Masaki Otagiri
Victor Tuan Giam Chuang *Editors*

Albumin in Medicine

Pathological and Clinical Applications

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Editors
Masaki Otagiri
Faculty of Pharmaceutical Sciences
Sojo University
Kumamoto, Japan

Victor Tuan Giam Chuang
School of Pharmacy,
Faculty of Health Sciences
Curtin University
Perth, WA, Australia

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Preface

Human serum albumin (HSA) is the most extensively researched plasma protein to date. Technological advancements in genetic engineering and molecular and structural biology have progressed in tandem with albumin research, especially in the area of applications, where rapid progress and development have resulted in massive favorable outputs for which albumin has clearly been proven to be a robust biomaterial.

Owing to its relatively long in vivo half-life of approximately 19 days, albumin is an attractive recombinant genetic fusion partner for extending the half-life of peptides and small proteins. The genetic modification of albumin also allows it to be applied to a wide variety of in vivo purposes including the targeting of specific types of cells or organs for the delivery of albumin-bound drugs. A recent landmark finding in the metabolism of HSA is the discovery of its pH-dependent interaction with the intracellular neonatal Fc receptor. The Fc receptor–albumin interaction can be intervened in a therapeutically useful manner to manipulate the half-life of albumin-bound drugs and albumin fusion proteins. The enormous ligand-binding properties of HSA can be applied in extracorporeal albumin dialysis, a procedure that involves the removal of toxins and drugs that are known to bind to albumin from the body via an external dialyzing solution that contains albumin.

This book summarizes medical and pharmaceutical applications of HSA in which current albumin-based products are presented in a significant number of chapters. The book is intended for use by pharmaceutical and medical scientists including pharmaceutical chemists, pharmacokineticists, toxicologists, and biochemists in both academia and the private sector.

We take this opportunity to thank all of the scientists who contributed to the successful publication of this book, and we hope that this work will provide useful insights that will stimulate further progress in the field of albumin research and development.

Kumamoto, Japan — Masaki Otagiri
Perth, Australia — Victor Tuan Giam Chuang
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Contents

Contributors

Jan Terje Andersen Centre for Immune Regulation (CIR) and Department of Immunology, Oslo University Hospital Rikshospitalet, Oslo, Norway

Department of Biosciences, University of Oslo, Oslo, Norway

Makoto Anraku Faculty of Pharmaceutical Sciences, Sojo University, Kumamoto-shi, Kumamoto, Japan

Malin Bern Centre for Immune Regulation (CIR) and Department of Immunology, Oslo University Hospital Rikshospitalet, Oslo, Norway

Department of Biosciences, University of Oslo, Oslo, Norway

Jason W. Castle General Electric Global Research, Niskayuna, NY, USA

Victor Tuan Giam Chuang School of Pharmacy, Faculty of Health Sciences, Curtin University, Perth, WA, Australia

Neil Desai Vice President, Strategic Platforms, Abraxis BioScience, A wholly owned subsidiary of Celgene Corporation, Los Angeles, CA, USA

Yu Ishima Department of Pharmacokinetics and Biopharmaceutics, Institute of Health Biosciences, The University of Tokushima, Tokushima, Japan

Yasunori Iwao Department of Pharmaceutical Engineering, School of Pharmaceutical Sciences, University of Shizuoka, Shizuoka, Japan

Alex Jackson GE Healthcare, Life Sciences, The Grove Centre, Amersham, Buckinghamshire, UK

Christina K. Kalli GE Healthcare, Life Sciences, The Grove Centre, Amersham, Buckinghamshire, UK

Teruyuki Komatsu Department of Applied Chemistry, Faculty of Science and Engineering, Chuo University, Tokyo, Japan

Ulrich Kragh-Hansen Department of Biomedicine, University of Aarhus, Aarhus C, Denmark

Peng Lei Centre for Immune Regulation (CIR) and Department of Immunology, Oslo University Hospital Rikshospitalet, Oslo, Norway

Department of Biosciences, University of Oslo, Oslo, Norway

Toru Maruyama Department of Biopharmaceutics, Graduate School of Pharmaceutical Sciences, Kumamoto University, Chuo-ku, Kumamoto, Japan

Center for Clinical Pharmaceutical Sciences, School of Pharmacy, Kumamoto University, Chuo-ku, Kumamoto, Japan

Jeannette Nilsen Centre for Immune Regulation (CIR) and Department of Immunology, Oslo University Hospital Rikshospitalet, Oslo, Norway

Institute of Clinical Medicine, University of Oslo, Oslo, Norway

Masaki Otagiri Faculty of Pharmaceutical Sciences, Sojo University, Kumamoto, Japan

