7**), 3,4-*O*-bis[3,4-(dimethoxy)cinnamoyl]-1,5-γ-quinide (**8**), 1,3,4-*O*-tris[3,4-(dimethoxy)cinnamoyl]-1,5-γ-quinide (**9**) and 3,4-dimethoxycinnamic acid (**10**).

Human serum albumin (HSA) is the most abundant protein in human plasma, a monomeric 585-residue protein containing three homologous helical domains (I–III), each divided into two subdomains (A and B) (He & Carter, 1992). Two main binding sites for small organic molecules are found, one located in subdomain IIA and one in IIIA, that are known as Sudlow I and Sudlow II sites, respectively (Sudlow, Birkett, & Wade, 1975a).

The protein is able to bind a large variety of endogenous ligands, as non-esterified fatty acids, bilirubin, heme, thyroxine, bile acids; many drugs with acidic or electronegative moieties (including phenols as paracetamol) also exploit the interaction with HSA to be carried in human body (Ghuman et al., 2005; Varshney et al., 2010). Recently an interactive association of multiple ligands with the same binding site inside subdomain II of HSA has been proposed (Yang et al., 2012). As to the binding of phenolic compounds from dietary sources, flavonoids as flavanol, flavonol, flavone, isoflavone, flavanones, and anthocyanidins are known to interact with HSA (Pal & Saha, 2014). The interactions of 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) with bovine albumin has been reported in this journal by Skrt, Benedik, Podlipnik, and Ulrih (2012). Resveratrol binds to HSA and its interaction is modulated by stearic acid (Pantusa, Sportelli, & Bartucci, 2012). Specific binding of caffeic, ferulic, and 5-CQA acids inside Sudlow site I of HSA has been studied, revealing K_D about 6 μM, 40 μM and 25 μM respectively (Kang et al., 2004; Min et al., 2004; Hu, Chen, Zhou, Bai, & Ou-Yang, 2012). In this study the aim was to extend the knowledge regarding the interaction between polyphenols present in coffee and HSA. For this purpose we have synthesised four quinides of caffeic acid and of 3,4-*O*-dimethoxycinnamoyl acid: 3,4-*O*-dicaffeoyl-1,5-γ-quinide **6**, 3-*O*-[3,4-(dimethoxy)cinnamoyl]-1,5-γ-quinide **7**, 3,4-*O*-bis[3,4-(dimethoxy)cinnamoyl]-1,5-γ-quinide **8**, and 1,3,4-*O*-tris[3,4-(dimethoxy)cinnamoyl]-1,5-γ-quinide **9** (Fig. 1).

Compound **6** is very abundant in roasted coffee, while the class of dimethoxycinnamoylquinic acids, precursors of compounds **7**, **8**, and **9**, was recently found and characterised in green coffee beans (Clifford, Knight, Surucu, & Kuhnert, 2006).

We have measured the dissociation constants to Sudlow site I of our compounds in physiological conditions by fluorescence spectroscopy following the quenching of the emission of the unique fluorescent tryptophan residue within the binding site in subdomain IIA (Trp-214) (Anna, 2002).

The absorption, distribution, albumin binding and excretion of phenolic derivatives **10** in human plasma after coffee consumption have been studied, but more complex derivatives were not considered yet (Farrell et al., 2012; Nagy et al., 2011). Our data may therefore be interesting to better understand the effects of coffee consumption on the human body, as to the binding to albumin and potential competition with drugs at the same site. Moreover, we are also interested in the development of biosensing tools for the rapid detection of coffee polyphenols in quality control of coffee beverages. HSA could represent a valuable binder to be used in