Published in final edited form as:

Curr Pharm Des. 2015 ; 21(14): 1862–1865.

Review: Modifications of Human Serum Albumin and Their Binding Effect

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Abstract

Human serum albumin (HSA) regulates the transport and availability of numerous chemical compounds and molecules in the blood vascular system. While previous HSA research has found that HSA interacts with specific varieties of ligands, new research efforts aim to expand HSA's ability to interact with more different drugs in order to improve the delivery of various pharmacological drugs. This review will cover fatty acid chain and post-translational modifications of HSA that potentially modulate how HSA interacts with various pharmacological drugs, including glycation, cysteinylolation, S-nitrosylation, S-transnitrosation and S-guanylation.

Keywords

Human Serum Albumin

As one of the most abundant proteins in blood plasma, human serum albumin functions to transport hormones, fatty acids and various compounds through the blood stream. Additionally, human serum albumin also helps maintain osmotic blood pressure.^[1] Human serum albumin has the ability to interact with a variety of ligands, including exogenous pharmacological drugs. Current human serum albumin research aims to enhance albumin's role from an endogenous ligand transporter to also having a role in facilitating the transport of a variety of exogenous compounds. The ultimately goal is to utilize human serum albumin to improve drug delivery of novel pharmacological approaches to treat various human diseases.

Human serum albumin research has a long history. Its reported that human serum albumin was precipitated from urine as early as 1500 A.D.^[2] Before the 20th century, non-human serum albumin proteins were already being crystalized.^[3] Clinical use of human serum albumin occurred as early as the 1940's, when a surgeon by the name of I.S. Ravdin clinically administered purified human serum albumin to seven wounded human patients during the Pearl Harbor attack.^[1] Successfully, all seven patients survived. And then in 1992, Xiao He and Daniel Carter solved the three-dimensional atomic structure of human serum albumin using X-ray crystallography to 2.8 angstroms.^[4]

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Human serum albumin is synthesized and secreted from the liver. Human serum albumin is translated from a single gene as preproalbumin.^[5] The protein is imported into endoplasmic reticulum for cleavage of its N-terminal prepropeptide by a serine protease. Afterwards, the protein is transported to the Golgi before eventually being secreted out of the cell. The final molecular weight of human serum albumin is roughly 66,700 daltons.^[6]

Structurally, the human serum albumin protein is mostly composed of α -helices with an overall structure that resembles a heart shape. Human serum albumin has nine double loops spanning three homologous domains.^[7] The domains are named Domain I, II and III. Each domain has two long loops with one shorter loop. The first two loops in each domain are denoted as subdomain A. The remaining loop in each domain forms subdomain B. Thus, human serum albumin has subdomain IA and IB in Domain I, subdomain IIA and IIB in Domain II and subdomain IIIA and IIIB in Domain III. Subdomains with separate helical structures mediate human serum albumin binding with various endogenous and exogenous ligands. However, while the domains have similar structure, each domain has been shown to have different ligand-binding affinities and functions. Two important binding sites on human serum albumin are Sudlow sites I and II. Sudlow site I is located in subdomain IIA and Sudlow site II is located in subdomain IIIA. Sudlow site I has a preferential binding affinity for bulky heterocyclic compounds such as azapropazone, phenylbutazone and warfarin. Sudlow site II seems to preferentially bind to aromatic compounds such as ibuprofen.^{[8][9]}

Drug interaction with human serum albumin generally enhances the distribution and bioavailability of the drug depending on the specific pharmacokinetic properties of the drug molecules. Additionally, because of its abundance, human serum albumin plays a significant role in the pharmacokinetic behavior of a variety of drugs, including: drug half-life in the bloodstream, regulating drug efficacy, decreasing drug toxicity, and improving drug targeting specificity.^[10]

Human serum albumin-drug interaction can be modified by small molecules such as fatty acid chains. Human serum albumin can directly bind to certain fatty acid chains. Human serum albumin normally interacts with up to two moles of unesterified fatty acids during physiological conditions.^[11] Human serum albumin has seven long-chain fatty acid binding sites throughout its 3 domains. The first five fatty acid binding sites seem to have the amino acid residues to facilitate polar interactions with the carboxylate head of a fatty acid chain. Each site has a different affinity for fatty acids. Fatty acid binding sites #1-5 bind to the carboxylate moiety of fatty acids with electrostatic/polar interactions. Interestingly, increased levels of fatty acid chains such as linoleic acid have been shown to decrease human serum albumin's binding affinity for sulfonylurea drugs, with gliclazide having the most dissociation from human serum albumin.^[12]

