Journal of Photochemistry and Photobiology B: Biology 138 (2014) 324–330

Contents lists available at ScienceDirect

Journal of Photochemistry and Photobiology B: Biology

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

Multi-spectral characterization & effect of metal ions on the binding of bovine serum albumin upon interaction with a lincosamide antibiotic drug, clindamycin phosphate

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ARTICLE INFO

Article history:
Received 26 February 2014
Received in revised form 28 May 2014
Accepted 31 May 2014
Available online 28 June 2014

Keywords:
Bovine serum albumin
Clindamycin phosphate
Quenching
3D spectra
Lifetime measurements

ABSTRACT

The interaction of clindamycin phosphate (CP) with bovine serum albumin (BSA) is studied by using fluorescence spectra, UV–visible absorption, synchronous fluorescence spectra (SFS), CD, 3D fluorescence spectra and lifetime measurements under simulated physiological conditions. CP effectively quenched intrinsic fluorescence of BSA. The binding constants K_A values are 2.540×10^5, 4.960×10^5, 7.207×10^5 L mol^{-1}, the number of binding sites n and corresponding thermodynamic parameters ΔG^o, ΔH^o and ΔS^o between CP and BSA were calculated at different temperatures. The interaction between CP and BSA occurs through dynamic quenching and the effect of CP on the conformation of BSA was also analyzed using SFS. The average binding distance r between the donor (BSA) and acceptor (CP) was determined based on Förster's theory. The results of fluorescence spectra, UV–vis absorption spectra and SFS show that the secondary structure of the protein has been changed in the presence of CP.

1. Introduction

Clindamycin is a lincosamide antibiotic (Fig. 1A). It is a regular topical treatment of acne and can be useful against some methicillin-resistant Staphylococcus aureus (MRSA) infections [1]. It is a semisynthetic derivative of lincomycin, a natural antibiotic produced by the actinobacterium Streptomyces lincolnensis. It is obtained by 7(S)-chloro-substitution of the 7(R)-hydroxyl group of lincomycin [2,3]. Clindamycin is used mainly to treat anaerobic infections caused by susceptible anaerobic bacteria, including dental infections [4] and infections of the respiratory tract, skin and soft tissue infections and peritonitis.

Serum albumins are the abundant proteins in plasma and the main transport proteins, whose principal function is to transport fatty acids. They contribute to colloid osmotic blood pressure and are mainly responsible for the maintenance of blood pH [5]. They are also capable of binding a diverse range of metabolites, drugs, dyes and organic compounds to act as a carrier [6,7]. One of the most significant biological functions of albumins is their capacity to carry drugs as well as endogenous and exogenous substances. The nature and magnitude of drug–albumin interaction significantly influences the pharmacokinetics of drugs. Among the serum albumins, BSA has a wide range of physiological functions [8].

BSA (Fig. 1B) has properties like stability, water solubility, versatile binding capacity and particularly its structure homology (80%) with human serum albumin. BSA has two tryptophan residues that have the characteristic of intrinsic fluorescence: Trp-134 in the first domain and Trp-212 in the second domain. Trp-212 is located within a hydrophobic binding pocket of the protein and Trp-134 is located on the surface of this molecule [9].

Therefore, the binding interaction of drugs to serum albumin in vitro has been considered as a model in protein chemistry to study the binding behavior of proteins. In this work, bovine serum albumin was selected as our protein model because of its low cost, ready availability and unusual ligand-binding properties and the results of all of the studies are consistent with the fact that bovine and human serum albumins are homologous proteins [10].

So, this kind of study provides key information regarding the structure, through which we can determine the therapeutic effectiveness of the drug and hence, this can become an important tool in research especially in the fields like chemistry, clinical medicine and life sciences and mainly in pharmacology and pharmacodynamics. Hence, we found it worth to investigate the mechanism of interaction CP with BSA in detail using multi spectroscopic techniques.

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http://dx.doi.org/10.1016/j.jphotobiol.2014.05.024
1011-1344/

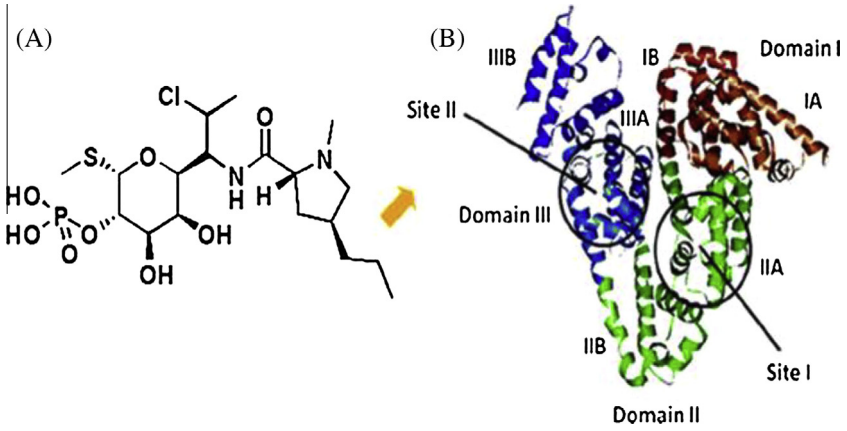

Fig. 1. Chemical structure of drug clindamycin phosphate (A) and a pictorial representation of the BSA model structure (B) obtained from homology modeling indicating the domains and the binding sites.

