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Amino Acid Distribution Analysis for A Model Protein Bovine Serum Albumin (BSA)

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Abstract:

Serum albumin, a soluble protein found in blood plasma, interacts with various drugs, including anticancer drugs, antibiotics, and antiviral medicines. BSA, a bio-tool, has been extensively studied for interactions between bio-compounds and proteins. Its medicinal importance, stability, neutrality, accessibility, cost-effectiveness, and abundance make it a popular choice for drug delivery research. BSA is also essential for the manufacturing of viral vaccines, which require a cell culture medium. This thesis investigates the interactions between BSA and drugs under physiologically simulated settings due to their medical importance and unusual ligand-binding capabilities. BSA is more stable in a wider pH range and at higher temperatures, making it a viable candidate for protein-based drug delivery systems. This study analyses amino acid composition and group-wise distribution of BSA.

Keywords: Bovine Serum Albumin (BSA), Amino acid composition, GroupWise distribution of amino acids

Introduction:

Serum albumin, a member of the globular protein family, is the most ubiquitous extracellular and well-known soluble protein found in blood plasma. All bodily tissues and fluids may contain this protein. It has been discovered that bovine serum albumin (BSA) interacts with several drugs, including anticancer drugs, antibiotics, and antiviral medicines. These interactions may have an impact on the medications' pharmacokinetics and pharmacodynamics, which may impair their efficacy and safety. Due to its well-characterized structure and accessibility in high purity, BSA has also been utilized as a model protein for research on Drug-Protein interaction (Loughney et al., 2014).
Among the several albumin resources, BSA is regarded as a bio-tool (model protein) and has been thoroughly investigated for interactions between bio-compounds and proteins. It was used in medical research due to its medicinal importance, ability to boost signal in assays, stability, and neutrality in many biochemical processes, accessibility, cost-effectiveness, and ability to be simply and abundantly obtained from bovine blood, around 76% sequence identity with human serum albumin (HSA), high homology, and unusual ligand-binding properties (Chang et al., 1997; Hood et al., 2005). Another reason is that BSA, an important component of Foetal Bovine Serum (FBS), is required for the manufacturing of several viral vaccines, which need the use of a cell culture medium. BSA was chosen in this thesis to investigate the interactions between BSA and drugs under physiologically simulated settings because of its medical importance and unusual ligand-binding

capabilities. Due to its availability, affordability, simplicity of purification, and abundance, albumin (BSA) was chosen as the model protein in the drug-protein interaction study. Because of its physiological affinity for several therapeutic compounds, BSA is a popular issue in drug delivery research. Because it is more stable throughout a wider pH range (pH 5-9) and at higher temperatures, BSA stands out from other proteins. This indicates how easily albumin can be produced and handled, which makes it a viable candidate for use as a protein-based drug delivery system.

BSA is the model protein used for HSA most frequently. Between HSA and BSA, there is around 76% homology, lots of cysteine, and an ongoing pattern of 8 pairs of disulfide connections are the same (Chang et al., 1997; Hood et al., 2005). Though HSA and BSA share many characteristics, the fundamental difference between the two is the total number of amino acid residues as well as the precise number and placement of tryptophan residues. BSA has two tryptophan (Trp213 and Trp 134), while HSA only has one (Trp214). Trp213 is concealed inside a hydrophobic binding pocket at subdomain IIA, whereas Trp134 is located close to the molecular surface of the BSA's subdomain IB, which is more accessible to the solvent.

After pH 4, there is more basic residue available to the solvent in HSA than in BSA. HSA comprises 585 amino acid residues, whereas BSA is composed of 583 residues in a single polypeptide chain (Chang et al., 1997; Hood et al., 2005). In the present work, amino acid composition analysis is carried out and discussed along with class-wise distribution which is responsible for BSA's structure, and functions.

Material and method:

2.1 Peptide information of BSA:

BSA's 3D structure was analyzed using X-ray crystallography with 3.2 resolution, revealing its complete chemical composition.