Most drugs that interact with unmodified human serum albumin are anionic, few cationic drugs have been shown to have detectable affinity for human serum albumin. [8] With fatty acid chains, human serum albumin shows increased affinity for cationic drugs. One example of such a fatty acid is myristate, which has been shown to affect human serum albumin-ligand binding.^[13] The laboratory of Hong Liang recently explored the possibility of human

serum albumin as a carrier of cationic ligands, using myristate fatty acid chains modifications on human serum albumin at the subdomain IIA to mediate the interaction between cationic ligands and human serum albumin.^[14] Using a combination of fluorescence quenching and X-Ray crystallography, they find that extensive myristoylation of human serum albumin can stabilize the binding of cationic compound amantadine to human serum albumin in subdomain IIA. Further, their data suggests certain anionic drugs can function as potential cationic drug carriers for human serum albumin. While this is still a long way away from reaching clinical settings, this research provides potential method for simultaneous delivery of anionic and cationic drugs using human serum albumin.

Human serum albumin can also undergo post-translational modifications, some of which affect human serum albumin's ability to interact with drugs. Of all the amino acids, most modifications of human serum albumin seem to be on cysteine 34 in domain I. These modifications include glycation, cysteinylolation, S-nitrosylation, S-transnitrosation and S-guanylation (Table 1).

Human serum albumin can undergo post-translational glycation. Glycation is the binding of a protein with a sugar molecule without the assistance of an enzyme.^[15] On human serum albumin, these sites include Arginine 114, 218 and 428 as well as Lysine 186.^[16] Glycation has been reported to alter human serum albumin structure.^[17] Glycation of human serum albumin seems to affect the binding of some, but not all drugs. While initial reports suggest that glycation may affect human serum albumin's binding ability to drugs such as warfarin, later publications did not find this significant change.^[18,19,20,21] Other compounds such as tolazamide, acetohexamide, glibenclamide, and tolbutamide have at least a 40% decreased binding affinity to glycated human serum albumin compared to non-glycated human serum albumin.^[22,23] Glycated human serum albumin has been shown to have five-fold increase in binding activity to L-tryptophan compared to non-glycated human serum albumin.^[24] While increased glycation of human serum albumin is usually correlated with increased blood glucose levels, as seen in diabetic patients, a recent *in vitro* study suggests that Zinc concentrations may also regulate human serum albumin glycation.^[25] Additionally, glycated human serum albumin has drawn recent interest as a supplementary monitor for monitoring glycemic levels in diabetic patients. While blood glucose provides an immediate and transient measurement of blood glucose at the time of measurement, glycated human serum albumin stays present in the bloodstream for 2-3 weeks.^[26] Therefore, tools measuring glycated human serum albumin may function as better monitors for glycemic levels over longer periods of time.

Human serum albumin can also undergo cysteinylolation, or the addition of another cysteine to Cysteine-34 on human serum albumin via a disulfide bond.^[27] This modification has been suggested to be facilitated by cystathionine β -synthase (CBS), as CBS deficient mice lack cysteinylated human serum albumin.^[28] This modification was found in patients with liver and kidney diseases, as well as patients with diabetes. Cysteinylated human serum albumin levels correlate with high-risk pregnancies and uteroplacental insufficiency (UPI), suggesting that measuring cysteinylated human serum albumin levels may be advantageous to monitor pregnancies affected by UPI.^[29] Increased human serum albumin cysteinylolation has also been observed at the end stage of renal disease patients.^[30] While several studies

have elucidated the potential role of cysteinylated human serum albumin in patients with diseases, little is known about how cysteinylolation may affect human serum albumin's ability to interact with ligands. One study from the researchers of the Maruyama lab find human serum albumin's binding affinity to bilirubin and tryptophan, as well as drugs such as warfarin and diazepam, was significantly decreased.^[31]

