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GLYCATION ENHANCERS AND INHIBITORS OF HUMAN SERUM ALBUMIN

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Abstract: The formation of unstable Schiff's base is the first step in the glycation reaction, followed by the conversion of Schiff's base into a stable reversible Admori product in the second step. Finally, AGEs are formed by further changes in Admori products by a series of reactions of dehydration and fragmentation. Glycation reaction mainly occurs at the amino group of lysine and arginine. As the glycation process involves proteins, it becomes indispensable to study proteins. There are various in-vitro techniques such as electrophoresis, fluorescence spectrometry, which can provide information about, whether the protein is glycated or not. But these methods are not sufficient to understand the process of glycation. That's why it is necessary to study the sequence and structure of a protein also, through the bioinformatic approach.

. We have analysed the total number of lysine and arginine which are accessible for glycation in each 3D structure available with us. To screen glycation enhancers, where we observed presence of more surface accessible lysine and arginine residues when compared to the crystal structure of human serum albumin. Similarly, glycation inhibitors were also screened in which number of lysine and arginine on the surface are less as compared to standard crystal structure human serum albumin. Some ligands after binding with human serum albumin do conformational change, in such a way that more or less buried lysine and arginine residues appear on the surface of a protein and become accessible for sugar molecules, making them more prone to glycation, such ligands can be called as glycation enhancers. Inversely, there are also some ligands which change the structure of human serum albumin in such a way that a smaller number of lysine and arginine appears on the surface of the protein, because some lysine and arginine get buried inside protein structure make them unavailable for glycation, such ligands can be called as glycation inhibitors

Keywords: human serum albumin, glycation enhancers, glycation inhibitors, HSA-ligand complex, Swiss-PDB Viewer

I. INTRODUCTION

The glycation of lysine and arginine, changes the structure and function of the protein (Thornalley et al.,2003). Surface accessibility is the deciding factor in the glycation of protein (Quan et al., 1999). In this study, the major focus is on the glycation of the most abundant protein i.e. HSA. In the recent few decades, there is more focus on research of HSA. The study of structural changes can be useful in determining the accessibility of lysine and arginine for glycation reaction.

In the diagnosis of diabetes and therapy of disease, glycated albumin can be useful (Armbruster,1987; Kohzuma et al,2011). The albumin shows higher glycation compared to hemoglobin, the rate of glycation is also more in albumin (Garlick and Mazer,1983). Analysis of glycated albumin as a good biomarker in diabetes is previously reported (Arasteh et al.,2014; Rondeau and Bourdon,2011).

Along with the transport function of HSA, there are many physiological processes related to it like, maintaining the pH of the blood. There are various binding sites in HSA, for fatty acids (Curry et al.,1998; Koyama et al.,1997), for small solutes (Peters,1996; Otagiri,2005), and for heterocyclic compounds (Peters,1996; Otagiri,2005). The antioxidation property depends on the protein structure, as HSA is present in large amount in plasma (Halliwell, 1988). The change in its structure results in damaged albumin which is removed from circulation and degraded (Halliwell and Gutteridge, 1990). The structure and function of HSA are affected by the glycation of protein (Shaklai,1984; Nakajou et al.,2003). The high level of glycation may interfere with the capacity of human serum albumin to interact with drugs. (Nakajou et al.,2003; Joseph et al.,2010; Joseph et al.,2011; Joseph and Hage,2010; Matsuda et al.,2011).

In Vivo and in vitro glycation shows close resemblance with respect to specific residues modified (Garlick and Mazer,1983; Arif et al.,2012; Barnaby et al.,2010; Barnaby et al.,2011; Wa et al.,2007; Barnaby et al.,2011; Anguizola et al.,2013). So in vitro glycation of human serum albumin can be useful to measure glycation pattern to analyse the information of specific modifications occurring at lysine and arginine residues. Under different structural variations due to glycation, drug binding affinity can increase (Fitzpatrick and Duggan,1987; Nakajou et al.,2003; Joseph and Hage,2010) or decrease (Voziyan et al.,2003). In previous reports, the binding capacity of solids with human serum albumin has also been examined (Shaklai et al.,1984; Fitzpatrick and Duggan,1987; Okabe and Hashizume,1994; Baraka-Vidot et al.,2012; Mereish et al.,1982; Gatti et al.,1987; McNamara et al.,1988; Ruiz-Cabello and Erill,1984; Koizumi et al.,1998; Doucet et al.,1993).

