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Probing the binding of (+)-catechin to bovine serum albumin by isothermal titration calorimetry and spectroscopic techniques

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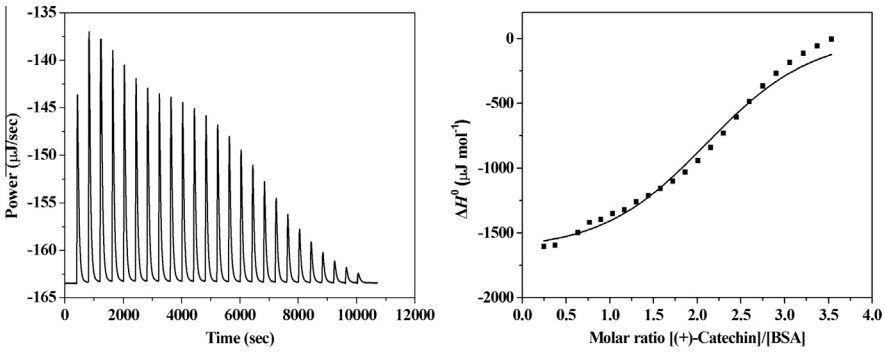
HIGHLIGHTS

- (+)-Catechin-BSA system is driven by enthalpy and entropy.
- (+)-Catechin can quench the fluorescence of BSA through a static quenching.
- (+)-Catechin can be stored and carried by serum albumin to reach its target organ.
- Binding site I is the primary binding site for (+)-catechin.
- (+)-Catechin may induce conformational and microenvironmental changes of BSA.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, the interaction between (+)-catechin and bovine serum albumin (BSA) was investigated using isothermal titration calorimetry (ITC), in combination with fluorescence spectroscopy, UV–vis absorption spectroscopy, and Fourier transform infrared (FT-IR) spectroscopy. Thermodynamic investigations reveal that the electrostatic interaction and hydrophobic interaction are the major binding forces in the binding of (+)-catechin to BSA. The binding of (+)-catechin to BSA is synergistically driven by enthalpy and entropy. Fluorescence experiments suggest that (+)-catechin can quench the fluorescence of BSA through a static quenching mechanism. The obtained binding constants and the equilibrium fraction of unbound (+)-catechin show that (+)-catechin can be stored and transported from the circulatory system to reach its target organ. Binding site I is found to be the primary binding site for (+)-catechin. Additionally, as shown by the UV–vis absorption, synchronous fluorescence spectroscopy and FT-IR, (+)-catechin may induce conformational and microenvironmental changes of BSA.

Introduction

Serum albumin is the most abundant protein in blood plasma (~60%), which has many physiological functions, such as maintaining the osmotic pressure and pH of blood and scavenging free radicals as an antioxidant [1]. It is an attractive macromolecular carrier, the lack of toxicity and immunogenicity make it an ideal candidate for drug delivery. In addition, serum albumin is the most multifunctional transport protein and plays an important role in the transport and deposition of a variety of endogenous and exogenous substances in blood [1]. Bovine serum albumin (BSA) has been one of the most extensively studied of this group of proteins, not only because of its medical importance, abundance, low cost, ease of purification, ready availability, unusual ligand-binding properties and it is widely accepted in the pharmaceutical industry

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[2–4], but also because of its structural homology with human serum albumin (HSA) [5,6]. The BSA molecule is made up of three homologous domains (I, II, III) that are divided into nine loops (L1–L9) by 17 disulfide bonds. The loops in each domain are made up of a sequence of large-small-large loops forming a triplet. Each domain in turn is the product of two subdomains (IA, IB, etc.) [5]. There are two famous binding sites of BSA for ligands, namely Sudlow's binding sites I (in subdomains IIA) and II (in subdomains IIIA) [7].

Knowledge of interaction mechanisms between drugs and serum albumin is very important for us to understand the pharmacokinetics and pharmacodynamics of them. First, the drug-serum albumin interaction plays a dominant role in the bioavailability of drugs because the bound fraction of drugs is a depot, whereas the free fraction of drugs shows pharmacological effects [8]. Second, drug distribution is mainly controlled by serum albumin, because most drugs circulate in plasma and reach the target tissues by binding to serum albumin [1,9]. If a drug is metabolized and excreted from the body too fast because of low protein binding, the drug will not be able to provide its therapeutic effect. Alternatively, if a drug has high protein binding and is metabolized and excreted too slowly, it may increase the drug's half-life in vivo and lead to undesired side effects [10]. Furthermore, very high affinity binding of a drug to serum albumin may prevent the drug from reaching the target at all, resulting in insufficient tissue distribution and efficacy. Third, the competition between two drugs for the binding sites on serum albumin may result in a decrease in binding and hence an increase of the concentration of the free biologically active fraction of one or both of the drugs. Co-administration of two drugs increases the free concentration of the drug with the lower affinity to serum albumin [11]. In addition, these hydrophobic binding pockets enable serum albumin to increase the apparent solubility of the hydrophobic drugs in plasma and modulate their delivery to the cells in vivo [12]. In a word, the absorption, distribution, metabolism, and excretion properties of a drug can be significantly affected as a result of its binding to serum albumin.

