Colloids and Surfaces A: Physicochem. Eng. Aspects 504 (2016) 471–478

Contents lists available at ScienceDirect

Colloids and Surfaces A: Physicochemical and Engineering Aspects

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

Thermodynamic and kinetic characterization of pH-dependent interactions between bovine serum albumin and ibuprofen in 2D and 3D systems

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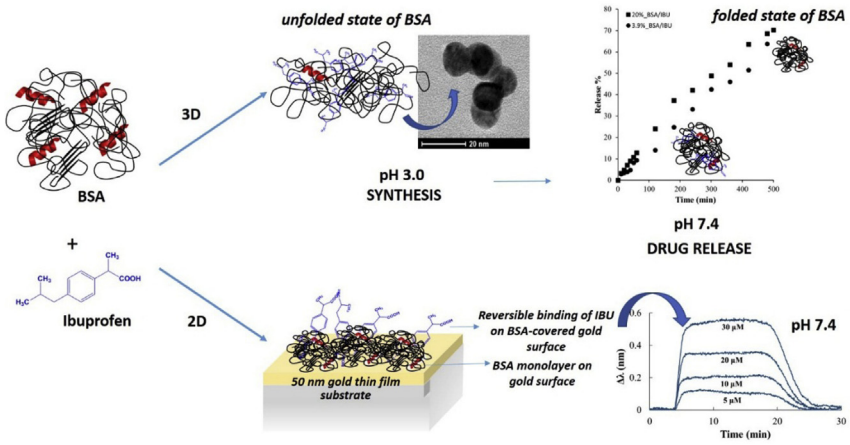
HIGHLIGHTS

- Design of BSA-IBU NPs was carried out by the results of several 2D and 3D experiments.
- The pH-induced structural changes of BSA were proven in 2D and 3D systems.
- Quantitative data of the BSA-IBU interactions were presented at different pH.
- Kinetic constants and thermodynamic state functions were determined by SPR and ITC.
- The pH-induced ibuprofen release of the nanosized composite particles was confirmed.

GRAPHICAL ABSTRACT

(not proportional representation)



ARTICLE INFO

Article history:
Received 19 October 2015
Received in revised form 27 May 2016
Accepted 28 May 2016
Available online 31 May 2016

Keywords:
Thermodynamics
Kinetic studies
BSA
Ibuprofen
Nanocomposite

ABSTRACT

The interactions between bovine serum albumin (BSA) and ibuprofen (IBU) were investigated at pH 3.0 and pH 7.4 by several two-(2D) and three-(3D) dimensional techniques to provide quantitative, kinetic and thermodynamic data on the BSA-IBU binding. Based on the results, the preparation of BSA-IBU composite nanoparticles (NPs) were successfully carried out for controlled drug release. The high resolution transmission electron microscopy (HRTEM), dynamic light scattering (DLS) and small angle x-ray scattering (SAXS) studies confirm the formation of nearly monodisperse NPs with $d_{average}$ = 10–13 nm depending on the protein concentrations and IBU contents. The kinetics of pH-induced drug release was studied by a vertical diffusion cell at pH 7.4 at 25 °C. The pH-dependent changes in the secondary structure of BSA were proven by SAXS, DLS and surface plasmon resonance (SPR) investigations. Depending on the protein conformations, the SPR results suggest that the bonded amounts of the drug molecule are 1239 mg IBU/g BSA and 174 mg IBU/g BSA at acidic and neutral pH, respectively. Besides quantification of the

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http://dx.doi.org/10.1016/j.colsurfa.2016.05.090
0927-7757/

interactions, the rate of association (k_a) and dissociation (k_d), the K_A and K_D standard equilibrium constants and the binding free energy (ΔG°) were also calculated on the basic of SPR measurements. The $\Delta G^\circ = -21.5 \pm 0.2\ kJ\ mol^{-1}$ obtained by SPR in 2D system is in good agreement with the $\Delta G^\circ = -17.38 \pm 0.54\ kJ\ mol^{-1}$ determined by isotherm titration calorimetry (ITC) in solution (3D).