2.2 The amino acid sequence of BSA:

Amino acids are key protein building blocks, influencing their structure, shape, and function. Uniport Database provides comprehensive information on protein sequence and function.

2.3 Amino acid composition of BSA:

Proteins typically contain 20 standard amino acids, but BSA contains all 583 amino acids in proportion, as shown providing insight into its biological characteristics and operations.

2.4 Group distribution study of amino acids in BSA :

Amino acids can be categorized into four main groups based on their structure and the chemical characteristics of their R groups.

Result and discussion:

3.1 Peptide information of BSA:

The 3D BSA structure was analyzed using X-ray crystallography with 3.2 resolution. By studying BSA, Brown and his associates were able to determine its complete composition (chemical). In 2012, BSA's crystal structure (PDB ID 3V03) was published by Majorek et al. [4] (Table 1). The three homologous α-helical domains (I, II, and III) that form the structure of BSA contain the principal sequence of albumin residues 1-190, 191-382, and 383-583 in the same order as the amino acid sequence.

The three domains of albumin are further subdivided into two subdomains, A and B. BSA has a heart-shaped organizational structure made up of nine loops (three in each domain), connected by 17 disulfide bridges. BSA mostly exhibits acidic properties, and its isoelectric point is pH 4.8. The BSA solution is uniform. It is slightly hydrophobic no non-protein molecules are found along with this protein (González et al., 2005; Lee et al., 2015; Vogi, 1977).

Table 1: Bovine serum albumin (BSA) peptide details

Bovine serum albumin (BSA) peptide details				
Sr. No.	Peptide	Residue Numbers	Amino Acid Length	Molecular Weight (d)
1	Full Length Precursor	01 to 607	607	69,324
2	Signal Peptide	01 to 18	18	2,107
3	Pro-peptide	19 to 24	6	478
4	Mature Protein	25 to 607	583	66,463

3.2 The amino acid sequence of BSA:

Amino acids are the building blocks of proteins and are responsible for the structure, shape, and function of the respective proteins. Uniport Database is a freely accessible source of comprehensive, high-quality information about protein sequence and function [Source: https://www.uniprot.org/] [https://www.uniprot.org/taxonomy/9913].

In all 607 amino acids are available on Uniport Database [Source: https://www.uniprot.org/uniprotkb/P02769/entry]. In the present analysis work out of 607 amino acid sequences, only 583 sequences of matured protein are used, and this data. Following is the sequence of BSA mature protein of 583 amino acids out of a total of 607 amino acids of full-length precursor protein and it is obtained from the Uniport database:

[Source: https://www.uniprot.org/uniprotkb/P02769/entry] (Figure 1).

[1DTHKSEIAHRFKDLGEEHFKGLVLIAFSQYLQQCPFDEHVKLVNELTEFAKTCVADESHAGCEKSLH
TLFGDELCKVASLRETYGDMADCCEKQEPERNECFLSHKDDSPDLPKLKPDPNTLCDEFKADEKKF
WGKYLYEIARRHPYFYAPELLYYANKYNGVFQECCQAEDKGACLLPKIETMREKVLASSARQRLRC
ASIQKFGERALKAWSVARLSQKFPKAEFVEVTKLVTDLTKVHKECCHGDLLECADDRADLAKYICD
NQDTISSKLKECCDKPLLEKSHCIAEVEKDAIPENLPPLTADFAEDKDVCKNYQEAKDAFLGSFLYEY
SRRHPEYAVSVLLRLAKEYEATLEECCAKDDPHACYSTVFDKLKHLVDEPQNLIKQNCDQFEKLGEY
GFQNALIVRYTRKVPQVSTPTLVEVSRSLGKVGTRCCTKPESERMPCTEDYLSLILNRLCVLHEKTPV
SEKVTKCCTESLVNRRPCFSALTPDETYVPKAFDEKLFTFHADICTLPDTEKQIKKQTALVELLKHKP
KATEEQLKTVMENFVAFVDKCCAADDKEACFAVEGPKLVVSTQTALA583].

