PREFACE

Human Serum Albumin (HSA) is the most abundant plasma protein. It has been widely used for drug delivery systems and has recently emerged as a versatile carrier for therapeutic agents against diabetes, cancer and infectious diseases. This book provides an overview of the expanding field of preclinical and clinical applications and developments that use albumin as a carrier of drug delivery systems. The authors' discuss the properties of drug binding sites within the structure of HSA, discuss new possibilities for the therapeutic potential of HSA and analyze recently reported HSA-drug complexes including HSA-antibody conjugates. Novel investigations on the applications of albumin fusion proteins are discussed as well, with a focus on tumor targeting and intracellular delivery. Other chapters examine the different aspects of albumin glycation and oxidation, the changes in the structure of human serum albumin determined from infrared spectroscopy and a review of CAPIDAN, a special fluorescent dye, which attaches to drug binding sites of human serum albumin.

Chapter 1 – Human serum albumin has recently emerged as a versatile carrier for therapeutic agents against diabetes, cancer and infectious diseases. Market approved products include fatty acid derivatives of human insulin or the glucagon-like-1 peptide (Levemir®) for the treatment of diabetes, a long-acting injectable human interferon alpha2b fusion protein (Albuferon®) for the potential treatment of hepatitis C virus infections, and the taxol albumin nanoparticle Abraxane® for the treatment of metastatic breast cancer, advanced non-small cell lung cancer and advanced pancreatic cancer. In addition, an increasing number of albumin-based drugs are currently in clinical trials. These include an antibody fusion protein for treating HER2/neu positive breast cancer, and a recombinant fusion protein linking factor IX with albumin for the Prophylaxis of hemophilia B. In the preclinical setting, next generation approaches include albumin binding bioactive gases (bio-Gas) such as nitric oxide or hydrogen sulfide (*S*-Nitrosated Albumin analogs and Albumin Persulfide) for treating ischemic/reperfusion injury, cancer and bacterial infections. This review provides an overview of the expanding field of preclinical and clinical applications and developments that use albumin as a carrier of drug delivery systems.

Chapter 2 – Human Serum Albumin (HSA) has been widely used for drug delivery systems. HSA binds to variety of drugs resulting in HSA-drug complexes. HSA influences the pharmacokinetic and pharmacodynamic properties of drugs and the complexes have demonstrated potential for improving drug tracking and efficiency. This review discusses the properties of drug binding sites within the structure of HSA. The authors analyzed recently reported HSA-drug complexes including novel HSA-antibody conjugates. According to the

literature reviewed, they present an overview of the HSA-drug complexes that may potentially lead to significant clinical applications by improving drug efficiency and control of distribution and release rate of drug. Moreover, the authors discuss the fabrication of HSA drug delivery systems and the factors that optimize particle sizes and distribution and the effect of pH on the HSA-drug complexes. Also, the authors discuss delivery of HSA-anticancer drug complexes to receptors expressed on the cancer cells surfaces. Research concerning *in vitro* and *in vivo* techniques used for the HSA-drug complex monitoring is also presented in this chapter.

Chapter 3 – Human serum albumin (HSA) is the most abundant plasma protein. It has been extensively studied and applied in medicine and pharmaceutics. Taking advantage of the recombinant DNA technology, fusion proteins with novel functions have been generated. The recent approval of the albumin-GLP-1 fusion protein drug, Tanzeum, will encourage researchers to explore many more advantages of HSA for therapeutic applications. Generally, these albumin fusion proteins possess long serum half-life, good solubility, and versatile ligand binding ability. In addition to being used as drugs, albumin fusion proteins can serve as carriers for drug delivery to improve drug efficacy and safety. Herein, novel investigations on the applications of albumin fusion proteins will be discussed, with focus on tumor targeting and intracellular delivery. These approaches carry the potential to co-deliver multiple drugs to the same cellular target for synergistic therapeutic effects.

Chapter 4 – Human serum albumin (HSA) is synthesized predominantly in the liver, and has an extraordinarily long circulatory half-life (~19 days). Since HSA is responsible for 80% of the colloidal osmotic pressure of plasma, the patients with hypoalbuminemia are typically given a plasma expander to increase the plasma volume. Clinically, using HSA as an extender is preferable to other options, such as dextran, hydroxylethyl starch, due to its long retention in plasma. However, the circulatory half-life of HSA is significantly-impacted by structural changes of HSA and pathological conditions. Recent scientific advances have revealed new information regarding the factors that influence the pharmacokinetic properties of HSA. In this chapter, the authors provide an overview of the impact of HSA structure and biotransformation upon the pharmacokinetic properties of HSA, and discuss new possibilities for the therapeutic potential of HSA based on its pharmacokinetic properties.

Chapter 5 – Glycative and oxidative stress are frequently observed in numerous disease states. From recent studies, it has become evident that protein glycation and oxidation play major roles in the activity, unfolding, and degradation of proteins, as well as for cell function. Human serum albumin (HSA), the major protein in the blood circulation, frequently undergoes increased glycation and oxidation in major diseases such as diabetes and renal failure. This chapter examines the different aspects of albumin glycation and oxidation by focusing on the impact of these processes on the structural, biological and physiological modification of the protein. Further, the importance of glycated and oxidized albumin as a biological marker for many diseases such as diabetes and renal failure is discussed.

Chapter 6 – The effect of organic solvent on the hydration and structure of human serum albumin was characterized using infrared spectroscopy in the thermodynamic water activity range from 0 to 0.98 at 25°C. Dioxane was used as a model organic solvent. This organic solvent may be considered as an informative molecular probe for analyzing the effect of hydrogen bonding and hydrophobic interactions on the hydration and structure of proteins.

The obtained results show that the hydration and structure of human serum albumin depend markedly on how the protein has been hydrated – whether in the presence or in the

absence of organic solvent. Two organic solvent effects on water sorption by human serum albumin were observed: At low water activity (a_w<0.5), the water adsorption branch obtained in the presence of organic solvent is similar to that measured in the absence of dioxane. At high water activity (a_w>0.5), the water sorption isotherm in the presence of dioxane lies above the corresponding isotherm for pure water. This result corresponds to the organic solvent – assisted effect on water binding by human serum albumin. Changes in the structure of human serum albumin were determined from infrared spectra by analyzing the structure of amide I band. It was found that, at low water activity, the protein-protein contacts in the dried protein largely govern its thermodynamic and structural properties. At high water activity, the protein state is determined by the protein-organic solvent and protein-water interactions. The results from the thermodynamic and structural measurements were analysed to give a unified picture of the hydration process in the absence and presence of organic solvent.

Chapter 7 –CAPIDAN, a special fluorescent dye, binds to drug binding sites of human serum albumin. About 90-95 per cent of its fluorescence in serum or plasma originates from albumin-bound dye. This specificity opens a new way to study albumin in unfractionated serum saving intact albumin structure. Due to these properties, CAPIDAN has shown that a variety of diseases is accompanied by some changes of the drug-binding sites. This is a new source of information useful for clinics. That sensitivity to site abnormalities is raised by using time-resolved measurements of fluorescence decay and three new methods of the data analysis: (1) estimation of absolute values of concentration of fluorescent species in heterogeneous biological objects; (2) measurement of dye rotational mobility in heterogeneous albumin binding sites; and (3) method of chloride/nitrate substitution to detect interaction of a drug negative charge with arginine positive charges in the albumin binding sites.