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Applications of Bovine Serum Albumin in Medical World

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ABSTRACT: Bovine serum albumin (BSA or "Fraction V") is a serum albumin protein derived from cows. It is often used as a protein concentration standard in lab experiments. The nickname "Fraction V" refers to albumin being the fifth fraction of the original Edwin Cohn purification methodology that made use of differential solubility characteristics of plasma proteins. By manipulating solvent concentrations, pH, salt levels, and temperature, Cohn was able to pull out successive "fractions" of blood plasma. The process was first commercialized with human albumin for medical use and later adopted for production of BSA.

KEYWORDS: BSA, serum, plasma, blood, temperature, protein, medical use, commercialized, purification, experiments

I.INTRODUCTION

The full-length BSA precursor polypeptide is 607 amino acids (AAs) in length. An N-terminal 18-residue signal peptide is cut off from the precursor protein upon secretion, hence the initial protein product contains 589 amino acid residues.¹ An additional six amino acids are cleaved to yield the mature BSA protein that contains 583 amino acids.^[1]

BSA has three homologous but structurally different domains². The domains, named I, II, and III, are broken down into two sub-domains, A and B.^[2]

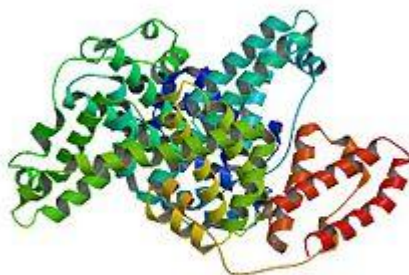
Peptide	Position	Length (AAs)	MW Da
Full length precursor	1 – 607	607	69,324
Signal peptide	1 – 18	18	2,107
Propeptide	19 – 24	6	478
Mature protein	25 – 607	583	66,463

Physical properties of BSA:

- Number of amino acid residues: 583
- Molecular weight: 66,463 Da (= 66.5 kDa)
- isoelectric point in water at 25 °C: 4.7^[3]

- Extinction coefficient of $43,824 \text{ M}^{-1} \text{ cm}^{-1}$ at 279 nm ^[4]
- Dimensions: $140 \times 40 \times 40 \text{ \AA}$ (prolate ellipsoid where $a = b < c$)^[5]
- pH of 1% Solution: 5.2-7^{[6][7]}
- Optical Rotation: $[\alpha]_{259}$: -61° ; $[\alpha]_{264}$: -63° ^{[6][7]}
- Stokes Radius (r_s): 3.48 nm ^[8]
- Sedimentation constant, $S_{20,W} \times 10^{13}$: 4.5 (monomer), 6.7 (dimer)^{[6][7]}
- Diffusion constant, $D_{20,W} \times 10^{-7} \text{ cm}^2/\text{s}$: 5.9 ^{[6][7]}
- Partial specific volume, V_{20} : 0.733 ^{[6][7]}
- Intrinsic viscosity, η : 0.0413 ^{[6][7]}
- Frictional ratio, f/f_0 : 1.30 ^{[6][7]}
- Refractive index increment (578 nm) $\times 10^{-3}$: 1.90 ^{[6][7]}
- Optical absorbance, $A_{279 \text{ nm}}^{1 \text{ g/L}}$: 0.667 ^{[6][7]}
- $\epsilon_{280} = 43.824 \text{ mM}^{-1} \text{ cm}^{-1}$ ^[9]
- Mean residue rotation, $[\text{m}']_{233}$: 8443 ^{[6][7]}
- Mean residue ellipticity: 21.1 $[\theta]_{209 \text{ nm}}$; 20.1 $[\theta]_{222 \text{ nm}}$ ^{[6][7]}
- Estimated α -helix, %: 54 ^{[6][7]}
- Estimated β -form, %: 18 ^{[6][7]}

