Food Chemistry 212 (2016) 434–442

Contents lists available at ScienceDirect

Food Chemistry

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

Multispectroscopic and docking studies on the binding of chlorogenic acid isomers to human serum albumin: Effects of esteryl position on affinity

Bin Tang [a], Yanmei Huang [a], Xiangling Ma [a], Xiaoxiang Liao [a,b], Qing Wang [a], Xinnuo Xiong [a], Hui Li [a,*]

[a] College of Chemical Engineering, Sichuan University, Chengdu 610065, Sichuan, PR China
[b] Technology Center, China Tobacco Yunnan Industrial Co., Ltd., Kunming 650204, Yunnan, PR China

ARTICLE INFO

Article history:
Received 10 March 2016
Received in revised form 2 June 2016
Accepted 4 June 2016
Available online 6 June 2016

Chemical compounds studied in this article:
Chlorogenic acid (PubChem CID: 1794427)
Neochlorogenic acid (PubChem CID: 5280633)
Cryptochlorogenic acid (PubChem CID: 9798666)
Phenylbutazone (PubChem CID: 4781)
Ibuprofen (PubChem CID: 3672)

Keywords:
Affinity
Chlorogenic acid
Food additive
Human serum albumin
Polyphenol
Spectroscopy
Structure

ABSTRACT

Structural differences among various dietary polyphenols affect their absorption, metabolism, and bioactivities. In this work, chlorogenic acid (CA) and its two positional isomers, neochlorogenic acid (NCA) and cryptochlorogenic acid (CCA), were investigated for their binding reactions with human serum albumin (HSA) using fluorescence, ultraviolet–visible, Fourier transform infrared and circular dichroism spectroscopies, as well as molecular docking. All three isomers were bound to HSA at Sudlow's site I and affected the protein secondary structure. CCA presented the strongest ability of hydrogen-bond formation, and both CA and NCA generated more electrostatic interactions with HSA. The albumin-binding capacity of these compounds decreased in the order CCA > NCA > CA. The compound with 4-esteryl structure showed higher binding affinity and larger conformational changes to HSA than that with 3- or 5-esteryl structures. These comparative studies on structure–affinity relationship contributed to the structural modification and design of phenolic food additives or new polyphenol-like drugs.

1. Introduction

Chlorogenic acid (CA; IUPAC name, 5-*O*-caffeoylquinic acid) (IUPAC, 1976), is one of the most abundant and widespread plant polyphenols in human diets and a valuable active component in traditional Chinese medicine (Hu, Chen, Zhou, Bai, & Ou-Yang, 2012; Liu, Liu, Shi, & Zhou, 2010). As a natural antioxidant in many foods (e.g., cabbages, potatoes, and apples) and beverages (e.g., coffee and green tea), commercially available CA is often used as an important food additive or pharmaceutical intermediate. Monomeric CA can bind to some enzymes and multisubunit proteins, such as lysozyme (Rawel, Kroll, & Riese, 2000), calcineurin (Yin et al., 2009), pepsin (Zeng, Liang, You, & Qu, 2014), bovine serum albumin (BSA) (Rawel, Rohn, Kruse, & Kroll, 2002; Wang, Zhang, Xu, & Du, 2011), and human serum albumin (HSA) (Hu et al., 2012; Kang et al., 2004; Wang et al., 2011), to modify their structural properties and alter their biological activities. The binding characteristics of small molecules toward these functional biomacromolecules can provide useful information about their actions and pharmacokinetic properties (Wang, Sun, Ma, Rao, & Li, 2015; Xiao et al., 2014).

Two monoacyl isomers of CA, neochlorogenic acid (NCA) and cryptochlorogenic acid (CCA), can be obtained from the esterification of caffeic acid with 3- and 4-hydroxyl of quinic acids, respectively (Fig. 1A) (Bajko, Kalinowska, Borowski, Siergiejczyk, & Lewandowski, 2016). Based on their highly similar chemical structures, both NCA and CCA also exhibit strong antioxidative activity like CA (Xu, Hu, & Liu, 2012). However, systematic comparative studies on these isomers are few. In recent years, dietary polyphenol analogs, which can bind to functional proteins and lead to

* Corresponding author.
E-mail address: lihuilab@sina.com (H. Li).