Figure 1: The amino acid sequence of BSA

3.3 Amino acid composition in BSA:

Generally, protein contains 20 standard amino acids, in BSA all 583 amino acids are present as per their respective proportion which is detailed in Table 2. It explains the total number of amino acid and % residues in overall BSA protein. All 20 of the standard amino acids are available in the bovine serum albumin (BSA) protein (Figure 2). This can aid in the comprehension of its biological characteristics and operations.

Table 2: Detailed amino acid composition of BSA [Source: (Weijers & Peters, 1977)

Amino Acid Composition of BSA					
Sr. No.	Amino acid	3 - Letter Abbreviation	1 - Letter Abbreviation	No. of Residues in BSA	% of Residues
1	Alanine	Ala	A	47	8.06
2	Arginine	Arg	R	23	3.95
3	Asparagine	Asn	N	14	2.40
4	Aspartic acid	Asp	D	40	6.86
5	Cysteine	Cys	C	35	6.00
6	Glutamic acid	Glu	E	58	9.95
7	Glutamine	Gln	Q	20	3.43
8	Glycine	Gly	G	17	2.92
9	Histidine	His	H	17	2.92
10	Isoleucine	Ile	I	14	2.40
11	Leucine	Leu	L	61	10.46
12	Lysine	Lys	K	59	10.12
13	Methionine	Met	M	4	0.69
14	Phenylalanine	Phe	F	27	4.63
15	Proline	Pro	P	28	4.80
16	Serine	Ser	S	28	4.80
17	Threonine	Thr	T	33	5.66
18	Tryptophan	Trp	W	2	0.34
19	Tyrosine	Tyr	Y	20	3.43
20	Valine	Val	V	36	6.17
Total				583	100.00

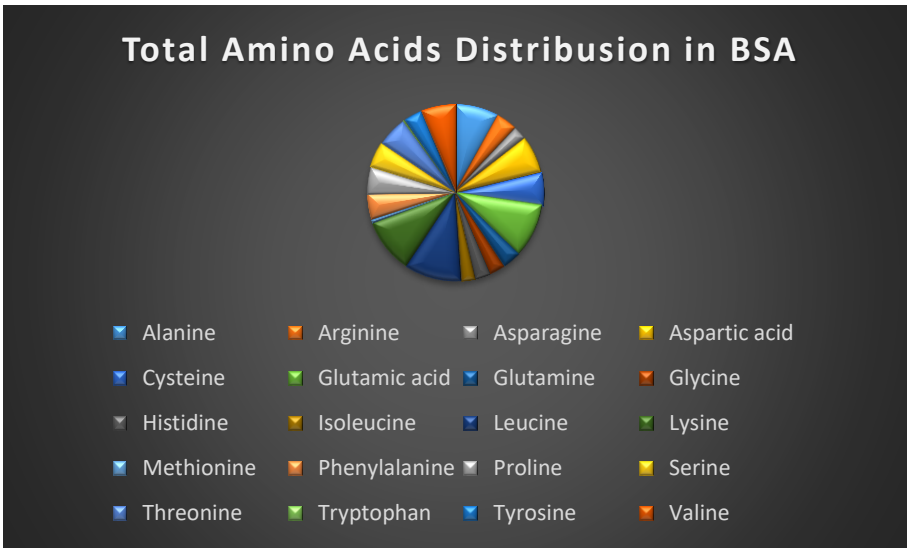

Figure 2: Total amino acids distribution in BSA

3.4 Goup distribution study of amino acids in BSA :

Amino acids can be categorized using a variety of techniques. However, according to their structure and the general chemical characteristics of their R groups, these molecules can be categorized into four main groups (Table 3). [Source: (Cox Michael M., 2015)].

