Journal of Photochemistry and Photobiology B: Biology 112 (2012) 16–22

Contents lists available at SciVerse ScienceDirect

Journal of Photochemistry and Photobiology B: Biology

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

Molecular spectroscopic studies on the interaction of morin with bovine serum albumin

Yan-Jun Hu *, Hua-Li Yue, Xiao-Ling Li, Si-Si Zhang, E. Tang, Li-Ping Zhang

Hubei Key Laboratory of Pollutant Analysis & Reuse Technology, Department of Chemistry, Hubei Normal University, Huangshi 435002, PR China

ARTICLE INFO

Article history:
Received 21 December 2011
Received in revised form 26 February 2012
Accepted 3 April 2012
Available online 19 April 2012

Keywords:
Morin
Bovine serum albumin
Conformational investigation
Binding parameters
Structure–affinity relationship

ABSTRACT

The interaction between morin and bovine serum albumin (BSA) was studied using molecular spectroscopic approach at different temperatures under imitated physiological conditions. Quenching of intrinsic tryptophanyl fluorescence of BSA with increasing morin concentration is the actuating tool in the analysis. The obtained quenching mechanisms, binding constants, binding sites and corresponding thermodynamic parameters at different temperatures indicate that the hydrophobic interaction play a major role in the morin-BSA association. Binding affinity between morin and BSA was determined using Scatchard equation and the modified Stern–Volmer equation, and the corresponding Structure–affinity relationships of flavonoids were discussed. Site marker competitive displacement experiments demonstrated that morin binds with high affinity to site II (subdomain IIIA) of BSA. Furthermore, the circular dichroism spectral results indicated that the conformation of BSA changed in the presence of morin. In addition, the effect of some common metal ions on the binding constant between morin and BSA was examined.

1. Introduction

Morin, (3,5,7,2′,4′-pentahydroxyflavone; molecular structure: inset of Fig. 1; CAS Registry Number: 654055-01-3), is a flavonoid that has been identified in fruits, vegetables, tea, wine, and many Chinese herbs [1]. It exhibits a variety of pharmacological activities such as antiinflammatory activity [2,3], antioxidant activity [4,5], antiviral activity [6], antiallergic activity [7], and antinociceptive activity [8]. Furthermore, morin exhibits an anti-tumor activity [9,10]. Although these broad pharmacological uses of morin have been mentioned above, its effects on plasma proteins and the corresponding mechanistic scenario of action has been poorly understood. The fundamental study of the molecular basis of such interaction could be highly advantageous.

The present study is focused on an endeavor to explore the important and pertinent issue of binding interaction of morin with a model transport protein, bovine serum albumin (BSA). BSA is used as the model protein for drug delivery because of its medically important, abundance, low cost, ease of purification, unusual ligand-binding properties and it is widely accepted in the pharmaceutical industry [11–15]. The crystal structure analyses indicate that the principal regions of ligand binding sites in albumin are located in hydrophobic cavities in subdomains IIA and IIIA. According to Sudlow's nomenclature, two primary sites (I and II) have been identified for ligand binding to serum albumin. Warfarin, an anticoagulant drug, and ibuprofen, a nonsteroidal antiinflammatory agent, have been considered as stereotypical ligands for Sudlow's site I and II, respectively [16–18]. Warfarin, as other bulky heterocyclic anions, binds to Sudlow's site I located in subdomain IIA, whereas ibuprofen, as other aromatic carboxylates with an extended conformation, prefers Sudlow's site II, located in subdomain IIIA.

To investigate the interaction between protein and drug is useful in studying the pharmacological response of drugs and design of dosage forms, and hence has become an essential research field in chemistry, life sciences and clinical medicine [19–23]. Therefore, the present programme aims at a spectroscopic characterization of the interaction of morin with the transport protein BSA using constant protein concentration and varying drug compositions. The interaction information regarding quenching mechanisms, binding parameters, thermodynamic parameters, binding mode and affinity, conformation investigation, binding location, and effect of metal ions are reported in this work. An attempt is also undertaken to unravel the corresponding Structure–affinity relationships of flavonoids.

2. Materials and methods

2.1. Materials

BSA and warfarin were obtained from Sigma-Aldrich (St. Louis, MO, USA); the buffer Tris was obtained from Amresco (Ohio, USA; the purity no less than 99.5%); morin (CAS Num: 654055-01-3)

* Corresponding author. Tel.: +86 714 6515602; fax: +86 714 6573832.
E-mail addresses: huyj@whu.edu.cn, yjhu@263.net (Y.-J. Hu).

1011-1344/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jphotobiol.2012.04.001

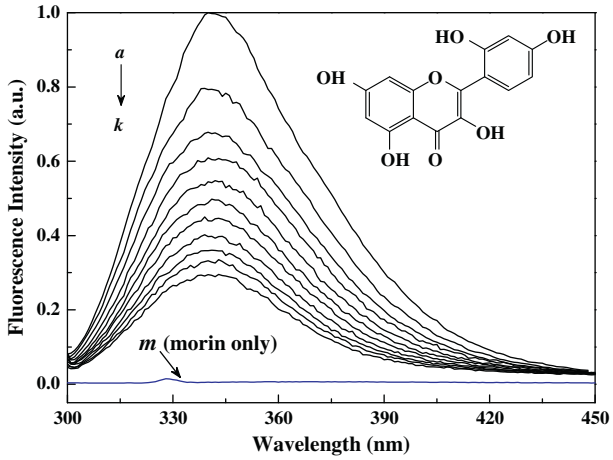

Fig. 1. (color in the web version) Emission spectra of BSA in the presence of various concentrations of morin. c (BSA) = 1.0×10^{-5} mol L^{-1}; c (morin)/(10^{-5} mol L^{-1}), a–k: from 0.0 to 4.0 at increments of 0.40; curve m (blue line) shows the emission spectrum of morin only (c (morin) = 1.0×10^{-5} mol L^{-1}), (T = 310 K, λ_{ex} = 295 nm). The inset corresponds to the molecular structure of morin.

