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Interaction of colchicine with human serum albumin investigated by spectroscopic methods

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Abstract

We investigated the interaction between colchicine and human serum albumin (HSA) by fluorescence and UV–vis absorption spectroscopy. In the mechanism discussion, it was proved that the fluorescence quenching of HSA by colchicine is a result of the formation of colchicines–HSA complex; van der Waals interactions and hydrogen bonds play a major role in stabilizing the complex. The modified Stern–Volmer quenching constant K_a and corresponding thermodynamic parameters ΔH, ΔG, ΔS at different temperatures were calculated. The distance r between donor (Trp^{214}) and acceptor (colchicine) was obtained according to fluorescence resonance energy transfer (FRET).

Keywords: Colchicine; Human serum albumin; Fluorescence quenching; Thermodynamic parameters; Fluorescence resonance energy transfer

1. Introduction

Protein-drug binding greatly influences absorption, distribution, metabolism, and excretion properties of typical drugs [1]. Human serum albumin (HSA) is the most abundant protein in the systemic circulation, with HSA comprising 60% in plasma [2]. Its principal function is to transport fatty acids, it is also capable of binding an extraordinarily broad range of drugs [3], and much of the clinical and pharmaceutical interest in the protein derives from its effects on drug pharmacokinetics [4]. The crystallographic analyses of HSA revealed that the protein, a 585 amino acid residue monomer, contains three homologous α-helical domains (I–III), and a single tryptophan (Trp^{214}) [5]. Many drugs and other bioactive small molecules bind reversibly to albumin and other serum components, which implicates HSA's role as carriers [6–9]. Serum albumin often increases the apparent solubility of hydrophobic drugs in plasma and modulates their delivery to cells in vivo and in vitro; they can play a dominant role in drug disposition and efficacy [10].

Colchicine (molecular structure: Fig. 1; formula: $C_{22}H_{25}NO_6$; CAS registry number: 64-86-8; molecular weight: 399.44) is a naturally occurring alkaloid used in human and veterinary medicine. It has been used as a model antimitotic drug, which was frequently used as an antimitotic agent in cancer research involving cell cultures [11,12]. Furthermore, colchicine is used for alleviation of inflammatory process during podagra and for reducing pain [13,14], and it has been proposed as an anti-inflammatory and anti-fibrotic drug to alleviate chronic lung inflammation and liver diseases in cystic fibrosis patients [15–17]. Hence, it is important to understand and predict ligand/drug displacement interactions for variety of endogenous and exogenous ligands/drugs. However, detailed investigations of the interaction of HSA with colchicine are scanty. Because of its medical relevance, our work should be valuable.

Fluorescence spectroscopy is a powerful tool for the study of the reactivity of chemical and biological systems since it allows non-intrusive measurements of substances in low concentration under physiological conditions. In the present work, our aim was to determine the affinity of colchicine to HSA, and to investigate the thermodynamics of their interaction. We tried also to find the stoichiometry of colchicine and HSA binding. In order to attain these objectives, we planned to carry out detailed investigation of colchicines–HSA association using fluorescence spectroscopy and UV–vis absorption spectroscopy. Through fluorescence resonance energy transfer (FRET), we planned to further investigate the energy transfer parameters of HSA for transfer to colchicine.

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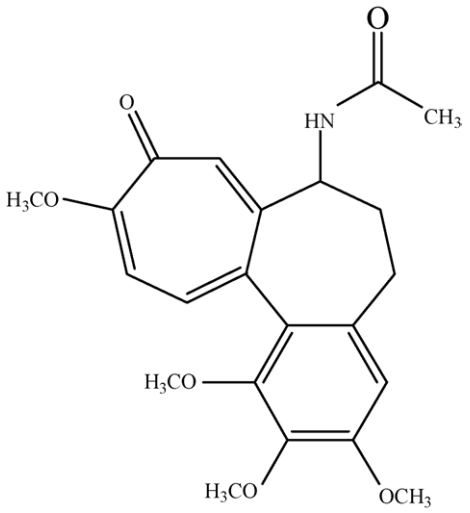

Fig. 1. Molecular structure of colchicine.