Kine Marita Knudsen Sand Centre for Immune Regulation (CIR) and Department of Immunology, Oslo University Hospital Rikshospitalet, Oslo, Norway

Department of Biosciences, University of Oslo, Oslo, Norway

Inger Sandlie Centre for Immune Regulation (CIR) and Department of Immunology, Oslo University Hospital Rikshospitalet, Oslo, Norway

Department of Biosciences, University of Oslo, Oslo, Norway

Adrian Smith GE Healthcare, Life Sciences, The Grove Centre, Amersham, Buckinghamshire, UK

Hiroshi Watanabe Department of Biopharmaceutics, Graduate School of Pharmaceutical Sciences, Kumamoto University, Chuo-ku, Kumamoto, Japan

Center for Clinical Pharmaceutical Sciences, School of Pharmacy, Kumamoto University, Chuo-ku, Kumamoto, Japan

Keishi Yamasaki Faculty of Pharmaceutical Sciences, Sojo University, Kumamoto-shi, Kumamoto, Japan

Chapter 1
Human Serum Albumin: A Multifunctional Protein

Ulrich Kragh-Hansen

Abstract Human serum albumin is synthesized in the liver and continuously secreted into the bloodstream. Several receptors are strongly involved in the following distribution and metabolism of the protein. The receptor-albumin interactions can be modified by specific mutations, a finding which could be of pharmaceutical and medical interest.

The largest pool of albumin is found in the extravascular spaces although at a lower concentration than in the bloodstream. The higher concentration in the circulation is the main contributor to plasma's colloid osmotic pressure and to the Gibbs-Donnan effect in the capillaries.

Albumin seems to be the quantitatively most important circulating antioxidant, and it has enzymatic properties which are so pronounced that they most probably are of biological importance. The protein's ability to bind ligands and thereby to serve as an important depot and transport protein for numerous endogenous and exogenous compounds is well studied. Recent work has given much new information about the location and structure of binding sites and about potential ligand interactions. Structural information is also useful when designing new drugs whether the aim is to avoid binding or to make use of the protein's depot function. Nonbinding therapeutics can get improved stability and benefit from the long biological half-life of albumin by forming complexes with it. The complex formation can take place by enriching the therapeutic with an organic molecule which can bind reversibly or covalently to the protein. If the therapeutic is a polypeptide or protein, fusion proteins can be produced.

Albumin also shows promises for targeted drug delivery. This process can be passive and based on the enhanced permeability and retention effect. The effect can be increased by using dimers, polymers, or albumin-based nanoparticles. The targeting process can also be active and based on an interaction between albumin carrying a targeting ligand and cellular receptors.

Keywords Albumin-receptor interactions • Ligand binding • Stability • Half-life • Drug targeting

U. Kragh-Hansen (✉)
Department of Biomedicine, University of Aarhus,
Ole Worms Allé 6, Building 1180, DK-8000 Aarhus C, Denmark
e-mail: ukh@biomed.au.dk

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1.1 Introduction

Human serum albumin (HSA) is a multifunctional protein exclusively synthesized by liver hepatocytes and continuously secreted into the circulation. Here, it is the most abundant protein and comprises 60–65 % of total plasma protein. Many observations propose the existence of an important link between the concentration of HSA and health (Peters 1996). Due to a large number of acidic (98 Glu + Asp) and basic residues (83 Lys + Arg), the protein is highly soluble in aqueous media. Thus, its concentration in plasma is ca. 0.6 mM (4 % w/v), but solutions of 20 % can be made for clinical use. Actually, it is possible to make preparations of up to 50 % (Peters 1996). The presence of the many titratable amino acid residues also implies that HSA has an important buffering capacity. The uneven content of acidic and basic residues results in a net charge of ca. −15 at physiological pH, a fact that renders HSA important for the Donnan effect in the capillaries. Finally, under certain circumstances, the protein can serve as a source of amino acids or energy.

1.2 Synthesis and Structure

HSA is a member of the albumin superfamily, which also includes the transport proteins α-fetoprotein, vitamin D-binding protein (Gc-globulin), and afamin (α-albumin) (Kragh-Hansen et al. 2013). In addition, the superfamily includes the α-fetoprotein-related gene, but due to multiple mutations, this gene is an inactive pseudogene in humans. All the genes are single-copy genes, and the four active ones in the human are expressed in a codominant manner, ie, both alleles are translated. The genes lie on chromosome 4, near the centromere for the long arm, at position 4q11−13. The albumin gene is 16,961 nucleotides long from the putative cap site to the first poly(A) addition site. It is split into 15 exons that are symmetrically placed within the three domains thought to have arisen by triplication of a single primordial domain.

