Chemistry behind Serum Albumin: A Review

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Abstract: This review informs about the chemical composition of plasma proteins majorly albumin and globulin. Blood proteins, also called plasma proteins, are found in blood plasma. While, serum proteins are present in the human body in very high quantities for other proteins. Hundreds of proteins are dissolved in the plasma but only two major protein groups are present i.e. Albumin and Globulin. Albumin is a very important component (55% of blood proteins) and it is made by the liver. There is an immediate correlation between albumin turnover and body size. Globulin is formed from different proteins called alpha, beta, and gamma types (38% of blood proteins) but a number of the globulins are mainly made by the liver, while others are made by the immune system. The average serum protein level existing in the human body is 6 to 8g/dl but 3.5 to 5.0g/dl is making up only albumin and globulin makes up 2/3g/l. Different aspects of the proteins are discussed below.

1 Introduction

Amino acids are the chemicals of all proteins. In our body, all tissues and cells are made up of proteins hence they are also called building blocks. It is the key elements of enzymes, hormones, antibodies, and clotting agents, etc. Proteins play a very important role in maintaining the fragile acid-alkaline balance of your blood. Proteins are of three types i.e. albumin, globulin, and fibrinogen but serum contains only albumin and globulin (Fibrinogen is absent in serum because it's converted into fibrin during blood clotting) [1]. The albumin: globulin (A/G) ratio is generally between 1:2:1 to 1:5:1. [2]

Major types of protein group are:

- Albumin
- Globulin
- Fibrinogen

Fig.1. Different types of proteins are present in blood.

Table1. Overview of main serum proteins components, function and abundance [3]

Sr. no	Proteins	Normal value	Molecular weight	Function	Abundance
1	Albumin	3.5-5.0g/dl	66,500 D	1. Prevent blood vessel leakiness. 2. Grows and heal tissues.	55%
2	Globulin	2-3g/dl	90,000-150,000 D	Participate in immune system.	38%
3	Fibrinogen	0.3-0.4g/dl	4,00,000-500,000 D	Blood coagulation.	7%
4	Clotting factors	0.1g/dl		Control bleeding in the case of injury, cut.	<1%
5	Regulatory proteins/Transcription factors	0.1g/dl		Regulation on the gene expression of regulatory proteins.	<1%

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1.1 Albumin

The most abundant and familiar protein of blood plasma is *Albumin*. Unlike other serum proteins, albumin has various physiological roles. For transportation of fatty acids and bilirubin, the osmotic pressure of blood is a necessary factor and this pressure arises due to albumins present in it. It comprises about 580 residues connected via peptide bonds to form a single chain.[4]serum albumin shows multiple functions i.e. ligand – binding affinity, transport properties, antioxidant properties, and enzymatic functions. Its isolation can be done using the electrophoresis technique [5]. Serum albumin is conceived by the liver and soluble in water. It is a monomer in animation. Albumins containing substances are called albuminoids [4]. 3.5 to 5g/dl is a common range of serum albumin ingrown-ups and 2.5 To 5.5g/dl for children less than 3 years of age. Sometimes Low albumin [hypoalbuminemia] may be affected by heart failure, malnutrition, and the case of high albumin [hyperalbuminemia] is affected by vitamin deficiency, burn, etc. [6]

Fig.2. Types of albumin and serum albumin

1.1.1 Types of albumin

- Lactalbumin: It is also called whey protein. It is a protein fragment of milk. Essentially comprises a small part of Beta-lactoglobulin [7].
- Ovalbumin: This type of albumin constituents about 50% of the proteins of egg white. It is also called serpin [8,9]
- Plant 2s - albumin: It is the best albumin for the storage of proteins [10]

1.1.2 Types of serum albumin

- Human serum albumin: It is mainly discovered in human hemoglobin. That is the most abundant protein in the human body and it is composed of about half of serum proteins. Human serum albumin is manufactured up of the liver. It is solvent in h₂O and is monomeric. Human serum albumin is put in code by the ALB gene.

Albumin shows various energetic roles. For conveying fatty acids and bilirubin, the osmotic strength of blood is a significant factor and this pressure arises due to albumins existing in it. It comprises about 580 residues connected via peptide bonds to form a single chain [4].