Catechins are plant polyphenolic compounds belonging to a subclass, known as flavan-3-ols, in the flavonoid family. The naturally occurring compounds are widely distributed in various fruits, green tea, red wine, juices, and in chocolate [13]. Catechins have received much attention due to their biological and pharmacological effects including antioxidant, anti-mutagenic, anti-carcinogenic, anti-viral, anti-microbial, and anti-inflammatory properties [14–19]. It is well known that structural features, namely the number of the galloyl and hydroxyl groups in the molecule of catechins, play an important role in their biological activities, particularly antioxidant properties [20], providing the protection against diseases directly or partially involving accumulation of free radicals in the body, e.g. cancers, aging, diabetes, neurodegenerative and cardiovascular diseases [21]. However, catechins were noticed to show also negative effects such as pro-oxidative, cyto- and phytotoxic activities [22–24].

Some spectroscopic studies on the interaction between serum albumin and catechins have been published [20,25–29], as well as some non-spectroscopic studies, e.g. electrophoretic techniques [20,30], and molecular docking [31]. Isothermal titration calorimetry (ITC), which measures directly the heat evolved during a reaction, is the method of choice for obtaining thermodynamic information. This is because only ITC allows researchers to obtain directly the variations of enthalpy ΔH^0 and of entropy ΔS^0, as well as the association constant K and the stoichiometry of binding n, for an association process [32]. Unlike other methods, ITC does not require chemical modification or immobilisation of reactants since heat of binding is a naturally occurring phenomenon [33,34]. This sets the technique apart from fluorescence methods that often require labeling or are specific to proteins that contain a fluorophore that is accessible to a quencher. ITC can also be applied to systems where the complex formed is insoluble. This is a distinct advantage over many solution based techniques, including capillary electrophoresis, where complex insolubility can be problematic [35]. The binding study between epicatechin and bovine serum albumin (BSA) had been done previously by ITC [36]. One of the most widely studied catechins is (+)-catechin (molecular structure: inset of Fig. 2) due to its high abundance in the human diet and relevant antioxidant activity [29]. However, to our knowledge, an accurate and full basic data for clarifying the binding mechanisms of (+)-catechin to BSA remain unclear. In the present work, a comprehensive investigation was performed for the binding properties of (+)-catechin to BSA under the physiological conditions. Using ITC, in combination with different spectroscopic methods, the binding information, including thermodynamic parameters, quenching mechanism, binding parameters, the equilibrium fraction of unbound (+)-catechin, high-affinity binding site, and conformation changes of BSA was investigated. The study provides an accurate and full basic data for clarifying the binding mechanism of (+)-catechin with BSA and is helpful for understanding its effect on protein function during the blood transportation process and its biological activity in vivo.

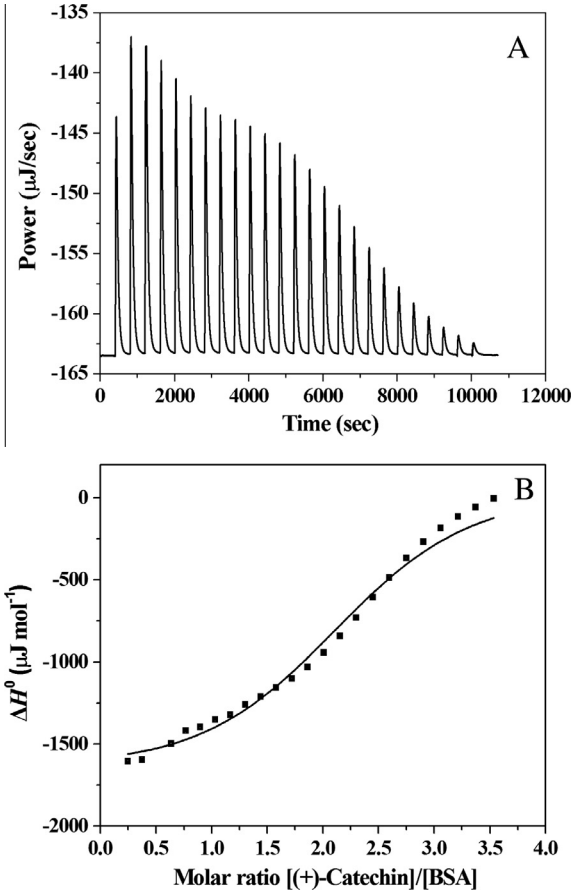

Fig. 1. (A) Raw data for the titration of 7×10^{-3} mol L^{-1} (+)-catechin with 6×10^{-4} mol L^{-1} BSA at pH 7.40 and 298 K, showing the calorimetric response as successive injections of (+)-catechin are added to the sample cell. (B) Integrated heat profile of the calorimetric titration shown in panel A. The solid line represents the best nonlinear least-squares fit to the independent binding sites model.