Food Chemistry 196 (2016) 550–559

Contents lists available at ScienceDirect

Food Chemistry

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

Interaction of cyanidin-3-O-glucoside with three proteins

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ARTICLE INFO

Article history:
Received 20 April 2015
Received in revised form 4 August 2015
Accepted 23 September 2015
Available online 28 September 2015

Keywords:
Cyanidin-3-O-glucoside
Hemoglobin
Myoglobin
Bovine serum albumin
Spectroscopic
Molecular modeling

ABSTRACT

We studied the binding of cyanidin-3-O-glucoside (C3G) with bovine serum albumin (BSA), hemoglobin (Hb) and myoglobin (Mb), using multi-spectral techniques and molecular modeling. Fluorescence and time-resolved fluorescence studies suggested that C3G quenched BSA, Hb or Mb fluorescence in a static mode with binding constants of 4.159, 0.695 and 1.545 $\times$ 10^4 L mol^{-1} at 308 K, respectively. The thermodynamic parameters represented hydrogen bonds and van der Waals forces dominated the binding. Furthermore, CD, UV–vis, and three-dimensional fluorescence spectra results indicated the secondary structures of BSA, Hb and Mb were partially destroyed by C3G with the α-helix percentage of C3G-Hb and C3G-Mb decreased while that of C3G-BSA was increased. UV–vis spectral results showed these binding interactions partially affected the heme bands of Hb and Mb. In addition, molecular modeling analysis supported the experimental results well. The calculated results of equilibrium fraction showed that the concentration of free C3G in plasma was high enough to be stored and transported from the circulatory system to reach their target sites to provide their therapeutic effects.

1. Introduction

Anthocyanins, a group of water-soluble flavonoids subgroup, are widely distributed in fruits and vegetables, but also found in flowers and other plant materials. They are responsible for red, blue and purple colours (Fleschhut, Kratzer, Rechkemmer, & Kulling, 2006). The most common naturally occurring anthocyanins are the 3-O-glucosides and the 3,5-O-diglucosides of malvidin, cyanidin, pelargonidin, delphinidin, petunidin and peonidin (Fleschhut et al., 2006). Cyanidin-3-glucoside (C3G), a typical representative of anthocyanins in food, is widespread in plants (Fig. 1). There are several reports mentioning its beneficial effects. For example, cyanidin-3-glucoside (C3G) exhibits free radical scavenging activity, suppresses inflammation, protects against endothelial dysfunction, vascular failure and myocardial damage, prevents obesity, ameliorates hyperglycemia and seems to help prevent cardiovascular disease (Kähkönen & Heinonen, 2003; Noda, Kaneyuki, Mori, & Packer, 2002; Seeram, Momin, Nair, & Bourquin, 2001; Serraino et al., 2003; Tsuda, Horio, Uchida, Aoki, & Osawa, 2003; Xu, Ikeda, & Yamori, 2004). In order to be transferred into a tumor, C3G needs to be bound by various proteins after ingestion, so knowing the details of the binding characteristics of C3G with these proteins in the therapeutic procedure at molecular level is of great importance (Xie et al., 2014).

Proteins are important biomacromolecules that play various roles in living beings (Mahato et al., 2010). Serum albumin is the most abundant drug carrier protein in blood plasma, which has many physiological functions, such as maintaining the osmotic pressure and pH of blood, and as carriers transporting a great number of endogenous and exogenous compounds such as fatty acids, amino acids, drugs and pharmaceuticals (Kratz, 2008). Thus, bovine serum albumin (BSA) was selected as a model protein. Hemoglobin (Hb) and myoglobin (Mb) are proteins which could involve in redox activities. They play an important role in physiological activities in human bodies, having numerous functions including the transport of oxygen, dispersion of hydrogen peroxide, and are involved in electron transfer reactions (Chen, Ikeda-Saito, & Shaik, 2008; Cheng, Liu, Bao, & Zou, 2011). Hb picks up oxygen from the tiny blood capillaries at the base of the lungs and carries it along the arteries to the body tissues, and carries CO_2 from the cells to the lungs for removal (Chatterjee & Kumar, 2014). Mb is found mainly in muscle tissues, and receives oxygen from red blood cells and transports it to the mitochondria of the muscle cells, where the oxygen is used in cellular respiration to generate energy. Hb is a tetrameric protein comprising of four polypeptide chains: two identical α-chains comprising of 141 amino acid residues each and two identical β-chains made up of 146 amino acid residues, while Mb is a single polypeptide chain protein of 153 amino acids (Chatterjee & Kumar, 2014). Although

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http://dx.doi.org/10.1016/j.foodchem.2015.09.089
0308-8146/