The mRNA for HSA encodes for a precursor protein (pre-pro-albumin) of 609 amino acids. The N-terminal pre-peptide of 18 amino acids guides the nascent albumin peptide chain from the ribosome, where it was synthesized, through a receptor on the membrane of the endoplasmic reticulum into the lumen of the reticulum. Afterward, it is rapidly cleaved off. The N-terminal, basic pro-peptide of six amino acids, is cleaved in one of the last steps before secretion of the mature protein into the space of Disse and the hepatic sinusoid. Thus, HSA consists of 585 amino acids; the molecular mass is ca. 66.5 kDa.

Normally, wild-type pro-albumin is not secreted from the liver cells in a detectable amount. However, it can be found in the circulation in certain pathological conditions (Kragh-Hansen et al. 2013).

HSA is produced as a simple, monomeric protein, ie, without prosthetic groups and covalently bound lipid or carbohydrate. The three-dimensional structure of the single polypeptide chain, and of its recombinant version (rHSA), has been determined crystallographically, and the structure is now known to a resolution of 2.3 Å (He and Carter 1992; Sugio et al. 1999; Hein et al. 2010). The polypeptide chain forms a heart-shaped protein with approximate dimensions of 80 × 80 × 80 Å and a thickness of 30 Å. It has about 67 % α-helix but no β-sheet and can be divided into three homologous domains (I–III). Each of these is comprised of two subdomains (A and B). The A and B subdomains have six and four α-helices, respectively, connected by flexible loops. All, but one, Cys34, of the 35 cysteine residues are involved in the formation of 17 stabilizing disulfide bonds. Small-angle X-ray scattering studies of HSA in solution show general agreement with the crystal structure (Olivieri and Craievich 1995). Also, a combined phosphorescence depolarization-hydrodynamic modeling study has proposed that the overall conformation of HSA in neutral solution is very similar to that observed in crystal structures (Ferrer et al. 2001).

In addition to HSA, the crystal structure of albumin from cattle, horse, rabbit, and hare has been determined (Bujacz 2012; Majorek et al. 2012). Although a number of differences were found in the binding pockets, as well as variations in surface structure and charge distribution, structural alignments of the crystal structures with HSA showed strong structural similarities between the albumins. This finding is probably mainly due to a conserved set of disulfide bridges.

At present, 70 mutations of the HSA gene are known which result in a circulating variant of pro-albumin or albumin (alloalbumins) (Kragh-Hansen et al. 2013; The Albumin Website). Because both alleles of the gene are translated, most genetic variants have been detected in heterozygotes, ie, in persons having both a variant and wild-type (normal) HSA. In addition to single-amino acid substitutions, glycosylated variants, N-terminally and C-terminally modified alloalbumins, have been found. Mutations can also compromise the protein synthesis to such an extent that HSA is completely absent or strongly decreased in affected individuals leading to the condition known as analbuminemia. To date, 22 such molecular defects have been reported (Minchiotti et al. 2013; The Albumin Website).

Because alloalbumins do not seem to be associated with disease, they can be used as markers of migration and provide a model for study of neutral molecular evolution. They can also give valuable molecular information about binding sites, antioxidant and enzymatic properties, as well as in vivo and in vitro stability. Mutants with increased affinity for endogenous or exogenous ligands could be therapeutically relevant as antidotes, both for in vivo and extracorporeal treatment. Variants with modified biodistribution could be used for drug targeting. In most cases, the desired function can be further elaborated by producing site-directed, recombinant mutants.

1.3 Distribution and Circulatory Half-Life

HSA is solely synthesized in the liver. By contrast, its sites of degradation are widespread. Most of the protein is hydrolyzed in the muscle and skin, but some leaks into the gut, some is taken up by Kupffer cells of the liver, and a small amount is degraded elsewhere or lost with shed dermis, saliva, sweat, tears, or milk (Fig. 1.1) (Peters 1996).

In healthy adults, ca. 13.8 g is made per day and secreted into the bloodstream. This amount corresponds to ca. 25 % of the protein synthesis activity of the liver. However, under physiological circumstances, only 20–30 % of the hepatocytes produce albumin, and synthesis can therefore be increased on demand by a factor of 200–300 % (Evans 2002). From the liver, HSA is distributed in the bloodstream but also to several extravascular spaces, some of which are poorly accessible; these are mainly found in the skin (Fig. 1.1). The total amount of HSA in the body is ca. 360 g, of which about two-thirds is outside the bloodstream and about one-third is in the bloodstream. However, the concentration of HSA is higher in the bloodstream, and that is why the protein can contribute with ca. 80 % of the colloid osmotic pressure of plasma (ca. 15 mm of Hg).

HSA leaves the intravascular space in different ways. For example, fenestrated capillaries and, especially, sinusoidal capillaries allow the protein to pass. The latter are mainly found in the liver and spleen but also in bone marrow, lymph nodes, and adrenal glands. In other situations, the escape is transcellular and mediated by a receptor.