Cysteine residues on human serum albumin can also undergo S-Nitrosylation or incorporation of nitric oxide.^[32] While nitrosating agents such sodium nitroprusside have low reactivity to the sulfhydryl groups in human serum albumin, S-nitrosoglutathione intermediates seem to interact with the sulfhydryl groups of albumin and facilitate S-nitrosylation.^[33] Currently, it is unclear how S-nitrosylation affects the binding of human serum albumin to its ligands. Nonetheless, a few papers offer some clues. S-nitrosylation of the Cys-34 on human serum albumin increases its affinity for Copper (II) and penolsulphophthalein (or PSP), both of which are normally circulated in the blood. This suggests S-nitrosylation may regulate the transport of organic anionic compounds and heavy metals through the circulatory system.^[34] A more recent paper published in 2008 by the Otagiri lab shows that S-nitrosylation of human serum albumin affects its ability to interact with fatty acids, specifically oleic acid.^[35] Mechanistically, they show when oleic acid binds to human serum albumin, it leads to greater accessibility of a single thiol group on albumin. They also conclude oleic acid fatty acid binding increases S-denitrosation and S-transnitrosation of S-nitrosylated human serum albumin. In addition to oleic acid, they find S-nitrosylated human serum albumin also strongly interacts with Bilirubin and weakly interacts with L-tryptophan, progesterone, ascorbate, Zinc, and iron.^[36] Besides Cys-34, Cys-410 has also been identified to undergo S-nitrosylation.^[35] Lastly, nitric oxide itself is a chemical messenger which is regulated by human serum albumin. The Nudler research laboratory shows that human serum albumin interacts with S-nitrosothiols, functioning as a nitric oxide sink to ultimately increase the formation of low-molecular weight S-nitrosothiols in order to regulate blood pressure.^[37]

Some new post-translational modifications of human serum albumin include S-guanylation and dehydroalanine conversion. S-guanylation of cysteine 34 is a recently reported modification of human serum albumin discovered when comparing blood samples between healthy patients and hemodialysis patients.^[38] S-guanylation modification occurs when an 8-nitroguanosine 3',5'-cyclic monophosphate group reacts with sulfhydryl groups of human serum albumin. While it is unclear how this modification may affect drug binding, research suggests that this protein may function as a endogenous antibacterial agent. Only minor structural conformational changes were observed with this modification. Dehydroalanine conversion is another recent post translational modification found on human serum albumin. Previously found in *in vitro* environments, cysteine residue conversion to dehydroalanine was found present in human patient blood plasma when healthy blood samples were compared to critically ill patient blood samples. However, it remains to be seen whether this modification significantly affects human serum albumin's biological function and its ability to interact with exogenous drugs.^[27]

In conclusion, while human serum albumin is a single non-glycosylated protein, accumulating research shows that more residues on human serum albumin can undergo

certain post-translational modification in specific environments. Human serum albumin can undergo a variety of modifications including: glycation, *S*-nitrosylation, *S*-guanylation, and dehydroalanine conversion. Current research shows that some of these modifications affect human serum albumin's ability to interact with exogenous drugs. Additionally, several reports show that fatty acids can impact how these post-translational modifications can regulate human serum albumin binding ability. Human serum albumin has an established role as a blood stream carrier, it will be interesting and exciting to test how these modifications to human serum albumin can affect and potentially help the drug delivery of various pharmacological treatments for different diseases.

