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Review on the loading efficacy of dietary tea polyphenols with milk proteins

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ABSTRACT

In this review, the structural analysis and the loading efficacies of dietary tea polyphenols (+)-catechin (C), (−)-epicatechin (EC), (−)-epicatechingallate (ECG) and (−)-epigallocatechingallate (EGCG) with milk proteins β-lactoglobulin (β-LG), α-casein (αs1-casein) and β-casein were compared. The multiple spectroscopic results and docking sresults showed that the polyphenol bindings are *via* hydrophilic, hydrophobic and H-bonding contacts, where larger polyphenols form more stable protein conjugates. The order of protein affinity towards polyphenol was β-casein> α-casein> β-LG. Loading efficacy was 30–50% for polyphenol-protein adducts. The comparison showed milk proteins are capable of transporting tea polyphenols, which increases the bioavailability of these dietary micronutrients in solution.

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Contents

1. Introduction

Major research has been focused on the use of polyphenols in the nutraceutical and pharmaceutical industries. Polyphenols found in tea show anticancer activity but their use is limited by poor availability in solution. Recent report demonstrated that epigallocatechin gallate (EGCG), a major extractable polyphenol in green tea, when is added to milk remains bioactive and reduces colon cancer cell proliferation at high polyphenol content (Haratifar, Mecking, & Corredig, 2014). Similarly, green tea polyphenols are used in chemoprevention of prostate cancer in preclinical and clinical investigations (Cao, Han, Xiao, Qiao, & Han, 2016; Khan, Adhami, & Mukhtar, 2009). Encapsulation of tea

Abbreviations: β-LG, beta-lactoglobulin; C, catechin; EC, epicatechin; ECG, epicatechingallate; EGCG, epigallocatechingallate; FTIR, Fourier transform infrared.

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polyphenols has shown to protect and increase bioavailability of these dietary compounds and to enhance their anticancer activity (Fang & Bhandari, 2010; Kalikalan, Kaur, & Manddal, 2013; Munin & Edward-Levy. 2011; Sanna et al., 2015; Singh et al., 2015). Encapsulation of tea polyphenols by milk phospholipids and milk protein has been reported (Gulseren, Guri, & Corredig, 2012; Haratifar, Meckling, & Correding, 2014; Liang & Xu, 2003). The effects of milk on the antioxidant capacity of different tea polyphenols were recently reported (Bourassa, Côté, Hutchandani, Samson, & Tajmir-Riahi, 2013; Dubeau, Samson, & Tajmir-Riahi, 2010). Recent reports show the complexation of tea polyphenols with milk proteins and the effect of such interaction on the protein secondary structure and the antioxidant capacity of tea polyphenols (Hasni et al., 2010; Kanakis et al., 2011). In solution polyphenols such as catechins (Fig. 1) can form insoluble complexes with milk proteins (Liang & Xu, 2003). This binding can affect the electron donation capacity of the catechins by reducing the number of hydroxyl groups available in the solution.

Milk bovine beta-lactoglobulin, the most abundant protein of milk whey, is a globular protein that contains 162 amino acids and has a molecular weight of 18.3 kDa. It is composed of nine β-strands and one α-helix, in which the hydrophobic sequences are mostly buried. At room temperature and neutral pH, it exists in the form of a dimer, which dissociates into monomers at acidic pH (Fox & McSweeny, 2003). It shows major binding affinity for hydrophobic and hydrophilic drugs (Essemine, Hasni, Caprpentier, Thomas, & Tajmir-Riahi, 2011; Hasni, Bourassa, & Tajmir-Riahi, 2011; Jameson, Adams, & Creamer, 2002; Kontopidis, Holt, & Sawyer, 2004; Loch et al., 2015). Under denaturing conditions, β-LG is able to form gels and aggregates or fibrillation, depending on the protein concentration and other conditions, such as pH and temperature (Bateman, Ye, & Singh, 2010; Lexis & Willenbacher, 2014; Veerman, Ruis, Sagis, & van der Linden, 2002; Zappone, De Santo, Labate, Rizzutia, & Guzzi, 2013; Zuniga, Tolkach, Kulozik, & Aguilera, 2010). Mammalian milks contain large quantities of protein-based particles such as casein micelles. These particles are stable, highly hydrated, polydisperse colloidal particles composed mainly by the mixture of phosphoproteins known as caseins, together with inorganic calcium phosphate (Dalgleish, 2011; Fang & Dalgliesh, 1999; Uversky, 2002). The three casein components are almost similar in size, molecular weight (αs1-casein 23.62, αs2-casein 25.50, β-casein 24.09 and κ-casein 19.00 kD) and net negative charge but differ in their degree of unfoldedness (Collini, D'Alfonso, & Baldini, 2000; Curley, Kumosinski, Unruh, & Farrell, 1988; Fox & McSweeny, 2003; Kumosinski, Brown, & Farrell, 1993; Phadungath, 2005; Thorn et al., 2005). Since major differences are observed in the hydrophobicity of beta-lactoglobulin, alpha-casein and beta casein, which can affect the binding affinities of these proteins, it was of interest to make a comparison on the loading efficacy of the tea polyphenols with milk proteins.

