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Characterization of drug-binding levels to serum albumin using a wavelength modulation surface plasmon resonance sensor

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Abstract

The drug binding to serum albumin is an area of intense research in evaluating drug candidates. A simple method based on a wavelength modulation surface plasmon resonance (SPR) sensor was developed for determination of the interaction for a drug and serum albumin. Seven kinds of drugs with different molecular masses and affinities were studied to illustrate the benefits of the wavelength modulation SPR sensor. With a known concentration of a drug, the percentage of the drug binding to human serum albumin (HSA) or bovine serum albumin (BSA) was then determined. The %bound values determined with a SPR sensor were compared with those obtained by other methods. In addition, the reproducibility and stability of the serum albumin immobilized on the sensor surface were studied under the experiment conditions. This method provides detailed information on affinities of the drug binding to serum albumin. The equilibrium constants of reactions for the HSA and penicillin V K, cefoperazone, cefotaximum natricum, oxacillin, amoxicillin, enoxacin, and norfloxacin, were 72.2 ± 3.0, 134 ± 21, 166 ± 25, 261 ± 63, 575 ± 38, 641 ± 36, and $980 \pm 16\ \mu mol\ L^{-1}$, respectively, while the equilibrium constants of reactions for the same seven drugs were 112 ± 3.0, 134 ± 19, 161 ± 23, 261 ± 18, 580 ± 85, 558 ± 19, and $675 \pm 31\ \mu mol\ L^{-1}$, respectively. The binding percentages range from $90.0 \pm 2.2\%$ to $40.8 \pm 5.2\%$ for drugs binding HAS, and from $85.9 \pm 1.2\%$ to $50.4 \pm 2.8\%$ for drugs binding BSA. These results illustrate how the wavelength modulation SPR sensor can be applied to the study of interaction of the small molecular drugs and serum albumin in real time.
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Keywords: SPR; Wavelength modulation; HSA; BSA; Drug; Affinity

1. Introduction

Serum albumin [1], the most abundant protein in the blood stream (accounting for about 60% of total plasma protein), is a major circulatory protein of known structure. The diversity of chemical functions presented at the surface of the protein has multiple lipophilic binding sites, which may combine with hydrophobic substances like drugs, especially neutral and negative charge lipophilic compounds [2]. Crystal structure analyses have revealed that the main drug binding sites on the human serum albumin (HSA) are located in subdomains IIA and IIIA. A large hydrophobic cavity is presented in the IIA subdomain. The geometry of the pocket in IIA is quite different from that found for IIIA. HSA has one tryptophan (Trp 214) in subdomain IIA. In addition, there are other sites where drugs (especially very small ones) can interact with HSA [3]. The bovine serum albumin (BSA) has two tryptophan moieties (Trp 135 and Trp 214), located in subdomains IA and IIA, respectively [4–8]. BSA is most commonly used in the study of the effect of the serum proteins on the P450 mediated metabolic reaction as well as in serum protein binding study.

Protein–drug binding plays an important role in pharmacokinetics (absorption, distribution, metabolism, and excretion of drugs in human body) and pharmacodynamics of the drug, because it affects both the activity of the drug and their disposition [9]. In the blood circulation, the presence of a drug has two forms: free and reversibly bound to a protein. It is widely accepted that the effect of a drug is related to the exposure of a patient to the free concentration of the drug in plasma rather than its total concentration [10]. Most drugs travel in plasma and reach the target tissue by binding to HSA. Therefore binding to HSA strongly influences the free drug concentration in plasma and has a large impact in the transfer of drugs to the tissue. Additionally, HSA allows solubilization of hydrophobic

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compounds, helps to a more homogeneous and buffers distribution of drugs thorough the body, and prolongs the duration of the drug action by preventing them from being metabolized. So, drug–HSA complexes in plasma serve as a reservoir for the free drug, as the free drug is easier to be removed from the body by various elimination processes. So the level of the drug binding to HSA is an important factor in the drug discovery processes. Quantitative investigation of drug–HSA binding is essential for the drug development and for determining the drug's safety in clinical. For the propose, a variety of techniques have been developed including high-performance affinity chromatography [3], capillary electrophoresis frontal analysis [11–13], a centrifugal ultrafiltration method [14], fluorescence spectroscopy [5,15,16], a chromatographic method [17,18], and equilibrium dialysis. Each technique has advantages and disadvantages. While these assays provide reliable binding constants, they require relatively large quantities of both drugs and proteins, grant low throughput, and cannot monitor drugs in real-time.