Table 3: Classification of amino acids

Classification of Amino Acids		
Group	Name of Group	Name of Amino Acids
I	Non-polar amino acids (Hydrophobic)	Glycine, Alanine, Valine, Leucine, Isoleucine, Proline, Phenylalanine, Methionine, Tryptophan.
II	Polar, uncharged amino acids (Hydrophilic)	Serine, Cysteine, Threonine, Tyrosine, Asparagine, Glutamine
III	Acidic amino acids	Aspartic acid, Glutamic acid
IV	Basic amino acids	Arginine, Histidine, Lysine

3.4.1 Group I amino acids in BSA:

The side chains of group 1 amino acids, commonly referred to as nonpolar (hydrophobic) amino acids, are typically nonpolar and hydrophobic. Through hydrophobic interactions, these amino acids tend to group within proteins, stabilizing protein structure. Because they are hydrophobic, these amino acids are usually found inside proteins, away from the watery environment (Figure 3).

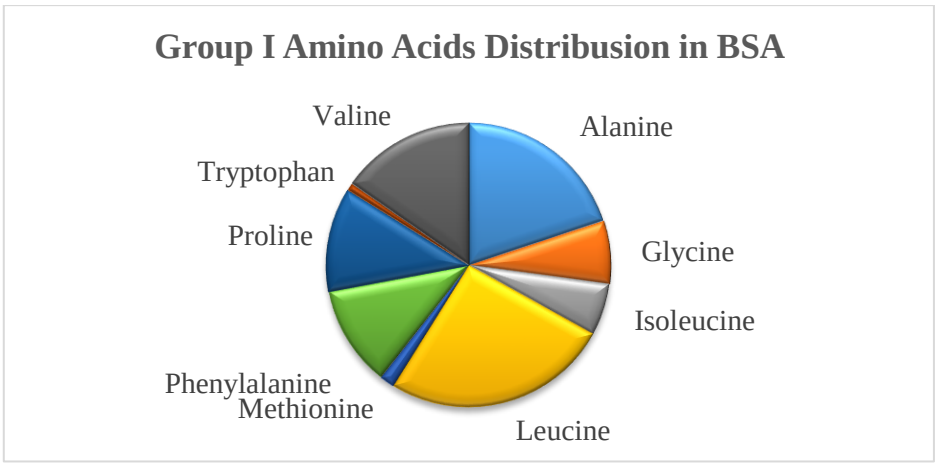

Figure 3: Group I amino acids in BSA

3.4.2 Group II amino acids in BSA:

The side chains of group II amino acids, sometimes known as polar amino acids, are polar but uncharged. These amino acids are frequently located on the surface of proteins, where they can interact with the aqueous environment because they are generally hydrophilic, or that is, they interact well with water. These amino acids are crucial for the synthesis of protein structure and function, including the active sites of enzymes, and they frequently take part in hydrogen bonding (Figure 4).

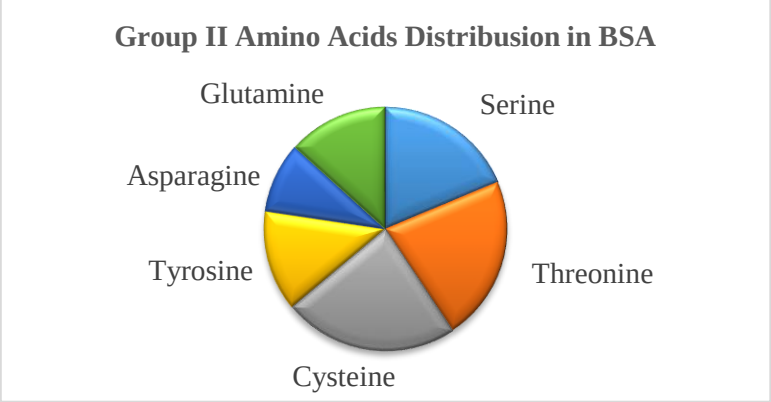

Figure 4: Group II amino acids in BSA

3.4 Goup distribution study of amino acids in BSA :

Amino acids can be categorized using a variety of techniques. However, according to their structure and the general chemical characteristics of their R groups, these molecules can be categorized into four main groups (Table 3). [Source: (Cox Michael M., 2015)].

3.4.3 Group III amino acids in BSA:

Group III amino acids, often referred to as charged amino acids, have side chains that are negatively charged at physiological pH. These amino acids are highly hydrophilic and are typically found on the surface of proteins or in active sites, where they can interact with other charged molecules or participate in catalysis. These amino acids play crucial roles in protein structure, stability, and interactions, as well as in enzymatic activities (Figure 5).