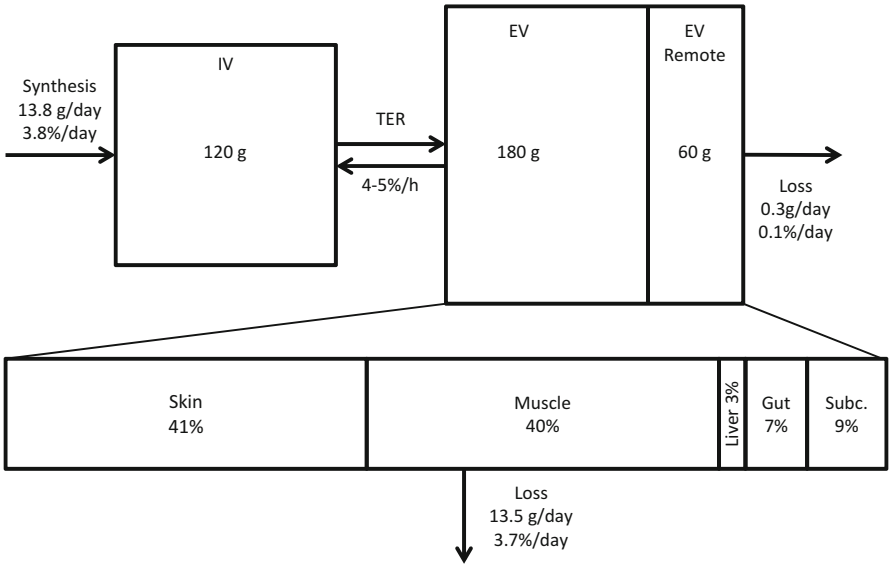

Fig. 1.1 Distribution and dynamics of HSA in a healthy person of 70 kg. *IV* intravascular, *EV* extravascular, *TER* transcapillary escape rate, *subc*, subcutaneous (The illustration is based on information found in Peters (1996))

Thus, the protein can interact with the receptor gp60 (60-kDa glycoprotein), also called albondin, situated in the plasma membrane of continuous endothelium (except for the brain) and alveolar epithelium (Sleep 2014; Merlot et al. 2014). Binding results in internalization of the complex by a caveolin-dependent endocytotic process and ultimately to transcytosis of albumin. It has been proposed that ca. 50 % of albumin leaves the capillary lumen in this way. Despite extensive studies, the gene for and the structure of gp60 are still unknown, and the molecular mechanisms of this transcytosis are still poorly understood.

HSA can most probably also leave the bloodstream by a process involving the intracellular receptor FcRn (neonatal Fc receptor) (Fig. 1.2a). This type of transcytosis is initiated by pinocytosis of HSA at the luminal membrane of the endothelial or epithelial cell (Bern et al. 2015). FcRn is placed in the membrane, but at physiological pH, there is no significant interaction between receptor and protein. After being taken up, HSA enters early endosomes. From there, the protein is transferred to acidified endosomes which have FcRn in their membrane, and at that pH (5–6), HSA binds strongly to FcRn. The endosomes with the protein-receptor complexes can now fuse with the basolateral side. This event results in exocytosis of HSA, because the pH of the cellular surroundings is neutral. Interestingly, IgG can be transcytosed by the same mechanism (Bern et al. 2015).

When albumin is saturated with fatty acids, transcytosis is two to three times higher than that of defatted albumin (Galis et al. 1988). The preferential transcytosis of cargo-carrying albumin is an interesting aspect, because it could help albumin to transport cargo into extravascular compartments.

HSA has an approximate plasma half-life of 19 days (Peters 1996). This half-life is extraordinary long for a circulating protein and is partly due to a return from the extravascular space to the circulation via the lymphatic system. The return amounts to 4–5 % of intravascular albumin per hour (Fig. 1.1), and the protein makes ca. 28 "trips" in and out of the lymphatic system during its lifetime (Peters 1996). Another contributing factor to the long circulatory half-life is that normally HSA is not lost in the urine. One reason for this is that, due to its size and charge, the filtration of HSA in the glomeruli is low. In addition, any protein filtered is reabsorbed in the proximal tubuli and transferred to the bloodstream via endocytosis by a receptor complex formed by cubilin and megalin (Merlot et al. 2014; Bern et al. 2015). Interestingly, results of animal studies have suggested an important role of FcRn for the renal retrieval of albumin (Sand et al. 2015; Bern et al. 2015).