RCSB-PDB is open-access resource providing the digital data of biomolecules (Berman et al.,2003; Berman et al.,2000; Berman,2008; Burley et al.,2017; wwPDB consortium Protein Data Bank,2019). Since the establishment of the protein data Bank in 1971 (Bank Protein Data,1971), thousands of data depositors submit the 3D biological molecules structure data. To nurture this macromolecular database the Research Collaboratory for structural Bioinformatics protein data Bank (RCSB– PDB) was established in 1999, which provides its data access through rcsb.org (Berman et al.,2000; Burley et al.,2018; Rose et.al,2017). The RCSB–PDB activities provide various services like data and access to the data for data exploration etc.The protein interacts with different drugs and ligands, RCSB also provide information about drug (Wishart et al.,2018) and ligands (Wassermann et al.,2011). RCSB– PDB archive services, regularly update their database of protein data through the integration of information from UniProt which deals with protein sequences and annotations. Through NGL viewer, we can visualize interactive 3D protein structures using computers and smart devices (Rose et al.,2018; Rose and Hildebrand,2015).

II. MATERIAL AND METHODS

Data collection from RCSB-PDB: The crystallographic structure of human serum albumin was obtained from the Protein Data Bank (PDB), a repository for the 3-D structural data of large biological molecules, such as proteins. The PDB ID for the crystallographic structure of human serum albumin was 1AO6. Ligand bound human serum albumin structures were also obtained from the RCSB protein data bank.

Data visualization by Swiss PDB Viewer: The file format initially used by the PDB was called the pdb file format. The structure files may be viewed using one of several open-source computer programs such as Swiss PDB Viewer. To understand the 3D structures of albumin, we have used molecular graphics software for data visualization i.e. Deep swiss PDB viewer is an interactive program for viewing 3D structures of the protein. The color mend of the viewer was used, which shows a palette of 20 colors and provides information about the

accessibility of amino acids. All downloaded files of the HSA structures were viewed one by one to study the accessibility of lysine and arginine residues.

III. RESULTS AND DISCUSSION

Study of the structure of human serum albumin crystallographic structure: To study possible conformations of human serum albumin we have used crystal structure of HSA having PDB ID: 1AO6 as a standard structure.

Study of HSA-Ligand crystallographic structures: Ligand is a substance that forms a complex with a biomolecule. Such ligand-bound human serum albumin structures were also obtained from the protein data bank. In order to get a holistic overview of the structure and conformation of human serum albumin and related change in the accessibility of amino acids, other structures of HSA ligand - complex containing different conformations of human serum albumin were accessed from Research Collaboratory for Structural Bioinformatics - Protein Data Bank. The structural changes after binding of a particular ligand were dtudied for following ligands and identification codes were used for this study:

LIGAND ID's: 1FL, 4EB, 9DN, 9DS, 9NE, 9NF, 9NR, 9NV, ACD, ALY, AZQ, AZZ, B3I, BAB, BAH, BAI, BAM, BLA, C1F, CA, CIT, CL, DAO, DIO, DKA, DZP, ESI, FUA, GOL, HEM, HLT, IBP, IDB, MN, IMX, IO3, IOS, IPX, IQX, LI8, LPX, LZQ, MCL, MYR, NA, NPS, OLA, OPB, P1Z, P28, PFL, PJZ, PLM, PO4, RWF, SAL, SEP, SO4, STE, SWF, T33, T44, T4A, TYS, ZN.

The identification codes: For each ligand-bound human serum albumin there is particular structural ID.