Fig.3. Human Serum Albumin [11]

- Bovine Serum Albumin: It is a Bovine serum albumin protein and it is found in animals (cow). It is also called Fraction “V”. It comprises about 583 amino acids and no carbohydrates. BSA is a small, stable, and non-reactive protein. BSA shows no biochemical applications. BSA is inexpensive so it is very commonly used in experiments. BSA is widely used for pharmaceuticals and tissue-related applications [12].

Fig.4. Bovine serum albumin [13]

1.1.3 Composition

Human serum albumin is the most plenty protein and contains only amino acid residues. Among all plasma proteins, it only does not contain any carbohydrates but BSA is a globular protein and it contains (581) only amino acid residues [4]. Both serum albumin contains components are shown in the given table [2].

Table 2. Composition of Human serum albumin and Bovine serum albumin [4]

S.No.	Amino Acids	HSA	BSA
1	Aspartic acid	39	41
2	Asparagine	15	13
3	Threonine	30	34
4	Serine	22	28
5	Glutamic acid	60	59

6	Glutamine	23	20
7	Proline	25	28
8	Glycine	12	15
9	Alanine	63	46
10	Cystine	34	34
11	Cysteine	1	1
12	Valine	39	36
13	Methionine	06	4
14	Isoleucine	08	14
15	Leucine	61	61
16	Tyrosine	18	19
17	Phenylalanine	30	27
18	Histadine	16	17
19	Lysine	58	59
20	Tryptophan	01	2
21	Arginine	23	23
22	Total	584	581
23	Amide-NH3	38	33
24	%N	16.523	16.459
25	Molecular Weight	66,248.3	66,210.2
26	Average residue weight	113.51	113.96

Fig.5. Amino acid sequence of Bovine serum albumin [14]

Fig.6. Amino acid sequence of human serum albumin [15]

1.1.4. Structure of albumin:

1.1.4.1. Primary structure:

Fig.7. Primary structure of HSA [11]

Creighton University Medical School & the University of Texas have studied the primary structure of serum albumin. It is very simplest protein composition of serum albumin generally involves the amino acid sequence in the single polypeptide chain. It comprises about 580 residues connected via peptide bonds to form a single chain. This chain also presents the 17-Disulphide bonds. The molecular weight of the primary structure of the amino acid composition is 66,500. Some scientists reported the 37 amides, 17 asparagine's & 21 glutamines sequences present in the molecule of albumin. The primary structure of albumin shows the single peptide chain but different in only one amino acid residues but the case of the primary structure of bovine albumin shows the difference in two amino acid residues from sheep & 5 to 6 amino acid residues differences from rat or chicken. Hence the primary structure of albumin does not show the conservative change and no effect of the functions especially secretory functions [16, 17].

1.1.4.2. Secondary structure:

- 55% α -helix and remaining 45% is the beta-structure.

Fig.8. Secondary structure of HSA [18]

It is a regular structure; it contains complex chains of polypeptides. The secondary structure shows two forms i.e. alpha-helix and beta-pleated sheet. Some amino acids show the tendency to form the alpha-helix and other amino acids show the form of beta-helix. Secondary structure composed of 55% of alpha-helix and 45% of the beta-helix. The secondary structure molecule shows a thickness of 30A and volume 88249 A. This structure is a little bit similar to the tertiary structure of albumin due to

both structures are three-dimensional structures & three homologous domains are present. The secondary structure shows the heart shape like that of the tertiary structure of albumin [19].

1.1.4.3. Tertiary structures:

- This structure of albumin is isolated by x-ray crystallography.
- The three domains coordinate with each other to set an ellipsoid pattern. These proteins usually bend and twist [20].

Fig.9. Tertiary structure of HSA [21]

The tertiary structure of albumin is arising from the secondary structure. It is firstly isolated with the help of x-ray crystallography. It comprises 585 amino acids, including some extent of tryptophan or methionine residues but a few required residues like lysine, aspartic acids, and no carbohydrate. This structure shows the heart shape due to the x-ray crystallography, but normally HSA is an ellipsoid structure. The tertiary composition of albumin is mainly formed of 67% of helices. It contains 3 homologous domains and two sub-domains. The tertiary structure shows the many sites for ligand- binding, drug binding, and enzyme activities. HSA contains 35 cysteine residues and it makes disulfide bridges that participate in the tertiary structure [21].

