In silico study: the antiglycation potency of aloin on the protein surface of human serum albumin

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Abstract. Advanced glycation end products (AGEs) is an end product of glycemic protein/fat/nucleic acid reactions with excessive glucose. One protein that is susceptible to glycemic reactions is the human serum albumin (HSA). Aloin is a proven natural substance in vitro can be used as an antiglycation, but not yet known as antiglycation target for HSA protein. Therefore, this study objective is to produce in silico prediction for antiglycation potency of aloin based on the pattern and binding affinity of aloin-protein HSA interactions. This study was performed by molecular docking methods using autodock vina program which integrated in PyRx 0.8 software. The ligands used are aloin, and glucose. The results of this study indicate that sub-domains 1B and 2A of HSA protein are the main target of antiglycation of aloin, with aloin’s antiglycation ability increased in further glycation.

Keywords: Aloin, Glycation, Antiglycation, Human Serum Albumin, In silico, Molecular Docking, Advanced Glycation End Products (AGEs)

1. Introduction

Advanced glycation end products (AGEs) is an end product of glycemic protein/fat/nucleic acid reactions with excessive glucose. The affected proteins undergo structural changes so that protein function changes [1]. Human Serum Albumin (HSA) is the most abundant protein in blood plasma. HSA protein plays a role in the transport of body metabolites such as metal ions, fatty acids and drugs [2].

Aloin is a bioactive compound found in Aloe vera plants that have various functions including antimicrobials, anticancer, and antioxidants [3]. Recent in vitro research shows that aloin and anthraquinone derivatives are capable of acting as antiglycation agents [4]. However, the current interaction that occurs between aloin toward proteins in the body is unknown. One of in silico method that can be used to predict protein-ligand interactions is molecular docking.

Molecular docking method which predicts the preferred orientation of non-covalent interactions between small molecule (ligand) and protein to form a stable complex [5-7]. The preferred orientation may be used to predict the strength of association or binding affinity between ligand and protein. Binding affinity is the interaction of ligands with their binding sites of protein. In general, high-affinity (more negative value) ligand binding results from the great intermolecular force and a high degree of occupancy for the ligand on its protein site. Ligand-protein affinities are influenced by non-covalent intermolecular interactions between the two molecules such as hydrogen bonding, electrostatic interactions, hydrophobic and van der Waals forces [8].
Molecular docking is an important method for drug discovery that is used to predict interactions between drug candidate with protein target [9]. One of the drug compounds that have been studied in vitro and in silico is quercetin. In silico study of quercetin using autodock vina program was able to show the non-covalent bonds formed between has protein and quercetin [10]. The interaction between aloin, glucose and the HSA protein will be elaborated in this study. Based on the interactions pattern and binding affinity between aloin and HSA protein used to prediction for antiglycation potency of aloin.

2. Materials and Methods

2.1. Materials and Tools
This research was conducted using hardware with specification Intel (R) Core (TM) i7 2670QM @ 2.20GHz 2.20 GHz RAM 8.00 GB. The programs used are VMD 1.9.2 [11], Chimera 1.11.2 [12], Autodock Vina integrated in PyRx 0.8 [7], Open Babel GUI 2.3.1 [13], and LigPlot+ 1.4.5 [14].

The 3-dimensional structure of aloin and glucose molecule are downloaded from the pubchem database [15]. The 3-dimensional structure of the human protein albumin serum is obtained from the protein data bank with the pdb code 4L8U, 4IW2 and 1AO6 [16, 17]. Glucose in this study consisted of 2 types of glucose, namely GLC (alpha-D-glucose cyclic) and GLO (alpha-D-glucose straight chain).

2.2. Methods

2.2.1 Preparation and Validation. The ligand preparation is done by downloading the ligand structure from the pubchem database [15]. Files saved with .sdf and .mol extensions are changed to .pdb. HSA protein used in this study there are 3 types that are downloaded from RSCB protein databank [18]. Three types of HSA proteins are called open-1 (pdb code: 4L8U), open-2 (pdb code: 4IW2), and close (pdb code: 1AO6). Proteins are separated by choosing protein structures without ligands, ions and solvents. Validation of protein structures through the molprobity server online [19, 20].

2.2.2 Molecular Docking. Docking is done by a random docking method using the integrated Vina AutoDock program in the PyRx 0.8 program. The result of the docking is the affinity binding value (BA) and the RMSD value. Random docking using the maximum gridbox. The docking result is 9 ligand conformation, docking result data used 10 repetitions and the selected data based on position, number of cluster and affinity binding value.

3. Results and Discussions

3.1. Pattern of glycation on the surface of HSA proteins
The pattern of glycation targets illustrates the predicted position of targeted glycation reactions that may occur when glucose attacks the HSA protein. The method used in this research phase is a random docking that uses a target of the entire surface of the HSA protein. A random docking method gives the opportunity for free-moving glucose molecules to find the best place to interact with proteins. Based on Sugio et al. [21] HSA is divided into 3 domains, each of which consists of 2 sub-domains, A and B, the depiction of the HSA sub-domain is illustrated in Fig. 1.
Figure 1. HSA sub-domains [21].