2BXE, 1YSX, 2XVU, 2XVV, 2XVQ, 2XSI, 2XWO, 2XVW, 2XW1, 1GNJ, 2I2Z, 2BX8, 2BXI, 2BXK, 3B9L, 3B9M, 1BKE, 2VUE, 2BXA, 1TFO, 1E7F, 2BXL, 1E7E, 2VDB, 2BXF, 2VUF, 1N5U, 109X, 1E7B, 1E7C, 2BXG, 2BXN, 2BXK, 2BXM, 2BXQ, 3LU6, 2YDF, 2BXH, 3LU7, 3LU8, 3CX9, 3JQZ, 3A73, 3SQJ, 1HK4, 1HK5, 1H9Z, 1BJ5, 1GNI, 2BXB, 2BXC, 1E7A, 1E7H, 2BXD, 3JRY, 1E7I, 1HK1, 1HK2, 1HK3, 1E7G, 1HA2, 2BXO, 2BXP, 2I3O

Data visualization by Swiss-PDB Viewer: Molecular Graphics Software i.e. DeepView –Swiss-PdbViewer (Version 4.04) was used for this study.The accessible amino acid residues which are present in protein were studied by checking the surface accessibility of amino acids in Swiss PDB Viewer with help of 3D structures accessed from RCSB-PDB. All downloaded files of HSA-ligand complex structures were studied, in addition to crystal structure of human serum albumin to check the accessibility of lysine and arginine residues. The color of mend of the Swiss PDB Viewer was used, which shows an orderly palette of 20 colors, which provides information about accessibility of amino acids.

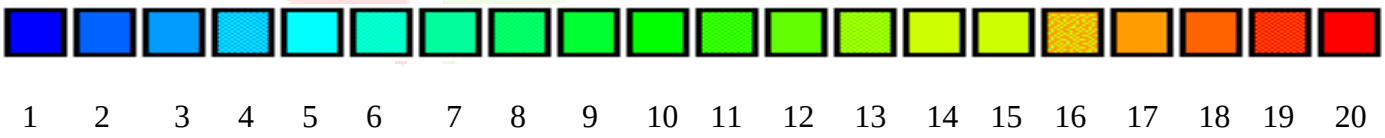

Figure 1: Orderly palette of 20 colors (providing 5% increments on a 0 to 1 scale).

Dark violet color denotes fully buried amino acid and red color is allotted to amino acids showing a minimum 75% surface accessibility. Generally, amino acids having more than 25% of accessibility can undergo glycation. So, with help of 20 colors as shown in figure 4.2.1. These colors provides accessibility information by 5% increments from the first violet color to the last red color with increment on 0 to 1 scale. That's why the first five colors i.e. 1,2,3,4,5 means amino acid has 0 to 25% accessibility and is buried, so not involved in glycation. The remaining 15 colors from 6 to 20, have access ability of 30 to 100 % and are involved in glycation.

Some ligands after binding with human serum albumin do conformational change, in such a way that more or less buried lysine and arginine residues appear on the surface of a protein and become accessible for sugar molecules, making them more prone to glycation, such ligands can be called as glycation enhancers. Inversely, there are also some ligands which change the structure of human serum albumin in such a way that a smaller number of lysine and arginine appears on the surface of the protein, because some lysine and arginine get buried inside protein structure make them unavailable for glycation, such ligands can be called as glycation inhibitors.

Table 1: Analysis of accessibility of Lysine and Arginine on HSA-Ligand structures of human serum albumin

Sr. No.	Ligand	Structural hits	Surface accessible lysine	Surface accessible Arginine	Total surface accessible lysine and arginine
1	1FL	2BXE	44	6	50
2	9DN	2XVU	43	7	50
		2XVV	43	6	49
3	9DS	2XVQ	42	5	47
4	9NE	2XSI	43	6	49
5	9NF	2XWO	41	5	46
6	9NR	2XVW	46	5	51
7	9NV	2XW1	45	7	52
8	ACD	1GNJ	49	8	57
9	ALY	2I2Z	48	9	57
10	AZQ	2BX8	46	7	53
		2BXI	47	8	55
		2BXK	45	7	52
11	AZZ	3B9L	44	7	51
		3B9M	45	7	52
12	B3I	1BKE	47	8	55
13	BLA	2VVE	44	4	48