Per day liver produces approximately 12 g of albumin & about 25% of total hepatic protein synthesis. For this reason, measurement of human serum albumin concentration is used to assays liver function tests. Albumin is originally synthesized as a preprotein. Several circumstances affecting albumin synthesis i.e. protein, amino acid nutrition, colloidal osmotic pressure, and disease, etc. The vital role of serum albumin is buffering and regulating the concentration of different low molecular weight substances [22]. In blood, albumin protein is present in large amounts then it is passed by urine from the body. The heterogeneity and micro heterogeneity of the protein, conformational transformations that the proteins undergo in solution are important aspects of albumin.

1.1 Globulin

Globulin is formed from different proteins called alpha, beta, and gamma types (38% of blood

proteins) but a number of the globulins are mainly made by the liver, while others are made by the immune system. Some of the globulins show the binding with hemoglobin. Other globulins transport function, and fight infection [23]. Globulin contains the most important binding protein is called Corticosteroid-binding globulin. Its conformational ability changes an unusual cortisol-binding connection form to a low binding affinity [24].

Fig.10. Structure of Globulin [25]

1.1.1 Types of globulin [23]

- Alpha-1 globulins: It is major globulin. It is mainly made by the lungs & liver & increases with inflammation.
- Alpha-2 globulins: It has a specific function i.e. inhibitor of coagulation by inhibiting amino acids.
- Beta globulins: it has many functions i.e. liver function, blood clotting and fight against infection, etc.
- Gamma globulins: it is also called antibodies. They help prevent and fight against viral and antibacterial infections.

1.2 Fibrinogen

Fibrinogen is a glycoprotein complex and it is made in the liver. It is also called factor I assay. Fibrinogen is one of 13 coagulation factors responsible for blood clotting. Fibrinogen is absent in serum because it's converted into fibrin during blood clotting [26].

1.2.1 Structure of fibrinogen

The structure of Fibrinogen molecules are mainly composed of two sets of disulfide bridged chain and its chains contain the of Alpha, beta, and gamma bridged chains. Each molecule of fibrinogen structure contains the two central E linked to the outer D domains by a coiled-segment [27].

Fig.11. Structure of Fibrinogen [28]

2 Literature review

The word albumin is origin was Latin word Albus (white), and Albus means white part of the egg that's mean outer covering of yolk. Albumen word is used for the white part of the egg, for urinary proteins, and the-in finishing to the specific protein from blood plasma with similar properties. Firstly albumin, fibrin & hemoglobin protein of the body are studied. Firstly, Hippocrates albumin was noted in the aphorisms of foamy urine and this urine is produced by the presence of albumin shows chronic organ disease [29]. Serum albumin history is very long so it is briefly described in the form of table [3].

Table 3. Chronological history of serum albumin.

S.No	Year	Scientist Name	Comments
1	400	Hippocrates	Noted from on urine with renal disease[29]
2	1500	Paracelsus	Precipitated protein from urine with vinegar[30]
3	1837	Ancell	Distribution of protein in the body[31]
4	1840	Denis	'albumin' is separated by the help of dialysis
5	1886	Kander	Albumin is separated with the help of ammonium sulfate
6	1894	Gruber	Crystallized horse albumin
7	1896	Starling	use of albumin in circulation
8	1921	Howe	Clinical albumin/globulin assay via the help of sodium sulfate
9	1923	Bennhold	albumin shows the binding qualities.
10	1932	Race	Albumin separated with the help with acid acetone
11	1934	Hewitt	Studied the Crystallized human albumin & long fatty acid
12	1937	Tiselius	Albumin separated with the help of the electrophoresis process[32]
13	1940,46	Cohn	Prepared bovine and then human albumin for intravenous use