2. Materials and methods

2.1. Materials

HSA was obtained from Sigma; colchicine was obtained from Duchefa, the purity of colchicine no less than 98%. The buffer Tris had a purity of no less than 99.5% and NaCl, HCl, etc. were all of analytical purity. The samples were dissolved in Tris–HCl buffer solution (0.05 mol L^{-1} Tris, 0.15 mol L^{-1} NaCl, pH 7.4 ± 0.1). All solutions were used with doubly distilled water.

2.2. Equipments

The UV spectrums were recorded at room temperature on a TU-1901 spectrophotometer (Puxi Analytic Instrument Ltd. of Beijing, China) equipped with 1.0 cm quartz cells. All fluorescence spectra were recorded on F-2500 Spectrofluorimeter (Hitachi, Japan) equipped with 1.0 cm quartz cells and a thermostat bath; sample masses were accurately weighted using a microbalance (Sartorius, ME215S) with a resolution of 0.1 mg.

2.3. Spectroscopic measurements

The absorption spectroscopy of colchicine was performed at room temperature. The fluorescence measurements were performed at different temperatures (298, 302, 306 and 310 K). The widths of both the excitation slit and the emission slit were set to 2.5 nm. An excitation wavelength of 295 nm was used throughout to minimize the contribution of the tyrosine residues to the emission. Appropriate blanks corresponding to the buffer were subtracted to correct background of fluorescence.

2.4. Principles of fluorescence quenching

Fluorescence quenching is described by the well-known Stern–Volmer equation [18]:

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

where F_0 and F denotes the steady-state fluorescence intensities in the absence and in the presence of quencher (colchicine), respectively, K_{SV} is the Stern–Volmer quenching constant, and [Q] is the concentration of the quencher. Hence, Eq. (1) was applied to determine K_{SV} by linear regression of a plot of F_0/F against [Q].

3. Results and discussions

3.1. Fluorescence characteristics of colchicine on HSA

Fluorescence quenching refers to any process, which decreases the fluorescence intensity of a sample [18]. A variety of molecular interactions can result in quenching. These include excited-state reactions, molecular rearrangements, energy transfer, ground-state complex formation, and collisional quenching. Quenching can occur by different mechanisms, which usually classified as dynamic quenching and 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 order to discuss the results within the linear concentration range, we selected carrying out the experiment within the linear part of Stern–Volmer dependence (F_0/F against [Q]), and stabilized the concentrations of HSA at 1.0×10^{-5} mol L^{-1}. Concentration of colchicine varied from 0 to 3.6×10^{-5} mol L^{-1} at increments of 0.4×10^{-5} mol L^{-1}. As can be seen from Fig. 2, addition of increasing concentrations of colchicine caused a progressive reduction of the fluorescence intensity, accompanied by a decrease of wavelength emission maximum λ_{max} in the albu-

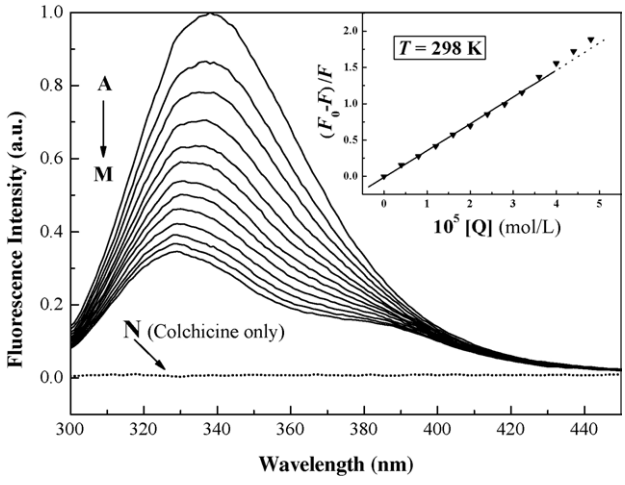

Fig. 2. Effect of colchicine on fluorescence spectrum of HSA (T = 298 K, λ_{ex} = 295 nm). A–M, c (HSA) = 1.0×10^{-5} mol L^{-1}, c (colchicine)/(10^{-5} mol L^{-1}): 0, 0.4, 0.8, 1.2, 1.6, 2.0, 2.4, 2.8, 3.2, 3.6, 4.0, 4.4, and 4.8, respectively; curve N shows the emission spectrum of colchicine only, c (colchicine) = 1.0×10^{-5} mol L^{-1}. The inset corresponds to the Stern–Volmer plot.