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Evaluation of non-covalent interactions between serum albumin and green tea catechins by affinity capillary electrophoresis

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ABSTRACT

The natural antioxidant-associated biological responses appear contradictory since biologically active dosages registered in vitro experiments are considerably higher if compared to concentrations found in vivo. The recent research indicates that natural antioxidants, including the major catechins of green tea epicatechin (EC), epigallocatechin (EGC), epicatechingallate (ECG) and epigallocatechingallate (EGCG) form non-covalent complexes with albumin, a crucial aspect that may modulate their plasma concentration, tissue delivery and biological activity. Affinity capillary electrophoresis (ACE) was used to characterize the binding of the four catechins to human serum albumin (HSA) and bovine serum albumin (BSA) at near-physiological conditions: 10 mmol/L phosphate buffer, HEPES 50 mmol/L (pH 7.5), temperature 37 °C. The studied flavonoids displayed affinities toward the albumin with binding constants in the range 10^3–10^5 M^{-1}, with a greater affinity of catechins toward HSA than BSA (between 3 and 3.5 fold higher). We also confirmed that catechins having a galloyl moiety (ECG and EGCG) have a higher binding affinity toward albumin than the catechins lacking the galloyl moiety (EC and EGC), and that for both albumins the order of affinity is EC < EGC < ECG < EGCG. We believe that our work can provide useful information for better understanding the intercurrent relationships between cathechins bioavailability and their elicited biological effects.

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1. Introduction

Naturally occurring antioxidants from vegetable foods and some beverages such as tea or coffee and red wine, have been associated with a reduced incidence of risk factors for a number of chronic and degenerative diseases including cancer and cardiovascular diseases [1]. Catechins (flavan-3-ols) are antioxidant polyphenolic compounds occurring at higher concentrations in different fruits and plants including green tea, which is the most important and rich natural source [1]. The major catechins of green tea extract are epicatechin (EC), epigallocatechin (EGC), epicatechingallate (ECG) and epigallocatechingallate (EGCG) (Fig. 1), which appear to have many health benefits due to their antioxidant and anti-inflammatory properties [2,3]. Structure-function relationship studies indicate galloyl and hydroxyl groups on the B-ring strongly implicated in modulating catechins biological activities [4,5]. For instance, catechins with a pyrogallol-type structure on the B-ring, such as EGC and EGCG, have strong antioxidant activity [6], while catechins with galloyl moiety, such as ECG and EGCG, are more biologically active than their homologues lacking the galloyl moiety [6,7].

Although the health benefits of catechins are largely recognized, doubts concerning their biologically active dosage have been raise since concentrations that appear responsible for their biological effects in vitro are remarkably higher as compared with the ones found in the plasma in vivo [8]. Such apparent contradiction could be explained by the fact that catechins tend to bind to serum albumin [9,10], a fundamental aspect that, as reported for the polyphenol Resveratrol [11,12], may modulate their plasma concentration, tissue delivery and biological activity. Consequently, investigating the different aspects related to the binding of catechins with serum albumin becomes of fundamental importance,

Abbreviations: ACE, affinity capillary electrophoresis; EC, epicatechin; EGC, epigallocatechin; ECG, epicatechingallate; EGCG, epigallocatechingallate; BSA, bovine serum albumin; HSA, human serum albumin.

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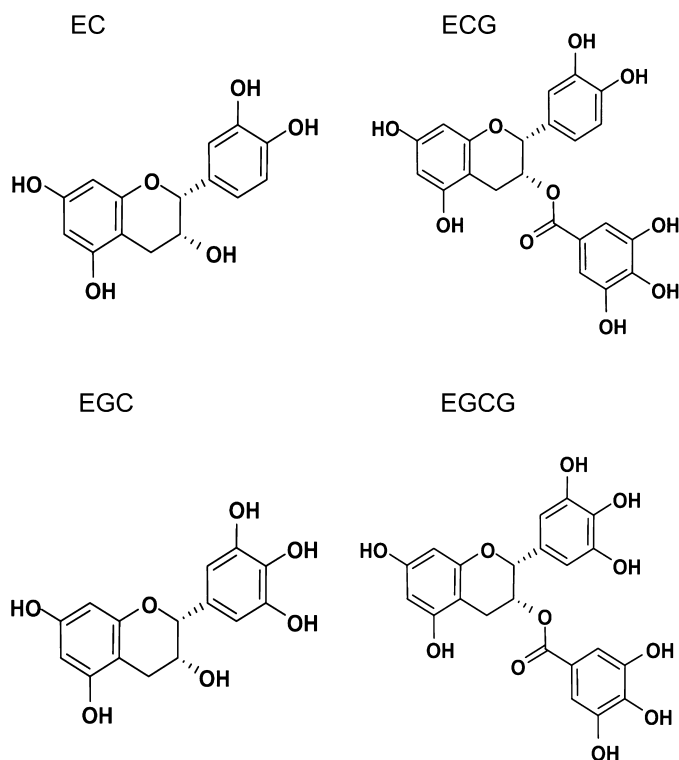

Fig. 1. Chemical structures of tea catechins.

in order to gain a better understanding of the intercurrent relationships between their bioavailability and the elicited biological effects.