14	1947(2)	Hughes, Klotz	Crystallized human albumin , studied the effect of albumin
15	1950	Peters	Noted biosynthesis of albumin in chick liver slices[33]
16	1954	Bennhold	Reported first two cases of analbuminemia
17	1956	Sober	Separated albumin by ion – exchange chromatography
18	1969	Bowman	Noted the vitamin D- binding to albumin
19	1971	Mc-menamy	Studied fragments of human albumin[34]
20	1974	Craven	The Crystal form of HAS
21	1979	Sargant	The Isolated gene for human albumin
22	1986	Dugaiczky	Reported the complete sequence of gene of human albumin[35]
23	1992	Carter	Study the crystal structure of human albumin[36]
24	1996	peter	Serum albumin is very important protein.[37]
25	1999	Sugio et al	Composed three homologous domains[37]
26	2002	Scott	Proteins are made up of amino acid[38]

Creighton University Medical School & the University of Texas have studied the primary shape of human serum albumin. Human Serum albumin is also used for the cases related to the investigations processes from the Institute for Impfstoffe (Dessau, G.D.R.)[39].

2.1 Serum albumin

Albumin is the universal transporter protein for different biomolecules and nutrients i.e. long chain fatty acids and metal ions etc.

2.1.1 Unique Properties of serum albumin

2.1.1.1. Albumin is a multiple functional protein:

The most abundant and familiar protein of blood plasma is *Albumin*. Unlike other serum proteins, albumin has various physiological roles. For

transportation of fatty acids and bilirubin, the osmotic pressure of blood is a necessary factor and this pressure arises due to albumins present in it. It comprises about 580 residues connected via peptide bonds to form a single chain. Serum albumin shows multiple functions i.e. ligand – binding affinity, transport properties, antioxidant properties, and enzymatic functions. Its isolation can be done using the electrophoresis technique. Serum albumin is produced by the liver and soluble in water. It is a monomer in nature. Albumins containing substances are called albuminoids. Per day liver produces approximately 12 g of albumin & about 25% of total hepatic protein synthesis. For this reason, measurement of human serum albumin concentration is used to assays liver function tests. Albumin is originally synthesized as a preprotein. Many factors affecting albumin organization are infinite i.e. protein, amino acid nutrition, colloidal osmotic pressure, and disease, etc. The vital role of albumin is buffering and regulating the concentration of different low molecular weight substances [40].

2.1.1.2 Binding affinity:

Binding affinity is the binding cooperation between a single biomolecule to its ligand/binding partner. Human serum albumin shows the unique property it is the binding capability for many ligands including amino acids, metal ions, and bilirubin. It also shows binding may be covalently, reversibly, a great number of distinct compounds. Most of the transport protein present in blood plasma but only albumin shows the binding affinity. It has a high connection for hem and is efficient for the transportation of different aggregates and drugs or different mediums [41].

Model of serum albumin:

In 1981 the author proposed the model of the serum albumin for binding of ligands. A basic model of serum albumin binding to qualify prophecy of HSA binding but it focuses on the very important role of HAS it is flexibility. HSA protein comprises 585 amino acids and it contains three homologous domains, two major selective small-molecule sites, and three homologous domains. Site –I am also called warfarin site and these sites are large, heterocyclic, and negative. It is primarily used for hydrophobic interaction. Site-II is usually small, aromatic,carboxylic acid and it appears hydrophobic, hydrogen–bonding, and electrostatic interaction to different ligands. Different composites are bind to both sites but some additional compounds can combine at supplementary sites on serum albumin at large groups of Fatty acids may assist with tiny fragments for coupling to HSA, and divining the field of their synergy with a definite ligand remains broadly unachievable [42].

Fig.12. Structure of HSA with binding sites are shown in figure [43]

In vitro assays perform the other method for binding the ligands i.e. quantitative structure- activity relationships (QSAR) is related to Binding minute molecules of serum albumin. Whenever both techniques have some important limitations. HSA cover may be measured by ultracentrifugation, liquid chromatography, charcoal adsorption, or micro dialysis. Both the techniques are work for the protein binding affinity and they generate different measures but not all of which are equally correct. Nonspecific adsorption of the micro dialysis, stability of drug during the experiment, sensitive pH, low-affinity binders, as well as investment and time necessities is all the difficulty in the resolution of HSA binding experiment [44].