A recently developed surface plasmon resonance (SPR) technology could provide an ideal method to monitor the association and disassociation of molecules in real-time without the use of labels. With this technique, one biomolecule is immobilized on a SPR sensor surface and another partner is introduced in the flow system. Binding is easily monitored in this arrangement due to the large change in refractive index at the sensor surface. The disadvantages of this method are heterogeneity of the sensor surface and diffusional limitation. Both of these factors may affect the association and dissociation rate constants [18]. Therefore, the binding constants can be derived from the SPR signal at equilibrium to eliminate the effects of the two factors mentioned above. However, the equilibrium signal should be corrected for less of free analytes at low concentration of the analyte [18]. SPR spectroscopy has become a widely applied technique to study antibody–antigen, DNA–NDA, receptor–ligand, protein–protein interaction, as well as a large variety of biomolecular application involving high-molecular weight molecules [19–21]. Recently, a few reports were published on biospecific interaction analysis on low-molecular weight compounds. Gambari et al. [22] detected different interactions between DNA-binding drugs and target DNA sequences by a SPR sensor. Baird et al. [23] and Danelian et al. [24] studied interactions of the drug and liposome. This technology is rapidly gaining increasing importance in the food, pharmaceutical, biotechnology, and biomedical industry. Studying interactions of the drugs and HSA has been demonstrated by using SPR sensors [25–28]. However, the most commonly used SPR sensors are Biacore systems (Biacore AB). These authors demonstrated how Biacore could be used to characterize the affinity of the drug for HSA in high-resolution and high-throughput modes.

SPR sensing devices using Biacore AB are an angle modulation SPR sensor that is based on fixing a discrete excitation wavelength and measuring the angle shift of incident light, and therefore the SPR reflected spectra are shown in terms of reflected light intensity versus the angle of the incident light. In practice, the surface plasmons are excited by a convergent beam of monochromatic light. It makes use of a coupling prism coated with a thin gold film and performs the sensing by varying the incidence angle with a goniometer. Such an instrument is more expensive than the one described here.

In the work reported here, we applied a SPR sensor, which is installed in our laboratory for simultaneous multi-wavelength measurement [21], to determine affinity of drug molecules and serum albumin (HSA and BSA), and calculate their binding percentage. The wavelength modulation SPR sensor developed is based on fixing the incident angle and measuring the resonant wavelength. Therefore, the SPR spectra are shown in terms of the reflected light intensity versus the wavelength of the incident light. The intensity of the reflected light is the minimum at the resonant wavelength. A change in the concentration or kind of an analyte in the flow cell will lead to changes in the refractive index of the layer of the sensing membrane. Changes in the refractive index cause a shift of resonance wavelength and the shift of resonance wavelength, which is the biosensor response. There is no moving part in the wavelength modulation sensor. First of all, the sensor is measuring reflected light intensities at all wavelengths in the range of 500–900 nm simultaneously. That is to say, it can monitor reaction in real time. Secondly, the wavelength modulation SPR sensor holds more potential for miniaturization as well as coupling the reflected light beam into an optical fiber and transmitting it for analysis to a remote site.

By using seven kinds of drugs, we described a method for determining affinities of the drugs for HSA and BSA. In addition, we investigated the reproducibility and stability of the HSA and BSA surfaces created within the wavelength modulation SPR biosensor. Together, these results illustrate how the wavelength modulation sensor can be used to provide detailed information about the drug and serum albumin interactions.

2. Experimental

2.1. Reagents

HSA and BSA were obtained from Shanghai Biology Product Research Institute. 3-Mercaptopropionic acid (MPA) was purchased from Sigma (St. Louis, USA). 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and *N*-hydroxysuccinimide (NHS) were obtained from Shanghai Lizhu Dongfeng Biotechnology Co. All other chemicals were of analytical reagent grate. All the biological reagents were stored at 4 °C. All solutions were prepared with ultrapure water (>18.3 MΩ cm^{-1}).

Penicillin V K, amoxicillin, oxacillin, cefoperazone, cefotaximum natricum, norfloxacin, and enoxacin were purchased from China Drug Biology Product Identification Institution. The molecules structures of the seven kinds of drugs were shown in Fig. 1. All drugs are weighed on a precision analytical balance and dissolved in appropriate amounts of a buffer of phosphate-buffered saline (PBS) in a glass vial to give definite concentration solutions. This was the stock solution, which was then diluted successively with the PBS buffer to yield drug solutions for the actual assay. The drug concentration series were from 10 to 2000 μmol L^{-1}.

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