BSA, like other serum albumins, is critical in providing oncotic pressure within capillaries, transporting fatty acids, bilirubin, minerals and hormones, and functioning as both an anticoagulant and an antioxidant.^[10] There are approximately 6 different long chain fatty acid binding sites on the protein, the three strongest³ of which are located one per each domain. BSA can also bind other substances such as salicylate, sulfonamides, bilirubin, and other drugs, which bind to “site 1” in subdomain IIA, while tryptophan, thyroxine, octanoate and other drugs that are aromatic in nature bind to “site 2” in subdomain IIIA.^[11]



Bos taurus (domestic cow)-from which bovine serum albumin is extracted⁴

BSA is often used a model for other serum albumin proteins, especially human serum albumin, to which it is 76% structurally homologous.^[12]

BSA has numerous biochemical applications including ELISAs (Enzyme-Linked Immunosorbent Assay), immunoblots, and immunohistochemistry. Because BSA is a small, stable, moderately non-reactive protein, it is often used as a blocker in immunohistochemistry.^[13] During immunohistochemistry, which is the process that uses antibodies to identify antigens in cells, tissue sections are often incubated with BSA blockers to bind nonspecific binding sites.^{[14][15]} This binding of BSA to nonspecific binding sites increases the chance that the antibodies will bind only to the antigens of interest.^[16] The BSA

blocker improves sensitivity by decreasing background noise as the sites are covered with the moderately non-reactive protein.^{[17][18]} During this process, minimization of nonspecific binding of antibodies is essential in order to acquire the highest signal to noise ratio.^[17] BSA is also used as a nutrient in cell and microbial culture⁵. In restriction digests, BSA is used to stabilize some enzymes during the digestion of DNA and to prevent adhesion of the enzyme to reaction tubes, pipette tips, and other vessels.^[19] This protein does not affect other enzymes that do not need it for stabilization. BSA is also commonly used to determine the quantity of other proteins, by comparing an unknown quantity of protein to known amounts of BSA (see Bradford protein assay). BSA is used because of its ability to increase signal in assays, its lack of effect in many biochemical reactions, and its low cost,⁶ since large quantities of it can be readily purified from bovine blood, a byproduct of the cattle industry. Another use for BSA is that it can be used to temporarily isolate substances that are blocking the activity of the enzyme that is needed, thus impeding polymerase chain reaction (PCR).^[20] BSA has been widely used as a template to synthesize nanostructures.^[21]

BSA is also the main constituent of fetal bovine serum, a common cell culture medium.⁷

II.DISCUSSION

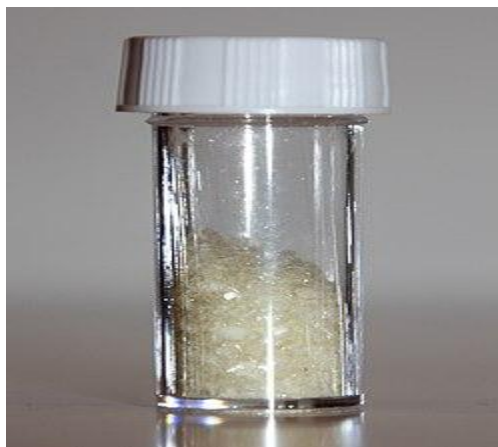
Serum albumin, often referred to simply as blood albumin, is an albumin (a type of globular protein) found in vertebrate blood. Human serum albumin is encoded by the ALB gene.^{[2][3][4]} Other mammalian forms, such as bovine serum albumin, are chemically similar.⁸

Serum albumin is produced by the liver, occurs dissolved in blood plasma and is the most abundant blood protein in mammals. Albumin is essential for maintaining the oncotic pressure needed for proper distribution of body fluids between blood vessels and body tissues; without albumin, the high pressure in the blood vessels would force more fluids out into the tissues. It also acts as a plasma carrier by non-specifically binding several hydrophobic steroid hormones and as a transport protein for heme and fatty acids. Too much or too little circulating serum albumin may be harmful. Albumin in the urine usually denotes the presence of kidney disease. Occasionally albumin appears in the urine of normal persons following long periods of standing (postural albuminuria).⁹

Albumin functions primarily as a carrier protein for steroids, fatty acids, and thyroid hormones in the blood and plays a major role in stabilizing extracellular fluid volume by contributing to oncotic pressure (known also as colloid osmotic pressure) of plasma.