2.1.1.3 Serum albumin is a transporter in Nano-drug delivery:

Some scientists reported the albumin is also a transporter of Nano-drug delivery because of its unique features due to high biocompatibility, biodegradable & non-immunogenicity. Secondly, the chemical structure of albumin shows the linkages with multiple distinct drugs & potentially protecting them from removal furthermore Finally, albumin can show the interaction towards the receptors of different sick tissues also cells and it giving an uncommon feature for rapid target sites of the disease & this locality help to the joining of definite ligands to the different drugs sites. Due to this fact, albumin, designated by a serum half-life of approximately 19 days, has the potential of increasing half-life extension and targeted the delivery of different drugs. Importance of albumin since a transporter of Nano-drug delivery for hydrophobic drugs [45].

Fig.13. Albumin shows the site of drug binding [46]

2.1.1.4 Albumin shows the free radical fixtueproperties:

Almost all plasma contains 50% of the albumin serum protein due to the albumin contains the most important reduced cysteine residue and thiols in the presence of the blood circulation. The quantity of this amino acid in albumin is 70 to 80 %. In adults, the albumin serum protein contains the sulfuric group and others maybe form the disulfide with different compounds i.e. glutathione and homocysteine, etc. According to the cys34 albumin gives the location to the scavenge hydroxyl radicals and this property help to thetrapping the free radicals.

For example: firstly, Cys34amino acid oxidized and it provided the sulfonic acid and it is again oxidized to form the two different acids sulfonic or sulphonic acid form. Both the reversible and unchangeable redox inflection processes follow the sulfonic acid used may be the central intermediate of the different reactive species. Recently Studied of HSA are included in various disulfide configurations also maintaining HSA-Cys34 as an essential redox control inintercellular parts of the body or react the no of different species i.e. nitrogen group or RNS group. Albumin also shows the no of antioxidant properties against the thiols groups it shown the oxidization of sulfonic acid. It is suggested by the highly engaging and valuable hypothesis that each oxidation and reduction cycle of Met residues in physiological systems. According to the cys34 albumin gives the location to the scavenge hydroxyl radicals and this property help to the trapping the free radicals [40].

2.2 Methods of serum albumin estimation

Many different methods for estimation of serum albumin are:

- Biuret assay
- Lowry assay
- Bradford assay
- BCA assay

Fig.14. Different methods of estimation ofserum albumin

All the methods are used for estimation of serum albumin but one of the widely used method for the separation of serum albumin i.e. biuret method.

2.2.1 Biuret method

It was first described in 1833 by Rose (egg albumen), 1848 by Weidman (Bovine serum albumin) But Rithausen &Poth firstly used the biuret reaction to the study of urine to determine the albumin by precipitating the proteins.

In this method firstly cupric salt is added to the alkaline solution of a serum protein then it produces a reddish-violet color and this reaction is called biuret reaction. Beer law is obeyed over a given concentration range of the sample. It is simple, stable, and easy to perform the measuring serum proteins. Its procedure firstly the pure serumalbumin, which can be weighed to give a solution ofknown strength and provide the best standard. While the albumin used as a standard lead to a small underestimate of the protein concentration butother techniques based on the polypeptide chain may be measuring. Bovine serum albumin is very stable and suitable to use than human serum albumin. Albumin solutions polymerize the sulfate groups in the protein. The National Bureau of Standards has said that the serum albumin in the dimer. So albumin is proposed as the most suitable standard for the biuret procedure, but it is only applicable for the albumin [47].

Table 4. Difference between the advantages and disadvantages of serum albumin estimation methods.

Sr.no	Methods	Advantages	Disadvantages
1	Biuret	(i) It is used in clinical chemistry. (ii) Amino acids and dipeptide do not give the reaction.	Sensitivity =100mg/dl (1mg/ml).
2	Lowry	Sensitivity=10g/dl.	(i) Interference by detergents and chelating agents. (ii) Proteins containing tyrosine and tryptophan give a biased reaction.
3	BCA	More sensitive and minimum interference required.	Interference by chelating agents.
4	Bradford	Fast and easy to perform	Proteins containing basic amino acids give a biased reaction.