FcRn is expressed in multiple cell types and tissues and is most important for the half-life of HSA, because it protects the protein (and IgG) from degradation in the lysosomes. Actually, FcRn rescues as much albumin as the liver produces (Bern et al. 2015). The initial steps in the protection are similar to those leading to transcytosis (Fig. 1.2a): the protein is taken up by the cell in question by pinocytosis and ends up as FcRn-bound in acidified endosomes. Now, in the present situation, the endosomes migrate to the membrane, where HSA originally was taken up, and release it by exocytosis. Thus, HSA is recycled back to the circulation. Albumin that does not bind to FcRn in the acidified endosomes, because it is conformationally

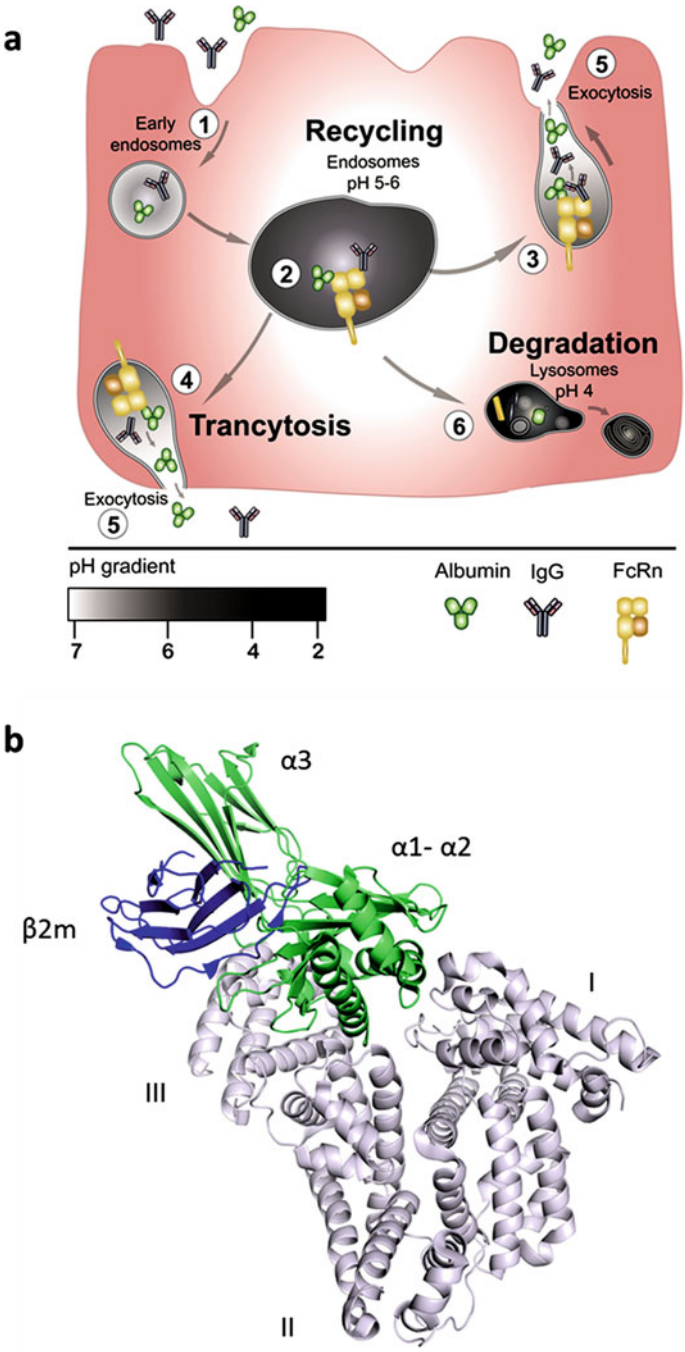

Fig. 1.2 (**a**) pH-dependent, FcRn-mediated cellular transport of HSA (and IgG). Initially, HSA is taken up by pinocytosis from the blood or luminal space of the cell and enters early endosomes (step *1*). From there it is transferred to acidified endosomes having FcRn in the membrane. At this pH of 5–6, the protein binds strongly to the receptor at a 1:1 stoichiometry (step *2*). At this point, three possible fates seem to exist for HSA. First, it can be exocytosed back to the blood or

modified or in surplus, will neither be transcytosed nor recycled but will be destined for lysosomal degradation.