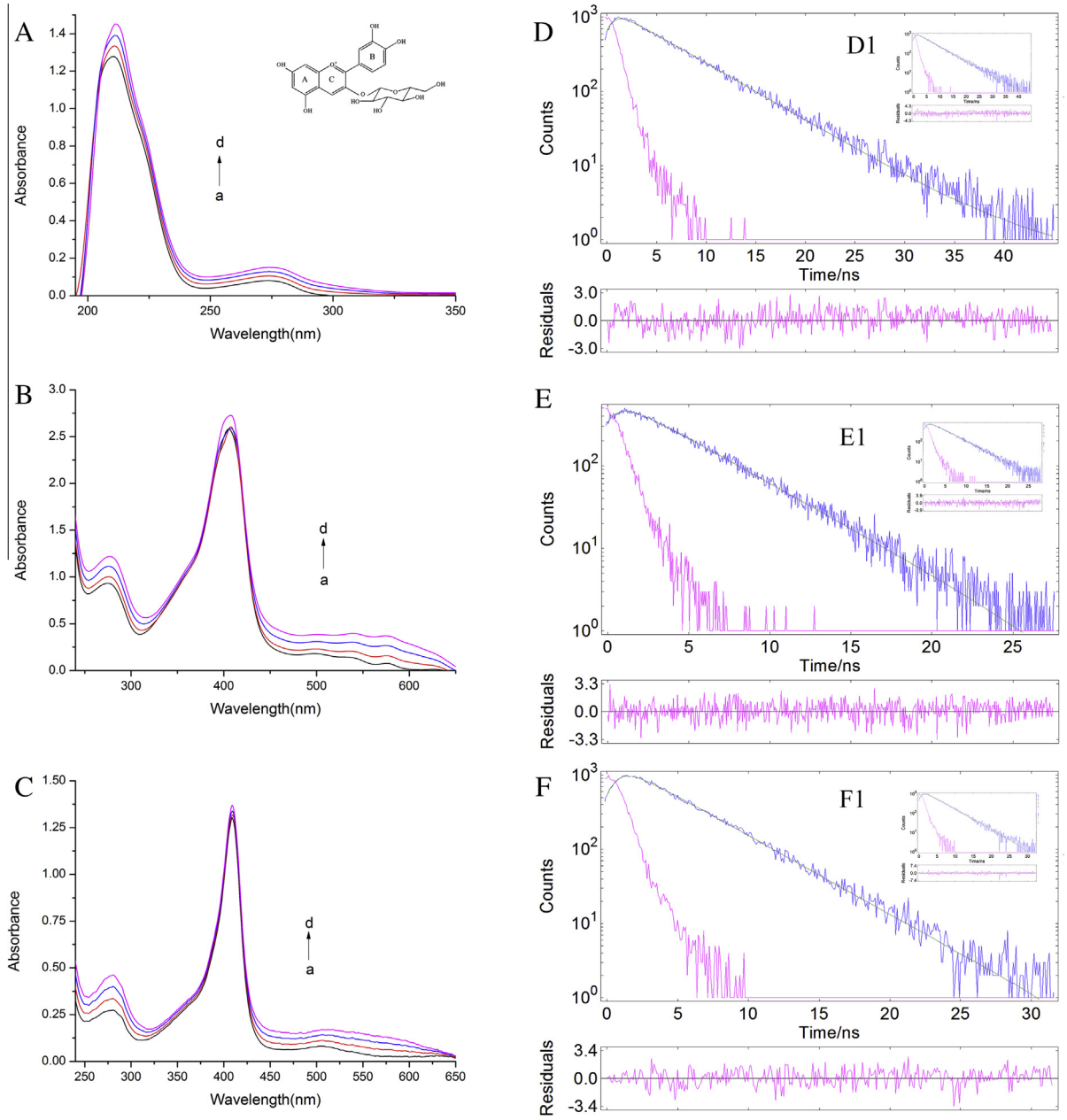

Fig. 1. Chemical structures of cyanidin-3-O-glucoside, UV absorption spectra of BSA (A), Hb (B) and Mb (C) without and with C3G. c(BSA) = 2.0 μmol L^{-1}, c(C3G)/c(BSA) = 0–3. c(Hb) = c(Mb) = 10.0 μmol L^{-1}, c(C3G)/c(Hb) = 0, 0.1, 0.2, 0.5, c(C3G)/c(Mb) = 0, 0.1, 0.2, 0.5. Fluorescence decay profiles and residual fit for BSA (D, D1), Hb (E, E1) and Mb (F, F1) (55 μmol L^{-1}) in the absence and presence of C3G (2 μmol L^{-1}).

Hb and Mb are not strictly plasma proteins, investigations on the interaction of drugs and small molecules with Hb and Mb are of great importance in terms of understanding their pharmacological actions.

Till now, most reports have focused on anthocyanins (Fleschhut et al., 2006; Kähkönen & Heinonen, 2003; Tang, Zuo, & Shu, 2014). However, to our best knowledge, the interactions of C3G with BSA, Hb and Mb have not been reported. Therefore, it is necessary to understand the level of binding of C3G with BSA, Hb and Mb, which will directly correlate with the efficiency of C3G *in vivo*.

The objective of this study is to investigate the *in vitro* binding characteristics of C3G with three proteins using multispectral and molecular modeling techniques. The multiple spectroscopic techniques include ultraviolet (UV)–visible absorption spectroscopy, time-resolved fluorescence, synchronous fluorescence spectroscopy, three-dimensional fluorescence spectra and circular dichroism (CD) techniques. These results could provide more detailed information to understand the delivery process of C3G *in vivo*.

2. Experimental

2.1. Materials

Bovine serum albumin, hemoglobin from bovine blood and myoglobin from equine heart were all purchased from Sigma Chemical Company (St. Louis, USA), and used without further purification. The cyanidin-3-O-glucoside with purity 95% was purchased from Extrasynthese (Genay, France). Tris with purity over