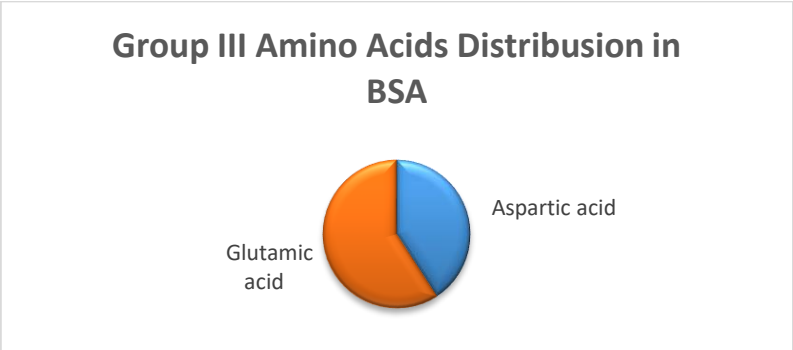

Figure 5: Group III amino acids in BSA

3.4.4 Group IV amino acids in BSA:

Group IV amino acids, often referred to as charged amino acids, have side chains that are positively charged at physiological pH. These amino acids play crucial roles in protein structure, stability, and interactions, as well as in enzymatic activities (Figure 6).

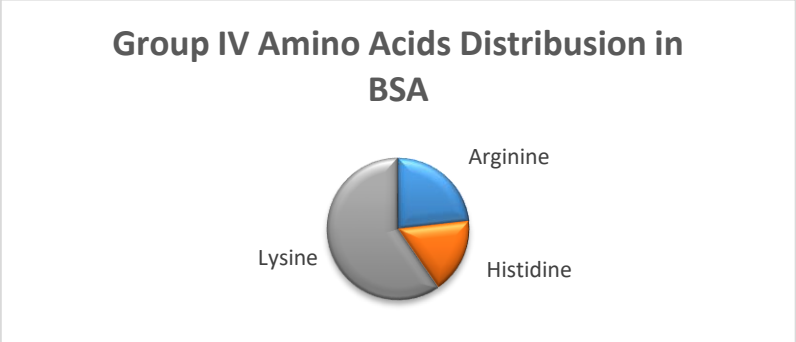

Figure 6: Group IV amino acids in BSA

3.4.5 Aromatic amino acids distribution analysis in BSA:
Absorption and fluorescence properties of BSA are because of aromatic amino acids. Tryptophan number is less but its role in intrinsic fluorescence is very important which is altimatly used in its spectroscopic analysis (Figure 7).

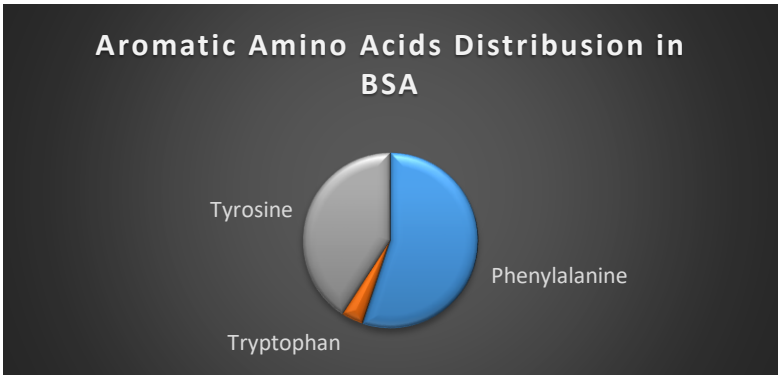

Figure 7: Aromatic amino acids in BSA

Conclusion:

The distribution of amino acids in BSA highlights its versatility and its capacity to interact with a broad variety of molecules while remaining stable and soluble in the bloodstream. The proper arrangement of amino acids facilitates the function of BSA as a carrier protein and its participation in several physiological activities.

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