Because smaller animals (for example rats) function at a lower blood pressure, they need less oncotic pressure to balance this and thus need less albumin to maintain proper fluid distribution.¹⁰

As an anionic protein, albumin binds readily to calcium in blood serum and contributes greatly to plasma calcium levels.¹¹ As such, in clinical applications it is necessary to adjust serum total calcium concentration upward or downward if hypoalbuminemia or hyperalbuminemia is present, respectively (measured serum total calcium decreases by 0.8 mg/dL per unit decrease in albumin concentration below 4 g/dL).^{[5][6]} Albumin is synthesized in the liver as prealbumin which has an N-terminal peptide that is removed before the nascent protein is released from the rough endoplasmic reticulum. The product, proalbumin, is in turn cleaved in the Golgi vesicles to produce the secreted albumin.^[4] Albumin is a globular, water-soluble, un-glycosylated serum protein of approximate molecular weight of 65,000 Daltons.¹²



Lyophilized Bovine Serum Albumin

Albumin (when ionized in water at pH 7.4, as found in the body) is negatively charged. The glomerular basement membrane is also negatively charged in the body; some studies suggest that this prevents the filtration of albumin in the urine. According to this theory, that charge plays a major role in the selective exclusion of albumin from the glomerular filtrate. A defect in this property results in nephrotic syndrome leading to albumin loss in the urine. Nephrotic syndrome patients are sometimes given albumin to replace the lost albumin.¹³

The general structure of albumin is characterized by several long α helices allowing it to maintain a relatively static shape, which is essential for regulating blood pressure.

Serum albumin contains eleven distinct binding domains for hydrophobic compounds. One heme and six long-chain fatty acids can bind to serum albumin at the same time.¹⁷

Serum albumin is widely distributed in mammals.¹⁴

- The human version is human serum albumin.
- Bovine serum albumin, or BSA, is commonly used in immunodiagnostic procedures, clinical chemistry reagents, cell culture media, protein chemistry research (including venom toxicity), and molecular biology laboratories (usually to leverage its non-specific protein binding properties).¹⁵

III.RESULTS

Plasma contains a large variety of proteins including albumin, immunoglobulins, and clotting proteins such as fibrinogen.¹¹ Albumin constitutes about 60% of the total protein in plasma and is present at concentrations between 35 and 55 mg/mL.¹² It is the main contributor to osmotic pressure of the blood and it functions as a carrier molecule for molecules with low water solubility such as lipid-soluble hormones, enzymes, fatty acids, metal ions, and pharmaceutical compounds.¹³ Albumin is structurally stable due to its seventeen disulfide bonds and unique in that it has the highest water solubility and the lowest isoelectric point (pI) of the plasma proteins.¹⁶ Due to the structural integrity of albumin it remains stable under conditions where most other proteins denature. Many of the proteins in plasma have important therapeutic uses.¹¹ Albumin is commonly used to replenish and maintain blood volume after traumatic injury, during surgery, and during plasma exchange.¹³ Since albumin is the most abundant protein in the plasma its use may be the most well known, but many other proteins, although present in low concentrations, can have important clinical uses.¹¹ When the ultimate goal of plasma processing is a purified plasma component for injection or transfusion, the plasma component must be highly pure. The first practical large-scale method of blood plasma fractionation was developed by Edwin J. Cohn during World War II. It is known as the Cohn process (or Cohn method).¹⁷ This process is also known as cold ethanol fractionation as it involves gradually increasing the concentration of ethanol in the solution at 5 °C and 3 °C.¹³ The Cohn Process exploits differences in properties of the various plasma proteins, specifically, the high solubility and low pI of albumin. As the ethanol concentration is increased in stages from 0% to 40% the [pH] is lowered from neutral (pH ~ 7) to about 4.8, which

is near the pI of albumin.^[3] At each stage certain proteins are precipitated out of the solution and removed. The final precipitate is purified albumin.¹⁸ Several variations to this process exist, including an adapted method by Nitschmann and Kistler that uses fewer steps and replaces centrifugation and bulk freezing with filtration and diafiltration.^{[1][3]}