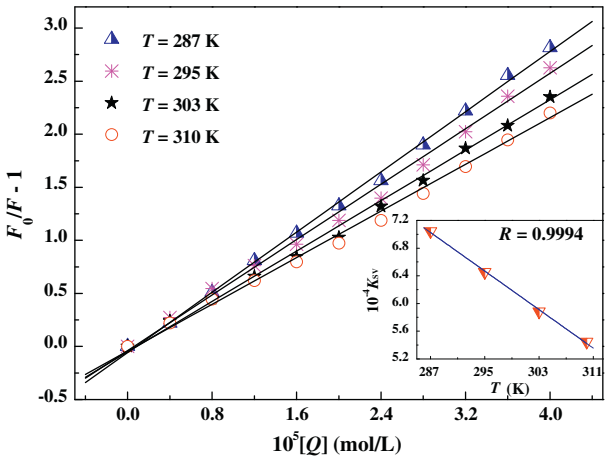

Fig. 2. Plots ($F_0/F - 1$) *vs.* [Q] for elucidation of the quenching constant (K_{SV}) for morin-BSA binding interaction from fluorescence data. The inset shows the relationship of the quenching constants K_{SV} *vs.* T.

was purchased from Acros Organics (New Jersey, USA); ibuprofen was obtained from Hubei biocause pharmaceutical Co., Ltd., (Hubei, China; the purity no less than 99.7%); NaCl, HCl, etc. were all of analytical purity. All samples were dissolved in Tris-HCl buffer solution (0.05 mol L^{-1} Tris, 0.15 mol L^{-1} NaCl, pH 7.4), Corresponding blanks, run under the same conditions, were subtracted from the sample spectra.

2.2. Equipments and spectral measurements

All fluorescence spectra were recorded on F-4500 Spectrofluorimeter (Hitachi, Japan) equipped with 1.0 cm quartz cells and a thermostat bath. The widths of both the excitation slit and the emission slit were set to 2.5 nm. An excitation wavelength of 295 nm was chosen due to the intrinsic property of tryptophan (Trp) fluorophore.

The UV spectrum was recorded at room temperature on a U-3010 Spectrophotometer (Hitachi, Japan) equipped with 1.0 cm quartz cells.

Circular dichroism (CD) spectra were measured with a Jasco J-810 Spectropolarimeter (Jasco, Tokyo, Japan) at room temperature over a wavelength range of 260–200 nm and under constant nitrogen flush. Quartz cells having path lengths of 1.0 cm were used at a scanning speed of 200 nm/min.

3. Results and discussions

3.1. Effect of morin on BSA fluorescence characteristics and quenching mechanism

Fluorescence quenching can proceed by different mechanisms, which are usually classified as either dynamic quenching or static quenching. Dynamic and static quenching can be distinguished by their differing dependence on temperature and viscosity. Dynamic quenching depends upon diffusion. Since higher temperatures result in larger diffusion coefficients, the bimolecular quenching constants are expected to increase with increasing temperature. In contrast, increased temperature is likely to result in decreased stability of complexes, and thus lower values of the static quenching constants.

In this work, the concentration of BSA solution were stabilized at 1.0×10^{-5} mol L^{-1}, and the concentrations of morin varied from 0 to 4.0×10^{-5} mol L^{-1} at increments of 0.4×10^{-5} mol L^{-1}. The effect of morin on BSA fluorescence intensity at 310 K is shown in Fig. 1. It was observed from Fig. 1 that a progressive decrease in the fluorescence intensity was caused by quenching. Curve m (Fig. 1, blue line[1]) shows the emission spectrum of morin only, which indicates that morin does not possess significant fluorescence features; thus, the effect of morin emission at the excitation wavelength of tryptophan (295 nm) would be negligible.

The phenomenon of fluorescence quenching is customarily described in terms of the following well-known Stern–Volmer equation [24,25]:

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

In which F_0 and F are the fluorescence intensities in the absence and presence of the quencher (Q), respectively, K_{SV} is the Stern–Volmer quenching constant and terms within square brackets represent concentrations of respective species. Herein, we have employed the Stern–Volmer equation (Eq. (1)) to estimate the Stern–Volmer quenching constant (K_{SV}) at four different temperatures (Fig. 2) and the data are compiled in Table 1. The results demonstrated the effect on fluorescence quenching by morin at each temperature studied, the result shows that the Stern–Volmer quenching constant K_{SV} is inversely correlated with temperature, which indicates that the probable quenching mechanism is the formation of morin-BSA complex (static quenching) rather than by dynamic collision (dynamic quenching).

One additional method to distinguish static and dynamic quenching is by careful examination of the absorption spectra of the fluorophore. Collisional quenching only affects the excited states of the fluorophores. and thus no changes in the absorption spectra are expected. In contrast, ground-state complex formation will frequently result in perturbation of the absorption spectrum of the fluorophore [24]. For reconfirming the probable quenching mechanism of fluorescence of BSA by morin is initiated by ground-state complex formation, we used the difference absorption spectroscopy to obtain spectra, the UV-Visible absorption spectra of BSA (blue curve b) and the difference absorption spectra between BSA-morin and morin (red curve c) at the same concentration could not be superposed (Fig. 3), this result reconfirm that the probable quenching mechanism of fluorescence of BSA by morin is a static quenching procedure.

[1] For interpretation of color in Figs. 1–7, the reader is referred to the web version of this article.