1. Introduction

Nanoscale drug delivery systems have been under investigations for several decades [1–3]. At present, numerous types of NPs are designed as feasible candidates for gene therapy and molecular imaging [4], but only very few have actually to mature to clinical applications. Polymer-, dendrimer-, lipid-, iron oxide-, quantum dots- or other organic- and inorganic-based NPs are synthesized in order to deliver a drug to the right place at the right time in adequate concentration [5–8]. The proteins are also widely used for encapsulation and transportation of different drug molecules. The albumin-(BSA or HSA)-based NPs play a determinant role in the development of novel nanocarrier systems because many binding sites are available to several drug molecules. Moreover, the albumins have various specific advantages in nano-scale range, such as biodegradability, biocompatibility and non-toxicity [8]. In the interest of the development of an effective drug delivery systems the interaction between the drug and the carrier should be strong enough to facilitate the transport but also weak enough to release the drug to the target. Thus, the quantitative study of the binding thermodynamics and the knowledge of the kinetics of the release process are necessary [9]. Contrary to the (radio)-labelled techniques in recent years a number of "label-free" techniques have been developed to report biomolecular interactions [10–12]. Two-dimensional SPR is a label-free technique and capable of measuring real-time quantitative binding affinities and kinetics for proteins interacting with biomolecules using relatively small (in nanomolar range) quantities of materials and has potential to be medium-throughput [13–15]. The conventional SPR technique requires that one binding component to be immobilised on a sensor chip while the other binding component in solution is flowed over the sensor surface; a binding interaction is detected using an optical method that measures remarkably small changes in refractive index at the sensor surface. By using this biosensor assay not only quantitative and kinetic information can be obtained, but the thermodynamic state functions of the interactions as well [16,17] because the experiments are carried out at different temperatures.

In the present work, BSA-IBU composite NPs were prepared at pH 3.0 for pH-induced controlled drug release and kinetics of the ibuprofen release process at pH 7.4 was studied in *in vitro* experiments. Since the preparation of composite NPs was carried out at pH 3.0 and the drug release was measured at pH 7.4 the interactions between the protein and drug molecule were investigated at the above mentioned two pH values by using several 2D and 3D techniques in order to provide deeper information on the binding and release processes. SPR and SAXS measurements were carried out to study the size and the structure of the nanosized particles and to determine the binding capability of protein at different pH. The thermodynamic binding constant (K_b), the state functions (ΔG°, ΔH°, ΔS°) and also the stoichiometry of the interaction (n) were determined by ITC [18], while the rate of association and dissociation and the K_A and K_D standard equilibrium constants were calculated by fitting of the SPR sensorgrams. The calculated kinetic constants obtained by SPR in 2D systems were compared with the results of the IBU release process measured in aqueous solution (3D).

2. Materials and methods

2.1. Materials

All chemicals and solvents were of analytical grade and were used without further purification. The BSA (fraction V), the IBU ($C_{13}H_{18}O_2$) and the components of the McIlvaine's buffer (pH 3.0) and the phosphate buffer (PBS, pH 7.4) were purchased from Sigma Aldrich, the sodium chloride (NaCl), the sodium sulphate (Na_2SO_4), the sodium hydroxide (NaOH) and the hydrogen chloride (HCl) from Molar Chemicals. The stock solutions were freshly prepared, using Milli-Q ultrapure water (18.2 MΩ cm at 25 °C).

2.2. Preparation of BSA-IBU nanocomposite particles

The studied nanosized protein-non steroidal anti-inflammatory (NSAID) composites were prepared according to the procedure published previously [19,20]. Briefly, 20 w/v% BSA was dissolved in 15 ml buffer solution (McIlvaine buffer, pH 3.0). When it completely dissolved IBU molecules were added to the BSA solution with continuous stirring in 1:1 and 1:10 molar ratios. We have stirred the solution for two additional hours at room temperature in order to form the BSA-IBU nanocomposites since more and more drug binding results more and more dissolved drug molecules. The BSA-IBU NPs were precipitated by 2 M Na_2SO_4. The product was obtained by freeze drying (lyophilized) after centrifugation (15000 rpm, 15 min).

2.3. HRTEM, DLS and SAXS measurements

HRTEM images were taken by a FEI Tecnai G^2 20 X-TWIN microscope with tungsten cathode at 200 kV. The parallel DLS measurements were performed with a Horiba, Nanopartica SZ-100 Nanoparticle Analyzer (He-Ne laser with 532 nm wavelength) in order to determine the size of NPs. Small angle X-ray scattering (SAXS) were used to analyze the morphology, size and inner structure of the prepared composites and also to study the conformation change of BSA at acidic and neutral pH values. SAXS curves were recorded with a slit-collimated Kratky compact small-angle system (KCEC/3 Anton-Paar KG, Graz, Austria) equipped with a position-sensitive detector (PSD 50 M from M.Braun AG. Munich, Germany). Cu Kα radiation was generated by a Philips PW1830 X-ray generator operating at 40 kV and 30 mA.

2.4. SPR investigations

SPR measurements were carried out to determine the size and the orientation of BSA adsorbed on the gold chip, and the binding capability of IBU on the BSA-functionalized gold surface at pH 3.0 and pH 7.4. A two-channel SPR sensor platform developed at the Institute of Photonics and Electronics (Prague) was used. The SPR chip is a thin gold layer (50 nm thick) deposited on a glass substrate. During investigations, a flow rate of 25 μl min^{-1} was applied at constant temperature (+20 ± 0.1 °C). The interaction of IBU with the BSA was studied in the concentration range 5.0–40.0 μM in PBS solution at pH 3.0 and 7.4 under physiological conditions