Although a number of techniques, such as Spectrofluorimetry, Circular Dichroism and Fourier Transform Infrared Spectrometry, have been employed to evaluate interactions between catechins and albumin [13–15], the affinity capillary electrophoresis (ACE) could be by far one of the best to evaluate binding constants between BSA or HSA and catechins. ACE has indeed been reported as an efficient tool in studying non-covalent interactions and determining binding constants of formed complexes [16–21]. In fact, besides its speed and flexibility, ACE shows several advantages such as: (i) only a small amount of protein and analyte is required; (ii) all interacting components can be studied in free buffer solution at physiological conditions; (iii) binding constants of several samples can be simultaneously estimated.

Capillary electrophoresis has been recently employed to study the interaction between epicatechin and albumin [22]. Hence, the present work was undertaken with the intent to develop the first ACE method for evaluating the affinity binding between the four major epicatechins present in green tea (EGC, EC, ECG, EGCG) and albumin. In addition, since BSA is one of the most extensively protein used for interaction studies [23–26], the current method has also been successfully employed to evaluate the difference in affinity of HSA and BSA for the different epicatechins.

2. Materials and methods

2.1. Chemicals

EGC, EC, ECG, EGCG, HSA, BSA, thymidine, Na_2HPO_4 $2H_2O$, NaH_2PO_4 H_2O, phosphoric acid, hydrochloric acid, sodium hydroxide, dimethylsulfoxide (DMSO) were obtained from Sigma (St. Louis, USA). 0.45 μm membrane filters (used to filter all buffer solution before CE analysis) were purchased from Millipore (Bedford, USA).

2.2. Standards preparation

Individual standard catechins EGC, EC, ECG, EGCG were dissolved in water at 500 μmol/L final concentration. BSA stock solution (750 μmol/L) was prepared by dissolving the powder of definite weight in deionized water. Thymidine, that served as the electroosmotic flow (EOF) marker, was also dissolved in water at 41 mmol/L final concentration. All the stock solutions were stored at −80 °C until used.

The varying concentrations of BSA in the running buffer were prepared from 0 to 4 μmol/L.

2.3. Capillary electrophoresis

A MDQ capillary electrophoresis system equipped with a diode array detector was used (Beckman instruments, CA, USA). The system was fitted with a 30 kV power supply with a current limit of 300 μA. The analysis was performed in an uncoated fused-silica capillary, 75 μm I.D. and 40 cm length (30 cm to the detection window), injecting 45 nl of sample (3.45 kPa × 5 s). The separation was carried out in a 10 mmol/L sodium phosphate (8.1 mmol/L Na_2HPO_4 and 1.9 mmol/L NaH_2PO_4), HEPES 50 mmol/L pH 7.5, 37 °C and 15 kV (80 μA) at normal polarity. The varying concentrations of BSA in the running buffer were prepared from 0 to 4 μmol/L.

After each run, the capillary was rinsed with, 1 min of 1 mol/L NaOH, 1 min of 0.5 mol/L NaOH, and equilibrated with the run buffer for 1 min. The migration of analytes was monitored by absorption at 214 nm.

The apparent electrophoretic mobility (μ) of the analytes was determined from their migration times, using the fraction $(L_{eff}L_{tot})/(tV)$, where L_{eff} is the effective capillary length from the injection end to the detector, L_{tot} is the total length of the capillary, t is the migration time and V is the applied voltage. The mobility ratio (R) of the analytes was calculated by using the equivalent relationship $R = \mu_{eof/}\mu_{analyte}$ [27], where μ_{eof} is the electroosmotic mobility (measured with EOF marker thymidine) analyzed during the same run used to measure $\mu_{analyte}$.

3. Results and discussion

3.1. ACE optimization

In this work, buffer type, concentration and pH used for ACE and EOF marker type along with concentration were first optimized. In order to evaluate the non-covalent interactions between catechins and protein under near physiological conditions, we first used the sodium phosphate buffer system of 50 mmol/L at pH 7.4 and DMSO as EOF marker. However, in the aforementioned conditions only catechins with the galloyl moiety, such as ECG and EGCG were completely separated from the EOF marker, while EC and EGC migrated along with DMSO. A partial separation (not necessarily at baseline) between analytes and the EOF marker is crucial to obtain a correct value of migration time to calculate the mobility ratio. The attempt to separate EC and EGC from the EOF marker by modifying the running buffer concentration from 10 to 100 mmol/L, and the pH from 7 to 8 did not allows peak separation, also because the DMSO produced a broad peak that made its separation from catechins impossible. Several known EOF markers have been then tested, including dimethylformamide, methanol, ethanol, acetone, acetonitrile, 2-propanol, guanidine and thymidine. The addition of HEPES (between 10 and 100 mmol/L) to sodium phosphate buffer has been also evaluated in the attempt to improve peaks resolution. A suitable separation between the EOF marker and EC or EGC was reached only when HEPES 50 mmol/L was added to sodium phosphate 10 mmol/L at the final pH of 7.5 and using thymidine

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