Abbreviations

HSA	Human serum albumin
MYR	Myristate
FA	Fatty acids

References

1. Peters, T. All about albumin. Academic Press; 1995.
2. Pagel, W. Paracelsus. Karger; 1982. p. 161
3. Gurber A. Sitzber. Physik. Med. Ges. 1894
4. He X, Carter D. Atomic structure and chemistry of human serum albumin. Nature. 1992
5. Hawkins JW, Dugaiczky a. The human serum albumin gene: structure of a unique locus. gene. 1982; 19(1):55–8. [PubMed: 6292049]
6. Squire PG, Moser P, O'Konski CT. The hydrodynamic properties of bovine serum albumin monomer and dimer. Biochemistry. 1968; 7(12):4261–72. [PubMed: 5750167]
7. Dockal M, Carter DC, Ruker F. The three recombinant domains of human serum albumin: structural characterization and ligand binding properties. J Biol Chem. Oct 8; 1999 274(41):29303–10. [PubMed: 10506189]
8. Otagiri M. A molecular functional study on the interactions of drugs with plasma proteins. Drug Metab Pharmacokinet. 2005; 20(5):309–23. [PubMed: 16272748]
9. Sudlow G, Birkett DJ, Wade DN. Characterization of two specific drug binding sites on human serum albumin. Mol Pharmacol. 1975; 11:824–32. [PubMed: 1207674]
10. Sleep D, Cameron J, Evans LR. Albumin as a versatile platform for drug half-life extension. Biochim Biophys Acta. Elsevier B.V. Dec; 2013 1830(12):5526–34.
11. Cistola DP, Small DM. Fatty acid distribution in systems modeling the normal and diabetic human circulation. A ¹³C nuclear magnetic resonance study. J Clin Invest. Apr.1991 87:1431–41. [PubMed: 2010553]
12. Anguizola J, Basiaga S, Hage D. Effects of fatty acids and glycation on drug interactions with human serum albumin. Curr Metabolomics. 2013; 1(3):239–50. [PubMed: 24349966]
13. Fanali G, Bocedi A, Ascenzi P, Fasano M. Modulation of heme and myristate binding to human serum albumin by anti-HIV drugs. An optical and NMR spectroscopic study. FEBS J. Sep; 2007 274(17):4491–502. [PubMed: 17725715]
14. Yang F, Lee P, Ma Z, Ma LI, Yang G, Wu X, et al. Regulation of amantadine hydrochloride binding with IIA subdomain of human serum albumin by fatty acid chains. 2013; 102(1):84–92.
15. Spiro RG. Protein glycosylation: nature, distribution, enzymatic formation, and disease implications of glycopeptide bonds. Glycobiology. 2002; 12(4):43R–56R.

16. Ahmed N, Dobler D, Dean M, Thornalley PJ. Peptide mapping identifies hotspot site of modification in human serum albumin by methylglyoxal involved in ligand binding and esterase activity. *J Biol Chem*. Feb 18; 2005 280(7):5724–32. [PubMed: 15557329]
17. Nakajou K, Watanabe H, Kragh-Hansen U, Maruyama T, Otagiri M. The effect of glycation on the structure, function and biological fate of human serum albumin as revealed by recombinant mutants. *Biochim Biophys Acta - Gen Subj*. Oct; 2003 1623(2-3):88–97.
18. Baraka-Vidot J, Guerin-Dubourg A, Bourdon E, Rondeau P. Impaired drug-binding capacities of in vitro and in vivo glycated albumin. *Biochimie*. Sep; 2012 94(9):1960–7. [PubMed: 22627382]
19. Okabe N, Hashizume N. Drug binding properties of glycosylated human serum albumin as measured by fluorescence and circular dichroism. *Biol Pharm Bull*. 1994; 17:16–21. [PubMed: 8148809]
20. Jackson AJ, Anguizola J, Pfaunmiller EL, Hage DS. Use of entrapment and high-performance affinity chromatography to compare the binding of drugs and site-specific probes with normal and glycated human serum albumin. *Anal Bioanal Chem*. Jul; 2013 405(17):5833–41. [PubMed: 23657448]
21. Chiou YJ, Tomer KB, C. Smith P. Effect of nonenzymatic glycation of albumin and superoxide dismutase by glucuronic acid and suprofen acyl glucuronide on their functions in vitro. *Chem Biol Interact*. 1999; 121:141–59. [PubMed: 10418961]
22. Tsuchiya S, Sakurai T, Sekiguchi S. Nonenzymatic glucosylation of human serum albumin and its influence on binding capacity of sulfonylureas. *Biochem Pharmacol*. 1984; 33(19):2967–71. [PubMed: 6487349]
23. Ascoli GA, Domenici E, Bertucci C. Drug binding to human serum albumin: Abridged review of results obtained with high-performance liquid chromatography and circular dichroism. *Chirality*. 2006:667–79. [PubMed: 16823814]
24. Joseph KS, Hage DS. The effects of glycation on the binding of human serum albumin to warfarin and L-tryptophan. *J Pharm Biomed Anal*. Elsevier B.V. Nov 2; 2010 53(3):811–8.
25. Seneviratne C, Dombi GW, Liu W, Dain J a. The in vitro glycation of human serum albumin in the presence of Zn(II). *J Inorg Biochem*. Elsevier B.V. Dec; 2011 105(12):1548–54.
26. Cohen MP. Perspective: measurement of circulating glycated proteins to monitor intermediate-term changes in glycaemic control. *Eur J Clin Chem Clin Biochem*. 1992; 30:851–9. [PubMed: 1489860]
27. Bar-Or R, Rael LT, Bar-Or D. Dehydroalanine derived from cysteine is a common post-translational modification in human serum albumin. *Rapid Commun Mass Spectrom*. 2008; 22:711–6. [PubMed: 18265430]
28. Bar-Or D, Curtis CG, Sullivan A, Rael LT, Thomas GW, Craun M, et al. Plasma albumin cysteinylolation is regulated by cystathionine beta-synthase. *Biochem Biophys Res Commun*. Dec 24; 2004 325(4):1449–53. [PubMed: 15555590]
29. Bar-Or D, Heyborne KD, Bar-Or R, Rael LT, Winkler JV, Navot D. Cysteinylolation of maternal plasma albumin and its association with intrauterine growth restriction. *Prenat Diagn*. Mar; 2005 25(3):245–9. [PubMed: 15791656]
30. Regazzoni L, Del Vecchio L, Altomare a, Yeum K-J, Cusi D, Locatelli F, et al. Human serum albumin cysteinylolation is increased in end stage renal disease patients and reduced by hemodialysis: mass spectrometry studies. *Free Radic Res*. Mar; 2013 47(3):172–80. [PubMed: 23215783]
31. Nagumo K, Tanaka M, Chuang VTG, Setoyama H, Watanabe H, Yamada N, et al. Cys34-cysteinylated human serum albumin is a sensitive plasma marker in oxidative stress-related chronic diseases. *PLoS One*. Jan.2014 9(1):e85216. [PubMed: 24416365]
32. Gaston BM, Carver J, Doctor A, Palmer LA. S-nitrosylation signaling in cell biology. *Mol Interv*. 2003; 3(5):253–63. [PubMed: 14993439]
33. Park J. S-Nitrosylation of sulfhydryl groups in albumin by nitrosating agents. *Archives of Pharmacol Research*. 1993:1–5.
34. Kashiba-Iwatsuki M, Miyamoto M, Inoue M. Effect of nitric oxide on the ligand-binding activity of albumin. *Arch Biochem Biophys*. 1997; 345(2):237–42. [PubMed: 9308895]