In this review, we compare the loading efficacies of several tea polyphenols catechin, epicatechin, epicatechin gallate and epigallocatechin gallate (Fig. 1) with milk beta-lactoglobulin, alpha-casein and beta-casein in aqueous solution at physiological conditions, using multiple spectroscopic results and docking studies. Structural analysis regarding catechin binding sites and the effects of catechin-protein conjugation on the protein stability and structure are discussed here.

2. Docking study and the binding sites of polyphenol with milk proteins

The docking studies were performed with ArgusLab 4.0.1 software (Mark A. Thompson, Planaria Software LLC, Seattle, Wa, http://www.arguslab.com). The β-LG and casein structures were obtained from literature report (Fox & McSweeny, 2003; Qin et al., 1998) and the polyphenol three dimensional structures were generated from PM3 semi-empirical calculations using Chem3D Ultra 6.0. The whole protein was selected as a potential binding site since no prior knowledge of such site was available. The docking runs were performed on the ArgusDock docking engine using regular precision with a maximum of 1000 candidate poses. The conformations were ranked using the Ascore scoring function, which estimates the free binding energy. Upon location of the potential binding sites, the docked complex conformations were optimized using a steepest decent algorithm until convergence, with a maximum of 20 iterations. Amino acid residues within a distance of 3.5 Å relative to the polyphenol were involved in the complexation (Hasni et al., 2010; Kanakis et al., 2011).

Docking results in which the tea polyphenols C, EC and EGCG molecules were docked to β-lactoglobulin, α-casein and β-casein preferred binding sites are shown in Fig. 2 and Table 1 (Hasni et al., 2010; Kanakis et al., 2011). The docking results show that polyphenols are surrounded by different amino acids in polyphenol-b-

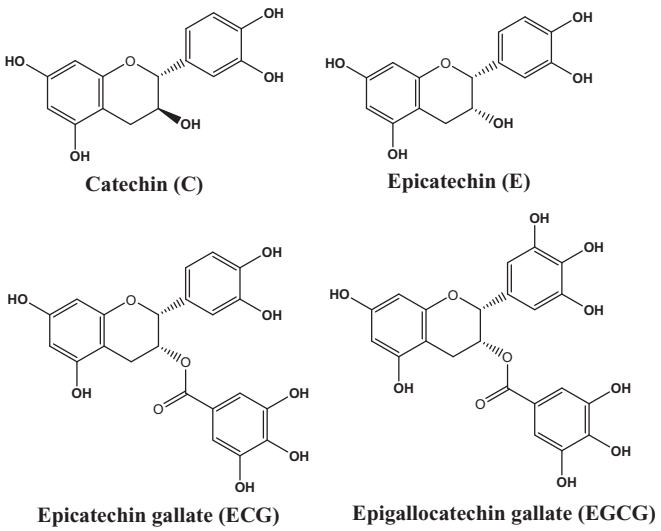

Fig. 1. Chemical structures of tea polyphenols.

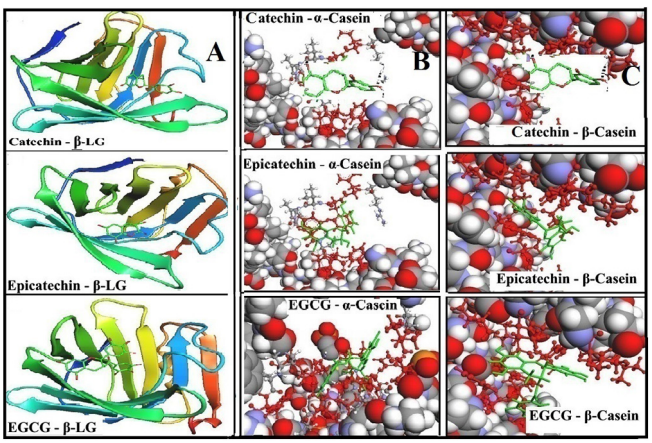

Fig. 2. Best docked conformations of tea polyphenols-protein conjugates. Residues of interest are shown in red color and the tea polyphenols in green color; **A**) β-LG, **B**) α-casein and **C**) β-casein. (Figures adapted from Hasni et al., 2010; Kanakis et al., 2011). (For interpretationof the references to colour in this figure legend, the reader is referred tothe web version of this article.)

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