Structural and Ligand-Binding Properties of Serum Albumin Species Interacting With a Biomembrane Interface

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ABSTRACT: In the process of drug development, preclinical testing using experimental animals is an important aspect, for verification of the efficacy and safety of a drug. Serum albumin is a major binding protein for endogenous and exogenous ligands and regulates their distribution in various tissues. In this study, the structural and drug-binding properties of albumins on a biomembrane surface were investigated using reverse micelles as a model membrane. In reverse micelles, the secondary structures of all albumins were found, to varying degrees, to be intermediate between the native and denatured states. The tertiary structures of human and bovine albumin were similar to those of the native and intermediate states, respectively, whereas those of the dog, rabbit, and rat were in a denatured state. Thus, bovine albumin is an appropriate model for studying structural changes in human albumin in a membrane-water phase. Binding studies also showed the presence of species difference in the change in binding capacity of albumins during their interaction with reverse micelles. Among the albumins, rat albumin appears to be a good model for the protein-mediated drug uptake of human albumin in a biomembrane environment. These findings are significant in terms of the appropriate extrapolation of pharmacokinetics and pharmacodynamics data in various animals to humans. J Pharm Sci 96:3117–3124, 2007

Keywords: albumin; reverse micelles; species; protein-binding

INTRODUCTION

Human serum albumin (HSA) is the most abundant protein in blood plasma, and serves as transport protein for many endogenous and exogenous compounds.[1,2] HSA is known to contain three domains (domain I, II, and III) and at least two distinct binding sites for several physiologically important compounds and a large number of strongly bound drugs, namely Site I (localized within domain II), also denoted as the warfarin binding site, and Site II (localized within domain III), also denoted as the indole and benzodiazepine binding site.[1,2]

Among mammalian serum albumins, the amino acid sequences are known for human, monkey, bovine, rat, equine, ovine, pig, dog, and rabbit. The homologies among these albumins are greatly similar (70–80%).[1] However, it was found that mammalian serum albumins contain specific drug-binding sites: rabbit and rat albumins contain a drug-binding site, corresponding to Site I of human albumin, and dog albumin contains a specific drug-binding site corresponding to Site II on the human albumin molecule.[3,4] Such species differences in drug-binding sites could be due to microenvironmental differences in

Abbreviations: HSA, Human serum albumin; CD, Circular Dichroism

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multi-dimensions, such as the size and/or hydrophobicity of the drug-binding sites rather than the presence of different amino acid residues.

The hypothesis that the membrane transport of a drug depends on the concentration of the nonbound drug is widely accepted. However, because this hypothesis does not fully explain the mechanism of uptake of certain drugs, a protein-mediated uptake system has also been proposed.[5–7] In such a system, structural changes in the protein due to their interaction with the membrane surface decreases the drug-binding capacity. ESR spectroscopic findings, indicating that the structure of HSA changes after interaction with the hepatocyte surface supports the existence of a protein-mediated uptake system.[8] It was also recently reported that HSA interacts with endothelial and tubular epithelial cells.[9–12] Although Reed and Burrington[13] also reported that HSA can exist in two conformations on the cell surface, each of which has a different binding affinity for the cell, detailed structures have not been forthcoming, probably because of the unavailability techniques for the structural evaluation of albumin in the presence of cells. Thus, reverse micelles and liposomes are frequently used as biomembrane models to examine the mechanism of interaction between a protein and a membrane.[14–16] Desfosses et al.[17,18] reported that human and bovine albumins undergo structural changes during their interaction with reverse micelles, leading to a decrease in drug-binding capacities. However, whether species differences exist in the structural and functional characteristics of various albumins on membrane surface remains unknown.

At present, the effectiveness and safety of medical supplies to the market are confirmed on the basis of animal bioassays. After accurately confirming their effectiveness and safety by extrapolating the data to humans, a clinical trial for humans is then carried out. Thus, a precise understanding of species differences with respect to pharmacokinetic parameters is indispensable for the appropriate extrapolation and clinical trials in humans. Moreover, considering the fact that the binding of drugs to albumin has an important role in the pharmacokinetics and pharmacodynamics of drugs, it becomes important to investigate the species differences in the uptake of albumin-mediated drugs.

In the present study, we report on an investigation of the structural and functional characteristics of albumins from various species on interacting with a biomembrane model, reverse micelles, to obtain information concerning species differences in albumin-meditated ligand uptake.

MATERIALS AND METHODS

Materials

Human, bovine, dog, rabbit, and rat albumins, oxyphenbutazone, dansylsarcosine, and diocthylsulfosuccinate (AOT) were purchased from Sigma Chemical Co. (St. Louis, MO). Prior to experimental use, these albumins were defatted according to the method of Chen.[19] All other chemicals and solvents were of analytical grade.

Preparation of Reverse Micelles

A reverse-micelle solution was prepared as described by Desfosses et al.[18] After adding isooctane to the surfactant, water was added via a microsyringe, and the solution was shaken until it became clear. Using this solution, an albumin-reverse micelle solution was prepared. As an index of the size of the reversed-phase micelle, the ratio of water concentration to surfactant concentration was calculated and designated as the Wo value (Wo = [water]/[surfactant]). A value of Wo of 20 (possibly with one or two albumin molecules incorporated in a micelle) [AOT] = 100 mM (complete uptake of albumin to micelles) was used in this study, because the interaction of α_1-acid glycoprptein (AGP), an another important drug carrier protein, with reverse micelles was previously carried out using same experimental conditions at this laboratory.[20]

Circular Dichroism (CD) Spectra

Circular dichroism (CD) spectra were recorded with a JASCO J-720 spectropolarimeter, using 10 μM albumins in 20 mM phosphate buffer at pH 7.4. Near-UV spectra were recorded in a 10-mm path length cell, and in a 0.1-mm path length cell for far-UV spectra. Prior to recording spectra, samples were mixed and incubated for 30 min at room temperature.

Tryptophanyl Fluorescence Spectrum

Fluorescence was measured using a Jasco FP-770 fluorometer (Tokyo). The excitation wavelength of