The representation of glycation pattern on HSA protein is presented in Table 1. Based on the table it is known that glucose attacks almost all sub-domains of HSA protein, but the position of 1B and 2A sub-domains located on the side of the HSA protein binding becomes the favored sub-domain of glucose, the three different HSA proteins PDB has a tendency to attack 1B and 2A sub-domains. Based on these patterns, both sub-domains are predicted to experience glycemic reactions. Based on the docking results in the know that quantitatively sub-domain 1B is the favorite glucose target with 54% glucose tend to attack sub 1B sub-domain. Further observations revealed that the pattern of glucose interactions in close and open-2 proteins tended to attack the 1B sub-domain, whereas in the open-1 protein attacked the 2A sub-domain. The affinity binding value corresponds to the interaction pattern that tends to be more negative in the 1B sub-domain than the 2A. Based on these trends, ligands capable of strong interaction on the 1B and 2A binding sides indicate to be good candidate for antidote medication.

Table 1. Patterns of glycation on HSA surfaces.

| Sub-domain | GLO (close) | GLC (close) | GLO (open-1) | GLC (open-1) | GLO (open-2) | GLC (open-2) | Average |
|------------|-------------|-------------|--------------|--------------|--------------|--------------|---------|
| 1A         | 0%          | 0%          | 0%           | 0%           | 0%           | 0%           | 0%      |
| 1B         | 87% (-5.08)*| 82% (-5.40)*| 18% (-4.50) | 13% (-4.90) | 70% (-4.70)* | 53% (-5.30)* | 54%     |
| 2A         | 2% (-5.00)  | 9% (-5.30)  | 29% (-4.55)  | 31% (-5.20)* | 18% (-4.60)  | 19% (-5.20)  | 18%     |
| 2B         | 11% (-4.69) | 9% (-5.20)  | 21% (-4.10)  | 7% (-5.00)   | 9% (-4.50)   | 20% (-5.10) | 13%     |
| 3A         | 0%          | 0%          | 27% (-4.61)* | 41% (-5.00)  | 0%           | 0%           | 11%     |
| 3B         | 0%          | 0%          | 0%           | 0%           | 0%           | 0%           | 0%      |
| Other      | 0%          | 0%          | 5%           | 8%           | 3%           | 8%           | 4%      |

* = the smallest value of binding affinity (kcal/mol);
** = the sub-domain favourite of glycation target
GLC = alpha-D-glucose cyclic and GLO = alpha-D-glucose straight chain.

3.2. Antiglycation Pattern of Aloin on HSA protein surface
The pattern of ligand-protein interaction was used to describe the prediction of antiglycation target position. Prediction is based on the similarity of interaction patterns of sample ligand (aloin and analogue compounds) with a pattern of glucose interactions on the surface of HSA proteins. The process of attacking the sample ligand in HSA protein is performed by the same method as glucose that is random
molecular docking. The depiction of the interaction patterns of the sample ligand in the HSA protein is presented in Fig. 2.

Figure 2. Pattern of aloin interaction on the surface of HSA protein.

Based on the data in table 2 shows the interaction patterns of all sample ligands similar to glucose, is occupying positions in the 1B and 2A sub-domains. Based on the more negative affinity binding value indicates that the strength of ligand interaction with protein in 1B and 2A sub-domains is stronger than other sub-domains. The prediction of the potential of ligand as antiglycation drug is determined based on two parameters namely the affinity binding value and the interaction with the target glycation residues of lysine, arginine and histidine.

Table 2. Patterns of aloin interaction on the surface of HSA proteins.

| Sub Domain | Percentage (%) and Smallest Binding Affinity (kcal/mol) |
|------------|-------------------------------------------------------|
|            | Aloin (close) | Aloin (open-1) | Aloin (open-2) | Average |
| 1A         | 11 (-7.5)     | 20 (-8.6) *    | 60 (-7.26)     | 42      |
| 1B         | 46 (-7.4) *   | 58 (-7.7)      | 28 (-7.5) *    | 41      |
| 2A         | 37 (-7.3)     | 6 (-7.1)       | 3              |
| 2B         | 8 (-7.4) *    | 4 (-7.05)      |
| 3A         | 9             | 11             | 2              |
| 3B         | 7             |
| other      |               |

* = the smallest value of binding affinity  
| = is the sub-domain favourite of aloin’s antiglycation target

Potency of antiglycation is determined based on the percentage value of aloin and glucose in each sub-domain of HSA. Based on Fig. 3 shows that sub-domain 2A protein HSA is a sub-domain that can be blocked completely by aloin. Whereas, sub-domain 2B cannot be protected properly by aloin. Based on the data in tables 1 and 2 shows that the 1B sub-domain is the main glycation target and the most interaction place of aloin. From the percentage value, aloin has not been able to block the 1B sub-domain perfectly. However, based on the more negative of binding affinity value indicates that aloin has potential as an antiglycation agent in the sub-domain 1B HSA protein. The percentage of aloin interaction on the surface of all HSA proteins “open” is higher than “close” (table 2). This shows that aloin has more potency to inhibit further glycation reactions than initial glycation reactions.
4. Conclusion

Based on the results of this study, it can be concluded that 1B and 2A are the main target sub-domains of the glycation reaction. Aloin is predicted to be able to protect 1B and 2A sub-domains around 40%. Based on the antiglycation pattern, aloin is able to protect 2A sub-domain better than other sub-domains. While, the 2B is a sub-domain that is less able to be protected by aloin. Based on analysis of aloin interaction on the surface of “open” and “close” HSA proteins, aloin has more potency to inhibit further glycation reactions.

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