In contrast to the other receptors interacting with albumin, the crystal structure of FcRn, and of its complex with HSA, is known (Fig. 1.2b). This detailed information is of great pharmaceutical and pharmacological interest, because on the basis of this knowledge, it is possible to construct albumin mutants with modified affinities for the receptor (Sand et al. 2015; Bern et al. 2015). Thus, it should be possible, for example, to design albumins with improved recycling and thereby increased circulatory half-life. Such a possibility is of great clinical impact, because in addition to increase the half-life of albumin itself, it should also increase very much the half-life of any bound cargo. An increased affinity for FcRn could perhaps also facilitate transcytosis of HSA with or without bound drugs or therapeutics. These possibilities are increasingly explored, but the influence of other factors has to be addressed. For example, the affinity of albumin for FcRn is strongly species dependent, a fact that has to be taken into account when using laboratory animals in preclinical investigations. In addition, engineered HSA mutants may become immunogenic, and the albumin-FcRn interaction could be influenced by protein-bound drugs or therapeutics. Thus, although very promising, there is still a long way to go before manipulating the interaction with FcRn can be used in the clinic.

1.4 Binding to Other Receptors

Several types of cancer secrete a glycoprotein known as secreted protein acidic and rich in cysteine (SPARC). Among its functions is albumin binding, a function which results in enhanced accumulation of albumin in the tumors, but apparently not in increased uptake of the protein into the tumor cells (Merlot et al. 2014). The albumin-receptor binding could also result in accumulation of albumin-bound drugs and therapeutics in the tumors and thereby result in better therapeutic effects. For example, the response of human head and neck cancers to nab-paclitaxel (nanoparticle albumin-bound paclitaxel) was reported to correlate with SPARC expression (Desai et al. 2009).

Fig. 1.2 (continued) luminal space from where it was taken up. Such an event results in recycling of the protein (steps *3* and *5*). Second, the exocytosis can take place at the basolateral membrane resulting in transcytosis (steps *4* and *5*). Finally, HSA which does not bind to FcRn in the acidified endosomes goes to degradation in the lysosomes (step *6*). Whether the HSA-FcRn complexes go to the luminal or to the basolateral membrane depends on, among other factors, binding of intracellular proteins such as adaptor protein-2 and calmodulin to sorting motifs of the cytoplasmic tail of the α-3 subunit (Sand et al. 2015). Principally the same mechanisms exist for IgG. IgG and HSA bind independently and noncooperatively to FcRn (The illustration is a modification of Fig. 2 in Bern et al. (2015).). (**b**) Crystal structure of the HSA-FcRn complex (Oganesyan et al. 2014). The domains (I–III) of HSA are indicated. FcRn is composed of a long α-chain of 44 kDa (domains α1–α3) and a short β2-microglobulin unit of 12 kDa (β2m). Of these structures, only α3 has a transmembrane fragment and a cytosolic part. Domain III of HSA plays the essential role for binding, but domain I is also necessary. The figure was made with PyMOL on the basis of the atomic coordinates (PDB ID: 4N0F) available at the RCSB Protein Data Bank

The membrane-bound receptors gp18 (18-kDa glycoprotein) and gp30 (30-kDa glycoprotein) are widely distributed scavenger receptors which bind and internalize chemically modified albumin. Thus, the receptors recognize damaged or changed albumin and target it for lysosomal degradation (Merlot et al. 2014).

1.5 Clinical and Pharmaceutical Uses

Huge amounts of HSA, ca. 500 ton per year worldwide, are used for improving clinical conditions such as shock, burns, trauma, surgical blood loss, hypoalbuminemia, decompensated cirrhosis, cardiopulmonary bypass, and acute respiratory distress (Mendez et al. 2005). HSA can also be used as a part of a hemodialysis regimen especially in patients with hepatic failure. Several such systems exist, but the molecular adsorbent recirculating system (MARS) is currently the most effective liver support device and can effectively remove protein-bound and water-soluble substances (Mitzner 2011). The efficiency of the system can most probably be increased by using tailor-made mutants of HSA itself or of one of its domains (Minomo et al. 2013).

HSA is also widely used as a stabilizing agent in pharmaceutical and biological products like vaccines, recombinant therapies, drug formulations, and coatings for medical devices (Chuang et al. 2002). Furthermore, the protein is used as a component in serum-free cell culture media, as a component for imaging agents, and, possibly, for therapeutic apheresis where plasma exchange might be desirable (Chuang et al. 2002).

Traditionally, HSA is obtained by fractionating human plasma. The risk by using this approach is that such preparations can be contaminated with blood-derived pathogens, for which reason the preparations have to be heated at 60 °C for 10 h in the presence of sodium octanoate and N-acetyl-L-tryptophanate. Of these ligands, octanoate has the greatest stabilizing effect against heat, whereas the presence of N-acetyl-L-tryptophanate diminishes oxidation of the protein (Anraku et al. 2004). However, recent studies have revealed that N-acetyl-L-tryptophanate should be replaced by N-acetyl-L-methionine, because the latter is a superior antioxidant and protects HSA against light and thereby photo-irradiation (Kouno et al. 2014). Although this pasteurization procedure is very effective, the potential risk of the presence of pathogens still exists, and the associated screening costs are substantial. In addition, in several countries, supplies of human plasma are limited. Therefore, many and large efforts are being made to produce rHSA as a substitute. rHSA can be highly expressed in various hosts including bacteria, yeast, and transgenic animals and plants (Chen et al. 2013). Of these, yeast (*Pichia pastoris*) and plants (Asian rice) seem to be the most promising for large-scale production. However, challenges like purity and production costs have to be met. For example, HSA expressed in rice has extensive glycation which showed supplier-to-supplier and lot-to-lot variability (Frahm et al. 2014). Therefore, it has not yet been possible to do large-scale production for clinical uses. Nevertheless, rHSA is currently used for