35. Ishima Y, Sawa T, Kragh-Hansen U, Miyamoto Y, Matsushita S, Akaike T, et al. S Nitrosylation of human variant albumin Liprizzi (R410C) confers potent antibacterial and cytoprotective properties. *J Pharmacol Exp Ther*. 2007; 320(3):969–77. [PubMed: 17135341]
36. Ishima Y, Akaike T, Kragh-Hansen U, Hiroshima S, Sawa T, Maruyama T, et al. Effects of endogenous ligands on the biological role of human serum albumin in S-nitrosylation. *Biochem Biophys Res Commun*. Dec 28; 2007 364(4):790–5. [PubMed: 18273439]
37. Rafikova O, Rafikov R, Nudler E. Catalysis of S-nitrosothiols formation by serum albumin: the mechanism and implication in vascular control. *Proc Natl Acad Sci U S A*. 2002; 99(19):5913–8. [PubMed: 11983891]
38. Ishima Y, Hoshino H, Shinagawa T, Watanabe K, Akaike T, Sawa T, et al. S guanylation of human serum albumin is a unique posttranslational modification and results in a novel class of antibacterial agents. *Journal of Pharmaceutical Sciences*. 2012; 101(9):3222–3229. doi:10.1002/jps. [PubMed: 22488009]

Table 1

Human Serum Albumin Modifications

Modification	Notable sites	Location
Glycation	Lysine-525 Lysine-199 Lysine-233 Lysine-281 Lysine-438. (Many other glycation sites not listed).
Cysteinylation	Cysteine-34
Nitrosylation	Cysteine-34	(See Above)
Guanylation	Cysteine-34	(See Above)
Dehydralanine	Cysteine-487

Atomic Coordinates were taken from PDB entry 1E78. Illustration of Human Serum Albumin was made with PyMOL.