

Virtual Screening of Human Serum Albumin Mutants to Optimize the Search for its Forms that Increase Affinity to Amyloid-B Peptide

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Abstract. A promising approach to the treatment of Alzheimer's disease (AD) is the removal of amyloid- β peptide ($A\beta$) from the patient's central nervous system by acting on human serum albumin (HSA). HSA carries 90% of $A\beta$ in blood serum and 40-90% of $A\beta$ in the cerebrospinal fluid (CNS). In this work, virtual screening of all possible mutant forms of HSA based on the data of the I-Mutant service made it possible to predict changes in HSA stability and identify the most "sensitive" regions of its polypeptide chain to substitutions. The data obtained will be used to optimize the search for HSA forms with increased affinity to $A\beta$, as well as to study the mechanisms underlying the modulating effects of HSA ligands on its interaction with $A\beta$, which can become the basis for the development of new approaches to therapy and prevention of AD.

1 Introduction

Alzheimer's disease (AD) remains the leading cause of dementia in older patients [1, 2]. This neurodegenerative disease leads to a deficiency of higher cortical functions and, as a result, to social and professional maladaptation. Over time, it progresses to the destruction of the mental activity of patients who require constant care at the stage of severe dementia.

The main pathomorphological features of AD localized in the brain of patients are deposits of amyloid- β peptide ($A\beta$) in the form of senile plaques, neurofibrillary tangles and mass neuronal death [3, 4]. The amyloid hypothesis of the development of AD suggests that it is the accumulation of $A\beta$ in the patient's brain that leads to the formation of neurofibrillary tangles [5], inflammation [6], synaptic dysfunction [7] and death of neurons [8].

To date, it has been shown that AD patients are characterized by an impairment of $A\beta$ clearance from central nervous system (CNS), as well as decrease in the concentration and activity of enzymes responsible for $A\beta$ proteolysis [9]. It is assumed that disturbance of $A\beta$ clearance from CNS leads to its accumulation and the onset of sporadic forms of AD, accounting for more than 90% of all cases [10]. For this reason, a lot of researches in recent years focus on mechanisms of $A\beta$ clearance from CNS [11], including clearance through

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peripheral transport proteins, the main one of which is human serum albumin (HSA) [12, 13].

HSA is abundant plasma protein (~ 0,4-0,6 mM). HSA binds 90% A β in blood plasma and 40-90% - in cerebrospinal fluid [12–16]. It is known that HSA binds monomers [17, 18], oligomers, protofibrils [14, 19] and fibrils of A β [20] on different fibrillation stages. HSA not only binds and isolates most part of A β in plasma, but also promotes A β transfer from CNS to blood [21]. Direct A β transport through blood-brain barrier is 25% of all clearance [22] for cognitively normal people. This fact suggests existence of equilibrium between A β in blood and A β in CNS. During AD, a new equilibrium emerges as a result of A β deposition in CNS [23]. This dynamic equilibrium could be changed with therapeutic approaches enhancing A β clearance from CNS and promoting its deposit as a complex with HSA in plasma [21, 24].

The affinity of HSA to A β can be modulated with HSA ligands [25]. For example, we have shown that serotonin (neurotransmitter) and linoleic acid increase the affinity of HSA to A β . The results obtained are consistent with the data of epidemiological and animal AD studies [17, 18]. Ibuprofen enhances the affinity of HSA to monomeric A β and intensifies inhibitory effect of HSA toward A β fibrillation, that in line with data on a reduction in the risk of AD development long-term ibuprofen intake [26].

Other promising direction for development of new therapeutic and preventive approaches for AD is search for HSA modifications increasing its affinity to A β . For example, it was shown, that the set of HSA Domain II mutants is characterized by high affinity to A β [27]. At the same time, mutant forms are potentially able of shifting the balance between the CNS and peripheral blood flow more efficiently than the wild type enhancing the A β clearance from the brain of AD patients. Also, the same effect could be achieved with basified HSA, in the structure of which some part of carboxyl groups are replaced by amino groups [28].

In this work, we realized an algorithm for virtual screening of all possible mutant forms of HSA based on the data of the I-Mutant service [29], which is one of the most used servers for assessing changes in the stability of protein molecules during point replacements [30, 31]. Visualization and analysis of the data obtained allowed identifying the most “sensitive” regions to substitutions, which is important for optimizing the search for HSA forms with increased affinity to A β , as well as for studying the mechanisms underlying the modulating effects of HSA ligands toward its interaction with A β . Such HSA forms can become the basis for the development of new approaches to the treatment and prevention of AD.

2 Materials and methods

2.1 HSA structure

HSA structure (corresponding to the human serum albumin sequence, UniProt ID: P02768) was taken from the Protein Data Bank (PDB, <https://www.rcsb.org/>), PDB code 1A06. Numbering in the HSA structure starts from the fifth amino acid residue.

2.2 Effect of point mutations on HSA stability

Free energy change (DDG) values for HSA for all possible mutations for each amino acid were calculated by the I-mutant v. 2.0 (<https://folding.biofold.org/i-mutant/i-mutant2.0.html>) at pH7 and 25°C. DDG is calculated as the difference between value of the Gibbs free energy of the mutant protein and the value of the Gibbs free energy of the wild-

type protein (kcal/mol). $DDG < -0.5$ means a significant decrease in protein stability, $DDG > 0.5$ corresponds to a significant increase in protein stability, $-0.5 \leq DDG \leq 0.5$ means no mutation effect on stability.