2. Experimental section

2.1. Reagents

Clindamycin phosphate and protease-free and essentially globulin free BSA (Fraction V) was bought from Sigma Chemical Co. (St. Louis, USA). The stock solution of BSA (Molecular weight of BSA is 65,000) and CP was prepared in 0.1 M phosphate buffer of pH 7.4 containing 0.15 M NaCl. All other chemicals were of analytical reagent grade and double distilled water was used throughout the experiment.

2.2. Instruments

- A Hitachi RF-5301 spectrofluorimeter (Tokyo, Japan) was used for fluorescence spectra measurements.
- A CARY 50-BIO UV–vis spectrophotometer (Varian, Australia) was used for absorption spectral analysis.
- A Jasco J-715 spectropolarimeter (Tokyo, Japan) was used for circular dichroism spectral analysis.
- Fluorescence lifetime measurements were carried out in an ISS'S ChronosBH fluorescence lifetime spectrometer.
- The pH was measured on a pH meter (Elico LI120 pH meter, Elico Ltd., India).

2.3. Procedures

2.3.1. Fluorescence quenching study

On the basis of preliminary investigations, intrinsic fluorescence was measured at 288, 298 and 308 K in the range of 300–500 nm upon excitation at 296 nm. The concentration of protein was fixed at 5 μM while that of drug was varied in the range of 5–45 μM.

2.3.2. UV–visible absorption studies

The UV–visible absorption measurements of BSA in presence and absence of CP were recorded in the range of 240–490 nm. Concentrations of BSA were fixed at 5 μM while that of the drug was varied from 5 to 45 μM in presence of phosphate buffer. The experiment was carried out at room temperature.

2.3.3. Synchronous fluorescence measurements

Synchronous fluorescence spectra of BSA (5 μM) were recorded with increasing concentrations of CP (0–45 μM) by setting $\Delta\lambda = 60$ nm and $\Delta\lambda = 15$ nm for tryptophan and tyrosine residues respectively. The spectra were recorded in the range of 260–320 nm.

2.3.4. Binding studies in the presence of site probes

The displacement experiments were performed varying probes viz, warfarin, ibuprofen and digitoxin for site I, II and III respectively [11]. The concentration of BSA and probe constant were kept constant (5 μM each) while concentration of CP was varied. The fluorescence emission spectra were recorded and the binding constant values of CP–BSA-probe were evaluated.

2.3.5. Energy transfer between CP and BSA

The absorption spectrum of the drug in the range of 240–490 nm and emission spectrum of protein were recorded in the range of 300–500 nm. Then, the overlap of the UV–visible absorption spectrum of CP with the fluorescence emission spectrum of BSA was used to calculate the energy transfer based on the Förster's theory.

2.3.6. Thermodynamics of CP–BSA interactions

Thermodynamic parameters for the binding of drug to BSA were determined by carrying out the binding studies at three different temperatures 288, 298 and 308 K in the range of 300–500 nm upon excitation at 296 nm by spectrofluorimetric method.

2.3.7. Effect of metal ions

The fluorescence spectra of CP-BSA were evaluated in presence of various ions viz Zn^{2+} ($ZnCl_2$), Ca^{2+} ($CaCl_2$), Cu^{2+} ($CuCl_2{\cdot}2H_2O$), Mg^{2+} ($MgSO_4{\cdot}H_2O$), Co^{2+} ($CoCl_2{\cdot}6H_2O$), K^{+}(KCl), Ba^{2+} ($BaCl_2{\cdot}2H_2O$), Mn^{2+} ($MnCl_2{\cdot}2H_2O$), Li^{+} ($Li_2SO_4{\cdot}H_2O$) and Ni^{2+} ($NiSO_4{\cdot}6H_2O$) in the range of 300–500 nm upon excitation at 296 nm. The concentration of BSA and respective ions was fixed at 5 μM.

2.3.8. 3D fluorescence spectra

3D fluorescence spectra of BSA were evaluated with CP and without CP at an excitation wavelength range of 200–350 nm and emission wavelength range of 200–600 nm.

2.3.9. Circular dichroism(CD) measurements

The CD spectral data of BSA (2.5 μM) in presence and absence of CP were made in the range of 200–250 nm. The BSA to CP concentration was varied in the ratio of 1:0, 1:1 and 1:3. The CD results are expressed in terms of Mean Residue Ellipticity (MRE) in deg cm^2 $dmol^{-1}$ according to the following equation:

$$\mathrm{MRE} = \frac{\text{observed CD (mdeg)}}{C_p n l \times 10} \quad (1)$$

where C_p is the molar concentration of the protein, n the number of amino acid residues (583 for BSA) and l the path-length (0.2 cm).