different pharmaceutical purposes. This is the case with, for example, Recombumin® from Novozymes Biopharma, Albagen™ from New Century Pharmaceuticals, and recombinant human albumin from Akron Biotech and Sigma-Aldrich.

1.6 Ligand Binding

HSA serves as a depot protein and transport protein for numerous endogenous and exogenous compounds. In this way, the protein has a major impact on the pharmacokinetics and pharmacological effects of drugs (Yamasaki et al. 2013). Albumin binding can also affect the ligands in a more individual manner. Thus, binding can result in an increased solubility in plasma of the compound, in a reduced toxicity, or in protection of the ligand against oxidizing agents.

Although the ligand-binding properties of HSA are very versatile, it mainly binds organic anions and inorganic cations. This unique ability of the protein is due to a favorable combination of hydrophobic pockets and side-chain charges and to a pronounced flexibility, which, to a large degree, is caused by fairly long interdomain and intradomain polypeptide linkers and flexible loops.

Detailed molecular information about the binding sites is very helpful in the assessment of displacement effects. However, it should be born in mind that displacement effects may as well be caused by ligand-induced conformational changes of the protein. Structural information is also useful when designing new drugs whether the aim is to avoid binding or to make use of the protein's depot function.

For comprehensive tabulations of ligands, see the reviews of, for example, Kragh-Hansen (1981), Peters (1996), Kragh-Hansen et al. (2002), and Fanali et al. (2012). In the following, the known binding sites and regions for high-affinity binding will be presented.

1.6.1 N-terminal End

Cu^{2+} and Ni^{2+} are strongly bound in a square-planar ligand arrangement formed by the three N-terminal amino acids Asp1, Ala2 and His3. The metal ions are held tightly in a chelate ring involving the α-amino nitrogen of Asp1, the first two peptide nitrogens, and the N^{1} imidazole nitrogen of His3. Co^{2+} binds with a lower affinity to principally the same site in an octahedral environment but with the β-COO^{-} group of Asp1 and the ε-group of Lys4 axially contributing to the metal ion coordination sphere (Fanali et al. 2012; Bal et al. 2013).

Co^{2+} binding to HSA from patients with cardiac ischemia is diminished. Perhaps it is not the site at the N-terminal end but other sites which are affected (Bal et al. 2013). Anyway, the observation has led to the development of assays for the condition. However, albumin's affinity for Co^{2+} can also be affected by several other conditions (Gaze 2009).

