Journal of Luminescence 169 (2016) 220–226

Contents lists available at ScienceDirect

Journal of Luminescence

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

Review

Interaction of tea polyphenols with serum albumins: A fluorescence spectroscopic analysis

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ARTICLE INFO

Article history:
Received 2 May 2015
Received in revised form
30 August 2015
Accepted 8 September 2015
Available online 25 September 2015

Keywords:
Tea polyphenols
Serum proteins
Fluorescence
Binding constant
Static quenching

ABSTRACT

Interactions of some tea polyphenols, namely (−) Catechin (C), (−)-epicatechin (EC), (−) epicatechin-3-gallate (ECG), (−)-epigallocatechin (EGC) and (−)-epigallocatechin-3-gallate (EGCG) are outlined with the serum albumin proteins. These interactions had all resulted in binding with the proteins with a concomitant static quenching of the protein fluorescence. A fluorescence technique has been considered as the tool to comprehend the polyphenol–protein interactions mainly and simultaneously other spectroscopic techniques used to verify the results have been discussed. In this mini review the different types of equations usually employed to calculate the binding constant values have been outlined, namely, modified Stern Volmer plot, Scatchard plot and Lineweaver Burk equation, with their corresponding results. The *n* values (number of binding sites) had always been close to unity suggesting a 1:1 complexation with the polyphenols and the protein. A structural change in the polyphenols has been found to alter the binding constant value and the galloyl moiety attached to the C ring of the polyphenols have been found to play a crucial role in this regard. It has been found that an increase in galloyl moiety increases binding of the catechins with proteins.

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1. Introduction

The history of tea began over 5000 years ago in ancient China. Currently, tea is a very popular beverage consumed by two-thirds of the world's population. Green tea, black tea, and Oolong tea are all derived from the leaves of Camellia sinensis plant and contain an assortment of compounds, the most significant components of which are catechins or polyphenols. Tea polyphenols, known as catechins, account for 30–42% of the dry weight of the solids in brewed green tea [1]. Catechins contain a benzopyran skeleton with a phenyl group substituted at the 2-position and a hydroxyl (or ester) function at the 3-position. Variations to the catechin structure include the stereochemistry of the 2,3-substituents and the number of hydroxyl groups in the B and D rings. Belonging to the flavan-3-ol class of flavonoids, major catechins found in tea

Abbreviations: C, Catechin; EC, Epicatechin; EGC, Epigallocatechin; ECG, Epicatechin-3-gallate; EGCG, Epigallocatechin-3-gallate; BSA, Bovine serum albumin; HSA, Human serum albumin; αHSA, Human salivary alpha amylase; CD, Circular dichroism; ITC, Isothermal titration calorimetry

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http://dx.doi.org/10.1016/j.jlumin.2015.09.018
0022-2313/© 2015 Published by Elsevier B.V.

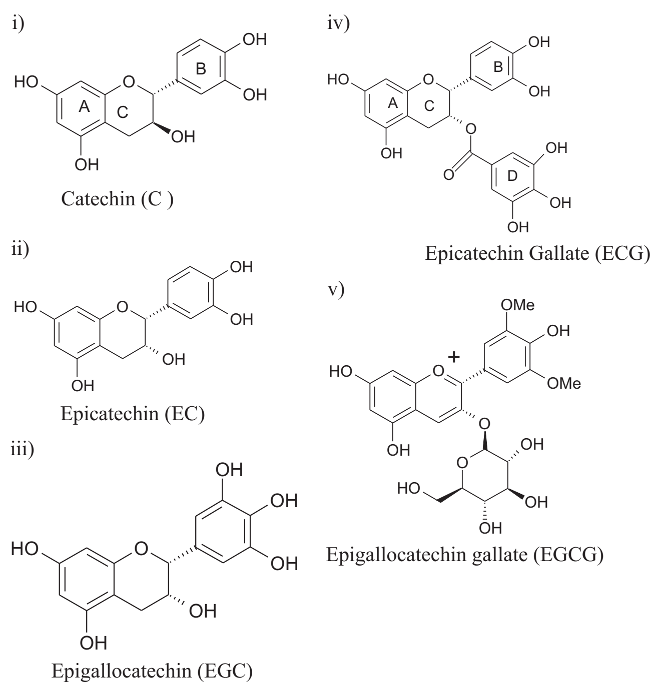

Fig. 1. Chemical structures of tea polyphenols, namely, (i) catechin (C), (ii) epicatechin (EC), (iii) epigallocatechin (EGC), (iv) epicatechin Gallate (ECG) and (v) epigallocatechin gallate (EGCG).

leaves are (−) Catechin (C), (−)-epigallocatechin-3-gallate (EGCG), (−)-epigallocatechin (EGC), (−) epicatechin-3-gallate (ECG) and (−)-epicatechin (EC) and their structures are shown in Fig. 1. Catechin (C), gallocatechin, epigallocatechin digallates, epicatechin digallate, 3-O-methyl EC and EGC, catechin gallate, and gallocatechin gallate are present in smaller quantities. Some flavonols, including quercetin, kaempferol, myricitin, and their glycosides are also present in tea.