Some newer methods of albumin purification add additional purification steps to the Cohn Process and its variations, while others incorporate chromatography, with some methods being purely chromatographic.^[3] Chromatographic albumin processing as an alternative to the Cohn Process emerged in the early 1980s, however, it was not widely adopted until later due to the inadequate availability of large scale chromatography equipment.^[3] Methods incorporating chromatography generally begin with cryodepleted plasma undergoing buffer exchange via either diafiltration or buffer exchange chromatography, to prepare the plasma for following ion exchange chromatography steps.^[3] After ion exchange there are generally further chromatographic purification steps and buffer exchange.^[3]

Lactalbumin, also known as "whey protein", is the albumin contained in milk and obtained from whey. Lactalbumin is found in the milk of many mammals. There are alpha and beta lactalbumins; both are contained in milk.

Targeted small scientific studies suggest that certain types of lactalbumin (whey protein) may improve immune responsiveness and increase levels of glutathione systemically in animals and which apparently possess antiviral (against viruses), anti-apoptotic (impede cell death) and anti-tumor (against cancers or tumors) activities in humans, but larger and better studies are needed to confirm these attributions.¹⁹

Ovalbumin (abbreviated OVA^[1]) is the main protein found in egg white, making up approximately 55% of the total protein.^[2] Ovalbumin displays sequence and three-dimensional homology to the serpin superfamily, but unlike most serpins it is not a serine protease inhibitor.^[3] The function of ovalbumin is unknown, although it is presumed to be a storage protein.^[4] Ovalbumin is an important protein in several different areas of research, including:²⁰

- general studies of protein structure and properties (because it is available in large quantities).
- studies of serpin structure and function (the fact that ovalbumin does not inhibit proteases means that by comparing its structure with that of inhibitory serpins, the structural characteristics required for inhibition can be determined).
- proteomics (chicken egg ovalbumin is commonly used as a molecular weight marker for calibrating electrophoresis gels).
- immunology (commonly used to stimulate an allergic reaction in test subjects; e.g., established model allergen for airway hyper-responsiveness, AHR).²¹

The ovalbumin protein of chickens consists of 385 amino acids, its relative molecular mass is 42.7 kDa,^[5] and it adopts a serpin-like structure.^[6] Ovalbumin also has several modifications, including N-terminal acetylation (G1), phosphorylation (S68, S344), and glycosylation (N292).^[5] It is secreted from the cell, targeted by an internal signal sequence (residues 21–47), rather than the N-terminal signal sequence commonly found in other secreted proteins. Ovalbumin's signal sequence is not cleaved off,¹⁶ but remains as part of the mature protein.^[7] When heated, ovalbumin undergoes a conformational change from its soluble, serpin structure into an insoluble all- β -sheet structure with exposed hydrophobic regions. This causes the protein to aggregate and cause the solidification associated with cooked egg white.^[8]

IV. CONCLUSIONS

In the context of the increasing incidence of drug resistance, the search for new therapeutic agents represents a continuous struggle, and metal complexes are among the promising types of molecules showing antibacterial, antiviral and antineoplastic potential. One of the crucial steps in developing a new and effective drug is studying and understanding its ability to bind to albumins, as they play a key role in its transport,²⁰ distribution, metabolism and excretion. With this in mind, we highlighted the essential notions related to bovine serum albumin from the point of view of its structure and functions, as well as the elements to be taken into consideration when designing metal complexes with protein binding abilities. Moreover, the most important instrumental techniques employed to study the type and strength of the metal

complexes – BSA interactions were discussed, as well as the type of information they provide: fluorescence spectroscopy and UV-Vis absorption spectroscopy.²¹

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