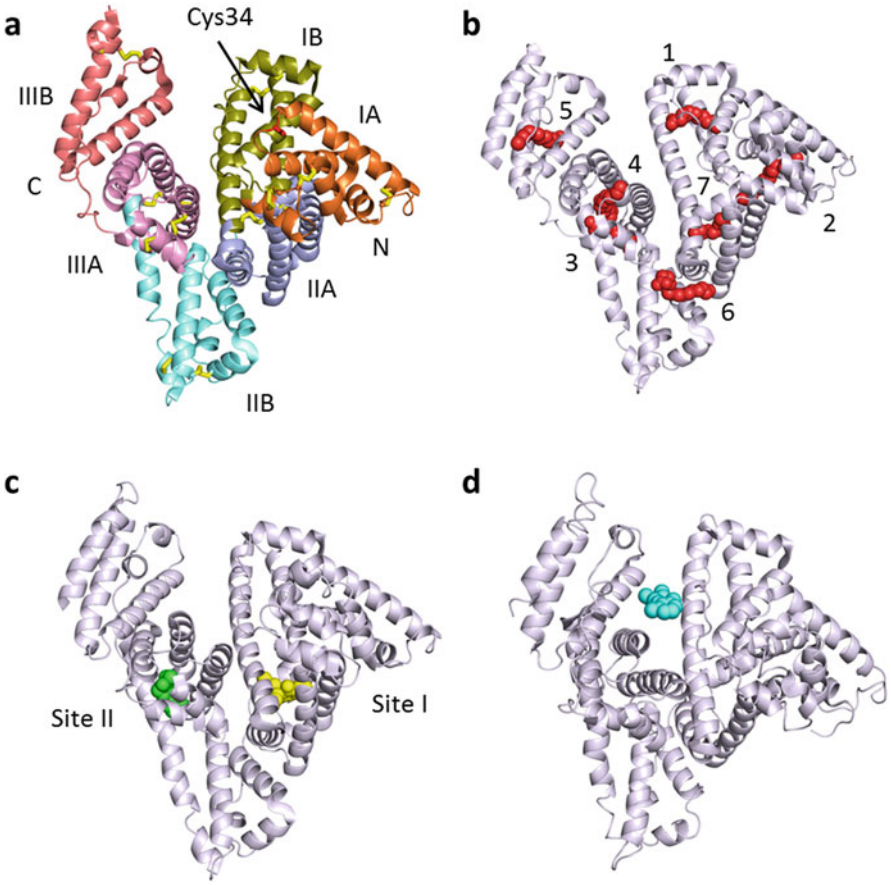

Fig. 1.3 (**a**) Crystal structure of HSA with the position of Cys34 indicated in *red* and the 17 disulfide bridges marked in *yellow*. The subdivision of the protein into domains (I–III) and subdomains (**a**, **b**) is shown. *N* and *C* represent the N-terminal and C-terminal ends, respectively. PDB ID: 1BM0. (**b**) Locations of the seven binding sites common to fatty acid anions using palmitate as an example (*red*). PDB ID: 1e7h. (**c**) Binding site for warfarin (PDB ID: 2bxd) in site I (*yellow*) and for diazepam (PDB ID: 2bxf) in site II (*green*). (**d**) Lidocaine binding (*magenta*) in the central, interdomain crevice. PDB ID: 3JQZ

1.6.2 Cys34

Cys34 in subdomain IA (Fig. 1.3a) is located in a crevice on the surface of the protein and does not participate in any disulfide bridges. Despite a limited accessibility, the sulfhydryl group can bind Hg^{2+}, Ag^{+}, Au^{+}, and Pt^{2+} (Fanali et al. 2012). The residue can also interact with nitric oxide and 8-nitro-cGMP and thereby form *S*-nitrosothiol and *S*-cGMP-HSA, respectively (Ishima et al. 2012a, b). In the circulation, about half of HSA has Cys34 coupled to low molecular weight thiols such as cysteine, glutathione, and homocysteine. The thiol group can also interact with

several drugs such as bucillamine derivatives, D-penicillamine, captopril, meso-2,3-dimercaptosuccinate, N-acetyl-L-cysteine, aurothiomalate, auranofin, and ethacrynate (Kragh-Hansen et al. 2002).

1.6.3 Subdomains IA and IIA

Palmitate has been reported to bind with a high affinity to an enclosed site located between subdomains IA and IIA (Simard et al. 2006). The methylene tail of the fatty acid anion binds in a nearly linear conformation within a narrow hydrophobic cavity formed by residues of the two subdomains, while the carboxyl forms specific salt bridge interactions with Tyr150, Arg257, and Ser287 (site 2 in Fig. 1.3b). Apparently, no other ligands, with the exception of other medium- and long-chain fatty acid anions, bind in this site.

HSA is the major Zn^{2+} transporter in plasma, and in the fatty acid-free protein, the metal ion binds primarily to an essentially preformed, 5-coordinate site located at the interface of domains I and II involving His67 and Asn99 from the former and His247 and Asp249 from the latter domain (Blindauer et al. 2009). The site is also a primary binding site for Cd^{2+} and a weak site for Cu^{2+} and Ni^{2+}. Therefore, the site is often called the multi-metal-binding site (Bal et al. 2013). Binding of a fatty acid to site 2 results in conformational changes which disrupt the site.

1.6.4 Subdomain IB

This subdomain houses a fairly large, L-shaped cavity with charged residues such as Arg117 and Arg186, capable of making hydrogen bondings, at its entrance (Zunszain et al. 2008). High-affinity binding of bilirubin and fusidic acid within this pocket causes only minor conformational changes in the site. By contrast, high-affinity binding of hemin and low-affinity binding of a fatty acid anion (site 1 in Fig. 1.3b) induce a significant conformational rearrangement of the subdomain. Wang et al. (2013) found that the subdomain is a major binding site for complex heterocyclic molecules such as the oncology agents camptothecin, 9-amino-camptothecin, etoposide, teniposide, bicalutamide, and idarubicin. The authors also observed that the large binding cavity has two access sites.

1.6.5 Subdomain IIA

The pioneering work of Sudlow et al. (1975), which was based on displacement of fluorescent probes, revealed that most drugs bind with a high affinity to one of two sites, called site I and site II. Of these, site I is placed in subdomain IIA (Fig. 1.3c).