http://dx.doi.org/10.1016/j.foodchem.2016.06.007
0308-8146/

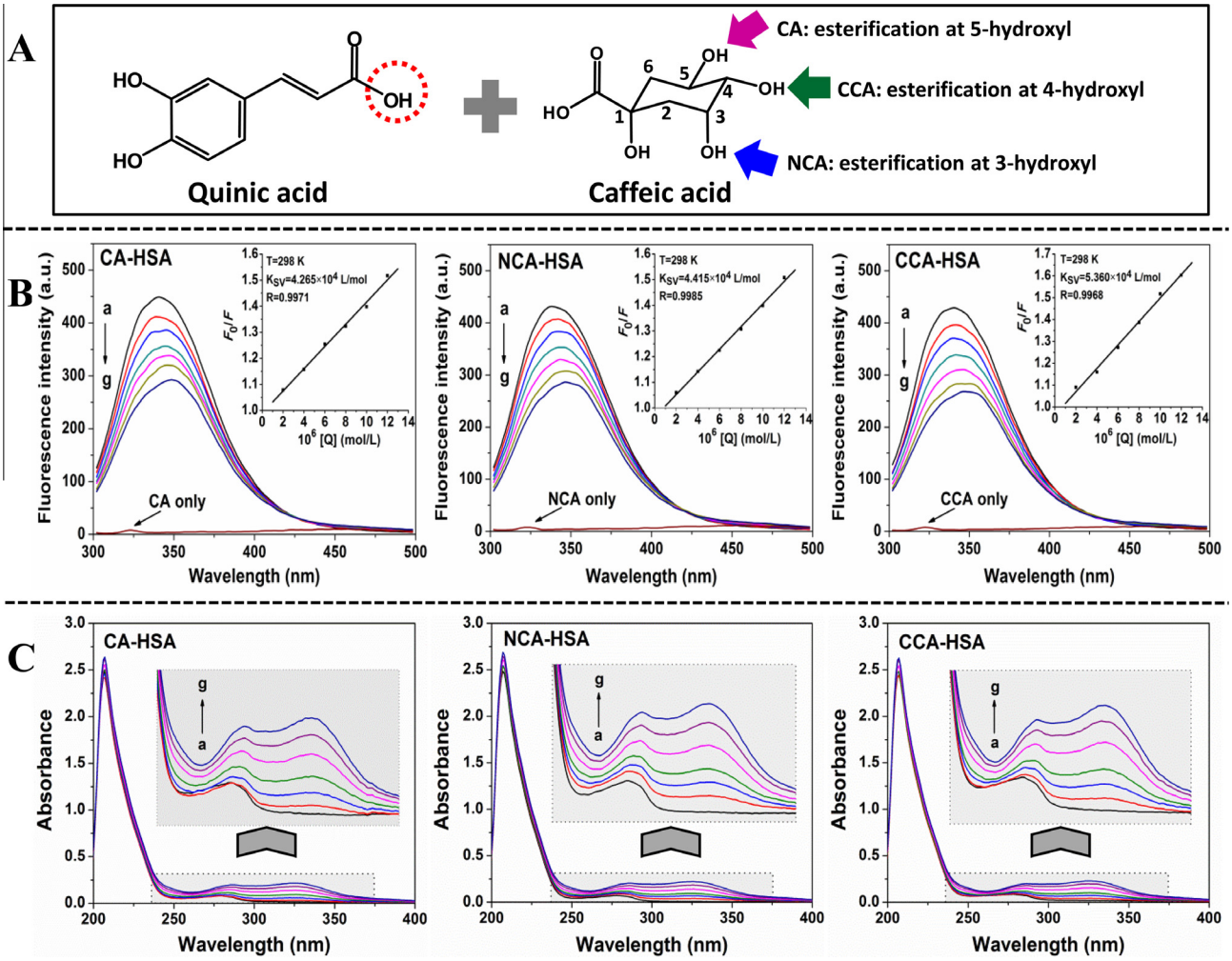

Fig. 1. (A) shows the esteryl positions in CA, NCA, and CCA molecules. (B) Shows the fluorescence emission spectra of 2.0×10^{-6} mol/L HSA in the presence of CA, NCA, and CCA. Ligand concentrations from "a" to "g" were 0, 2, 4, 6, 8, 10 and 12×10^{-6} mol/L from the top to bottom; individual CA, NCA, and CCA concentrations were 1.2×10^{-5} mol/L; the insets along emission spectra are Stern-Volmer plots at 298 K. (C) shows the UV–vis absorption spectra of 2.0×10^{-6} mol/L HSA with various concentrations of CA, NCA, and CCA. Ligand concentrations from "a" to "g" are the same as (B).

changes in the structural, functional, and nutritional properties of both polyphenol and protein, are gaining increased attention (Diniz et al., 2008; Li et al., 2010; Shi, Zhang, Chen, & Peng, 2011; Sinisi, Forzato, Cefarin, Navarini, & Berti, 2015; Skrt, Benedik, Podlipnik, & Ulrih, 2012). Structural differences among polyphenol analogs significantly affect their absorption, metabolism, and bioactivities in vivo, as well as their binding processes with proteins (Walle, 2004). Studies on interactions between structurally similar polyphenols and proteins have contributed to the understanding of structure–activity relationship. Many polyphenol–protein interactions have been summarized (Ozdal, Capanoglu, & Altay, 2013), but focus on the structure–affinity relationship is limited. The number of caffeoyl groups has been proven to moderately affect the binding of CA derivatives to BSA (Tang, Li, Li, Wen, & Qian, 2008). Furthermore, the esterification of carboxyl group could increase albumin affinity (Li et al., 2010). However, the effect of the esteryl-structure position in the mono-caffeoylquinic acid molecule on protein affinity is unclear. To our knowledge, differences in the protein-binding affinity between CA and its positional isomers have not been reported.

As the most abundant transport protein in human plasma, HSA can bind various endogenous ligands. Many drugs with acidic or electronegative moieties (including phenols as paracetamol) travel in the plasma and reach target tissues by binding to HSA (Sinisi et al., 2015). Phenolic compounds may exert their biological functions through a similar mechanism. In the present study, the interactions between HSA and three polyphenol analogs (CA and its two positional isomers) were simultaneously investigated under simulated physiological conditions. Multispectroscopic techniques including fluorescence, ultraviolet-visible (UV–vis), synchronous fluorescence, Fourier transform infrared (FT-IR), three-dimensional fluorescence, and circular dichroism spectroscopy were used to analyze the binding behaviors of HSA–ligand interactions and structural changes of protein. Molecular docking was also conducted to further examine interactions. This study reported the binding processes of NCA and CCA to HSA for the first time and comparatively discussed the differences among CA–HSA, NCA–HSA, and CCA–HSA interactions. The effects of esteryl position on protein affinity were clarified. This exploration on the structure–affinity relationship can elucidate the pharmacology and pharmacodynamics of CA isomers and aid the design of novel polyphenol-like food additives or drugs.

2. Materials and methods

2.1. Chemicals and reagents

Fatty-acid-free HSA (A1887-5G) purchased from Sigma-Aldrich (St. Louis, MO, USA) was directly used without further purification.