14	C1F	2BXA	46	5	51
15	DAO	1E7F	45	7	52
16	DIU	2BXL	45	7	52
17	DKA	1E7E	42	7	49
		2VDB	43	5	48
18	DZP	2BXF	45	6	51
19	FUA	2VUF	40	4	44
20	HEM	1N5U	48	8	56
		1O9X	50	7	57
21	HLT	1E7B	45	6	-
		1E7C	48	6	54
22	IBP	2BXG	48	8	56
23	IDB	2BXN	41	7	48
24	IMN	2BXK	45	6	51
		2BXM	47	6	53
		2BXQ	46	6	52
25	IMX	3LU6	46	8	52
26	IO3	2YDF	46	5	51

27	IPX	3LU7	46	8	54
28	IXQ	3LU8	47	7	54
29	LPX	3CX9	44	7	51
30	LZQ	3JQZ	39	6	45
31	MYR	1BJ5	49	8	57
		1BKE	47	8	55
		1E7C	48	6	54
		1E7G	46	7	53
		1H9Z	49	7	56
		1HA2	47	6	53
		1HK4	46	7	53
		1HK5	47	7	54
		1N5U	48	8	56
		1O9X	50	7	57
		2BXI	47	8	55
		2BXK	45	7	52
		2BXL	45	7	52
		2BXM	47	6	53
		2BXN	41	7	48
		2BXO	45	5	50
		2BXP	46	7	53
		2BXQ	46	6	52
		2I2Z	48	9	57
		2I3O	49	7	56
		2XSI	43	6	49
		2XVV	43	6	49
		2XVW	46	5	51

		3A73	43	8	51
		3B9L	44	7	51
		3B9M	45	7	52
		3CX9	44	7	51
		3SQJ	44	5	49
32	NPS	2VDB	43	5	48
33	OLA	1GNI	47	7	54
34	OPB	2BXB	46	6	52
		2BXO	45	5	50
35	P1Z	2BXC	47	8	55
		2BXP	46	7	53
		2BXQ	46	6	52
36	1E7A	1E7A	46	7	53
37	PJ2	3A73	43	8	51
38	PLM	1E7H	48	7	55
39	PO4	3LU7	46	8	54
40	RWF	1H9Z	49	7	56
		2BXD	44	7	51
41	SAL	2I2Z	48	9	57
		2I3O	49	7	56
		3B9M	45	7	52
42	SO4	3JRY	47	8	55

43	STE	1E7I	48	8	56
44	SWF	1HA2	47	6	53
45	T44	1HK1	44	6	50
		1HK2	44	4	48
		1HK3	46	7	53
		1HK4	46	7	53
		1HK5	47	7	54

Glycation inhibitors:

The ligand containing the lowest number of surface accessible lysine and arginine

1. 3JQZ =i.e. crystal structure of HSA complexed with Lidocaine.

   >25% Accessible lysine residues 39 and arginine residues 6

2. 2XWO= i.e. crystal structure of HSA complexed with dansyl l phenylalanine

   >25% Accessible lysine residues 41 and arginine residues 5

Glycation enhancers:

The ligand containing the highest number of surface accessible lysine and arginine

1.1O9X=i.e. human serum albumin complexed with tetradecanoic acid and HEMIN

>25% Accessible lysine residues 50 and arginine residues 7

2.1GNJ=i.e. human serum albumin complexed with arachidonic acid

>25% Accessible lysine residues 49 and arginine residues 8

There are numerous confirmations of human serum albumin when they bind to different ligands, due to which rate of glycation and related post-translational modifications also change. It means ligands can act as glycation enhancers or inhibitors depending on change in structure confirmation. As there is change in accessibility of lysine and arginine, due to conformational change in human serum albumin after its binding with other ligands. We have analysed the total number of lysine and arginine which are accessible for glycation in each 3D structure available with us. To screen glycation enhancers, where we observed presence of more surface accessible lysine and arginine residues when compared to the crystal structure of human serum albumin. Similarly, glycation inhibitors were also screened in which number of lysine and arginine on the surface are less as compared to standard crystal structure human serum albumin.

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