Dietary plant polyphenols have received attention for their biologically significant functions as antioxidants, anticarcinogens or antimutagens, which have led to their recognition as potential nutraceuticals. Recent interest in dietary polyphenols, specially tea polyphenols, has concentrated on their likely health benefits as reducing agents, more commonly termed antioxidants that play a major role in the protection of body tissues against oxidative stress [2–4]. Indeed, the consumption of foods rich in polyphenols has been associated with a decreased risk of cancers, stroke and coronary heart disease. All of these evidences are based upon the study done on experimental animals and cell culture and a few on human exposed to environmental chemicals. All of these studies revealed the beneficial properties of polyphenols [5]. This mini review is intended to investigate the interaction of some tea polyphenols on the most abundant proteins of plasma, the albumin proteins. Serum albumins are the most abundant proteins in the circulatory system of a wide variety of organisms. Being the major macromolecule contributing to the osmotic blood pressure they can play a dominant role in drug disposition and efficacy [6–9]. Many drugs and other bioactive small molecules bind reversibly to albumin and other serum components, which then function as carriers. Serum albumin often increases the apparent solubility of hydrophobic drugs in plasma and modulates their delivery to cells in vivo and in vitro. Consequently, it is important to study and understand the interaction of drugs and small drug like bioactive molecules with this protein.

2. Methods

The methods outlined in this work are briefly summarized below:

2.1. Fluorescence quenching

Fluorescence quenching is described by the Stern–Volmer equation

$$F_0/F = 1 + kqt_0[Q] = 1 + K_{SV}[Q] \quad (1)$$

where F_0 and F are the fluorescence intensities before and after the addition of the quencher (polyphenol), respectively, kq is the bimolecular quenching constant, t_0 is the lifetime of the fluorophore in the absence of the quencher, [Q] is the concentration of the quencher, and K_{SV} is the Stern–Volmer quenching constant. Hence, Eq. (1) was applied to determine K_{SV} by linear regression of a plot of F_0/F against [Q]. A linear Stern–Volmer plot is generally indicative of a single class of fluorophores in a protein, all equally accessible to the quencher; this also means that only one mechanism (dynamic or static) of quenching occurs. In the cases of a static mechanism, there is a complex formation, and in such cases, the bimolecular quenching constant is calculated; kq can be calculated by the ratio between K_{SV} and t_0. For BSA, the lifetime of the fluorophore is approximately 5 ns, and for R-amylase, the lifetime of the fluorophore is approximately 2.97 ns [10]. The maximum value possible for diffusion-limited quenching (dynamic mechanism) in water is $10^{10}\ M^{-1}\ s^{-1}$. When the value of the bimolecular quenching constant (kq) is higher, it could mean that there is a complex formation between protein and quencher, corresponding to a static mechanism. There have been several studies reporting BSA quenching due to specific interactions, and in these cases, the quenching constant has been much higher than the maximum value of diffusion-limited quenching in water. Smaller values of kq can result from steric shielding of the fluorophore. However, positive deviations from the Stern–Volmer equation are frequently observed when the extent of quenching is large. In that case, the Stern–Volmer plot exhibits an upward curvature, concave toward the y axis at high [Q]. These positive deviations may be an indication of two distinct situations. In many cases, this upward curvature indicates that the fluorophore can be quenched by both mechanisms with the same quencher. In other cases, the upward curvature indicates the presence of a sphere of action. This assumes the existence of a sphere of volume around a fluorophore within which a quencher will cause quenching with a probability of unity. In this situation, quenching occurs due to the quencher being adjacent to the fluorophore at the moment of excitation. These closely spaced fluorophore–quencher pairs are immediately quenched, but fluorophores and quenchers do not actually form a ground-state complex. This type of apparent static quenching is usually interpreted in terms of the model "sphere of action". Negative deviations from the Stern–Volmer equation are frequently found in the systems that have multiple fluorophores, such as BSA. The values of F_0/F, instead of increasing linearly with [Q] in the classic model, trend downward toward the x axis at high [Q], signifying differences in the accessibilities of fluorophores to the quencher [11]. The modified Stern–Volmer equation (Eq. (2)) includes a factor for fractional accessibility f_a, allowing calculation for the modified Stern Volmer constant K_{sv} for systems with more than one fluorophore; a method by Lehrer [12],

$$F_0/\Delta F = (1/(f_a \times K_{sv})) \times (1/[Q]) + 1/f_a \quad (2)$$

where $\Delta F = F_0 - F$; F_0 and F are the relative fluorescence intensities in absence and presence of the quencher, respectively, f_a is the fraction of fluorophore accessible to the quencher, [Q] is the concentration of quencher, and K_{sv} is the Stern–Volmer quenching constant. The plots of $F_0/\Delta F$ versus $1/[Q]$ yield $1/f_a$ as the intercept, and $1/(f_a K_{sv})$ as the slope. From the intercept and slope K_{sv} and f_a values were calculated.

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