2.3 Serum protein electrophoresis test

University of Bristol Feline Centre from 2002 & 2009 announced the result obtained by the serum protein electrophoresis. It is a lab & clinical experiment. It helps to distribute the serum protein based on the size & electrical charge. It is inexpensive & easy to complete screening procedures. All proteins are distributed with the help of the agarose gel and it has subsequent applications like an electrical charge, density is examined with the help of a densitometer and a graphical trace is described with the help of electrophoretogram. Electrophoretogram distributes the proteins into four fractions: albumin, and alpha, beta 2, and gamma globulins but alpha globulins are also divided into α -1 and α -2 figure 15.

Fig.15. Different fraction of serum protein by electrophoretogram [48]

The entire fraction doubled by the whole protein concentration gives approximate shows the protein concentration for every fraction and it is the final percentage of the protein [49].

2.4 Functions of serum albumin

Serum albumin is the fundamental factor for controlling the proper osmotic strength wanted for the complete delivery of body liquids from the other parts of the body, albumin further shows transport functions because this is the transporter of various biomolecules elements in the blood such as drugs, hormones, metals, etc., Albumin shows highest buffering capability over all the proteins and it has a total 16 histidine residues give the buffering action, albumin also performs a better role in stabilizing tool, it also inhibits the photo degradation of some acids, serum albumin also acts as nutritional roles and others functions of serum albumin are studied [50].

3 Discussion

Albumin shows two types of condition on the basis of the level of albumin:

3.1 Hypoalbuminemia

It means a low level of albumin present in the human blood. It is due to the albumin is less synthesized in the liver. This condition causes a number of diseases i.e. liver diseases, cancer, inflammatory diseases, renal disease, cardiovascular disease, malnutrition, etc. [51, 52]

- Cancer

There occur diverse ways of evaluating nutritional stations in cancer of which albumin is regularly used. Because it has a particular role i.e. starvation. While conclusion, it is wise to review the albumin has favor as a diagnostic sign of cancer durability in cancer. Out of 46 studies, only 29 studies examined cancers of the alimentary canal, all without three obtained higher albumin levels to activities linked to higher continuance in the analytical method. Out of 16 studies, 10 studies analyzed carcinoma, any excepting one attained higher albumin levels to activities linked to higher extension. In 6 investigations evaluated on women cancers and numerous cancers each, below levels of albumin held related with inferior survival. Finally, 8 studies evaluated on patients with distinct cancer areas, lower levels of albumin equaled correlated beside inferior survival. Serum albumin levels low and high are notable in cancer [53].

- Cardiovascular disease

Cardiovascular diseases are artery disease, heart failure, etc. serum albumin has properties that are: anti-inflammatory, antioxidant, anticoagulant activity as well as a colloid osmotic effect so hypoalbuminemia (it means a low level of the albumin in the body) could act as an unacknowledged modifiable risk factor for the cardiovascular disease [54].

- Inflammatory disease

Serum levels of albumin are used as “markers of nutritional status.” albumin is also suffering from inflammatory disease. It is maybe caused by hypoalbuminemia it means the low concentrations of albumin are present in the body so it results in a high risk for inflammatory diseases. Low albumin levels allow especially as “reverse acute phase reactants,” lowered during many inflammatory illnesses no matter nutrient intake [55].

- COVID-19

It is caused by hypoalbuminemia due to a low level of albumin in the body it is a high risk. This study is correct or not in the present study and its related study may be evaluated in future studies [56].

3.2 Hyperalbuminemia

It means a high level of albumin in the human blood. It is mainly caused by dehydration. Further, some other diseases are caused by it is studied [57].

Medical use:

Human albumin solution is practiced for medical ideas and operational obstacles and generally, it is used in the absorption of 5-25%. Human albumin is usually practiced to reinstate wasted fluid and further restore serum volume in shock, injuries, and cryosurgery sufferers. There is no definite pathological indication that albumin performance (linked to saline) protects experiences for somebody who has hypovolemic or concerning those who have unhealthy due to wounds or hypoalbuminemia and some other medical uses are studied [58].

4 Conclusion

The previous account revealed the importance of albumin in health and disease. Albumin finds applications in medicine. As research continues, more functions are going to be unraveled and more applications are going to be found.

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