2.3 Realization

The implementation of algorithms for screening, extraction and analysis/visualization of data was carried out in the freely distributed high-level programming language Python 3.6 (<https://www.python.org>) in the PyCharm v.2020 development environment. The structure of the HSA was visualized using the PyMOL v.1.6 graphic system (<https://pymol.org>).

3 Results and discussion

Virtual screening of all possible mutant forms of HSA made it possible to form a data table of DDG values, reflecting the change in the stability of HSA structure (Fig.1).

		point mutation																				
		A	V	L	I	M	F	W	Y	G	P	S	T	C	H	R	K	Q	E	N	D	
HSA residue number	5	S	-0.29	0.5	0.31	-0.03	0.23	0.22	0.22	0.46	-0.88	-0.99	-0.05	-0.46	-0.18	-0.23	-0.19	0.0	0.3	-0.32		
	6	E	-0.27	-0.07	0.03	0.0	0.14	0.43	0.08	0.48	-1.25	-1.67	-0.53	-0.41	-0.79	-1.31	-0.48	-1.32	-0.35	-0.28	-1.08	
	7	V	-0.89		0.09	-0.2	-0.63	-0.58	-0.58	-0.4	-2.57	-2.18	-1.59	-0.97	-1.44	-1.41	-1.79	-1.92	-1.21	-1.48	-1.65	-2.26
	8	A		0.09	-0.34	-0.37	-0.53	-0.01	-0.55	-0.28	-2.03	-1.7	-0.75	-1.2	-1.17	-1.56	-1.35	-1.55	-0.96	-1.19	-0.74	-1.7
	9	H	0.04	0.16	0.35	0.29	0.28	0.29	0.29	1.24	-0.47	-0.39	-0.42	-0.72	0.39		-0.3	-0.15	-0.19	0.07	-0.22	-1.31
	10	R	-0.47	0.19	0.12	0.03	-0.12	0.19	0.11	0.56	-0.89	-1.28	-0.92	-0.94	-0.68	-0.8		-0.78	-0.25	0.5	-0.39	-1.53
	11	F	-1.79	-0.93	-0.78	-0.71	-0.84		-0.22	0.55	-3.48	-2.01	-1.93	-1.38	-1.41	-1.86	-1.66	-1.14	-1.24	-1.47	-1.26	-2.4
	12	K	0.9	0.21	0.51	0.11	0.49	0.99	-0.08	1.24	-0.15	-0.4	0.13	-0.19	-0.04	-0.25	0.07		0.58	-0.03	0.54	-0.95
	13	D	0.68	0.48	0.56	0.3	0.87	0.58	0.37	0.98	0.34	-1.57	0.59	-0.13	0.27	0.53	0.54	0.34	1.41	0.84	1.07	

Fig. 1. An example of DDG values for the N-terminal region of HSA (5-13 a.a.) for all possible mutant substitutions. The difference in color corresponds to a decrease in protein stability at $DDG < -0.5$ and an increase in protein stability at $DDG > 0.5$.

In general, the analysis of the DDG values for the full HSA structure shows that the HSA structure is very “sensitive” to point substitutions. From the matrix of DDG values 578×20 (excluding mutually exclusive substitutions of amino acids with themselves (578 values)), only 2161 substitutions correspond to an increase in HSA stability; 8821 substitutions correspond to a significant decrease in stability. The most “sensitive” mutation areas correspond to HSA regions 22-27, 54-62, 96-116, 144-150, 166-180, 214-224, 257-284 in structural domain I, regions 298-303, 315-322, and 359-371 in structural domain II and regions 430-464, 551-562 in structural domain III (Fig. 2). Substitutions in regions 128-133 and 323-324 lead to an increase in HSA stability. The replacement with Tyr leads in the greatest number of cases to an increase in HSA stability. Replacing HSA residues with Gly, Pro, Ser, Thr, Cys, His, Arg, Lys, Gln, Glu, Asn, and Asp leads in most cases to a significant decrease in HSA stability.

Previously, analysis of the genomic data of exomes (WES) associated with AD (ADSP database), showed that Val506 located in the ibuprofen binding center corresponds to a single nucleotide polymorphism (SNP, <https://www.ncbi.nlm.nih.gov/snp/>) of the HSA gene rs571711778 (V>A). Residues Val70, Arg210, Arg233 of arachidonic acid binding sites correspond to SNPs rs368276725 (V>I), rs58624704 (R>Q), and rs756853717 (R>T); SNPs rs368276725 (V>I), rs58624704 (R>Q), rs75002628 (R>H), and rs201202407 (V>L) [32]. The data obtained using the virtual screening of HSA show that all found SNPs, except for rs756853717 (R>T) (Arg233), lead to a significant decrease in HSA stability. For rs756853717 (R>T) (Arg233), a missense mutation on Thr does not lead to a change in the protein structure. For the V47 residue located in the oleic acid binding site, we have

identified an unannotated SNP (V>A) that is characteristic only for the genomes of AD patients [32]. Mutation at this residue to Ala also leads to a significant decrease in HSA stability ($\Delta\Delta G = -1.92$).

Visualization of the obtained data made it possible to evaluate the spatial position of individual amino acids of HSA and HSA regions which are the most sensitive to point substitutions (Fig.2).

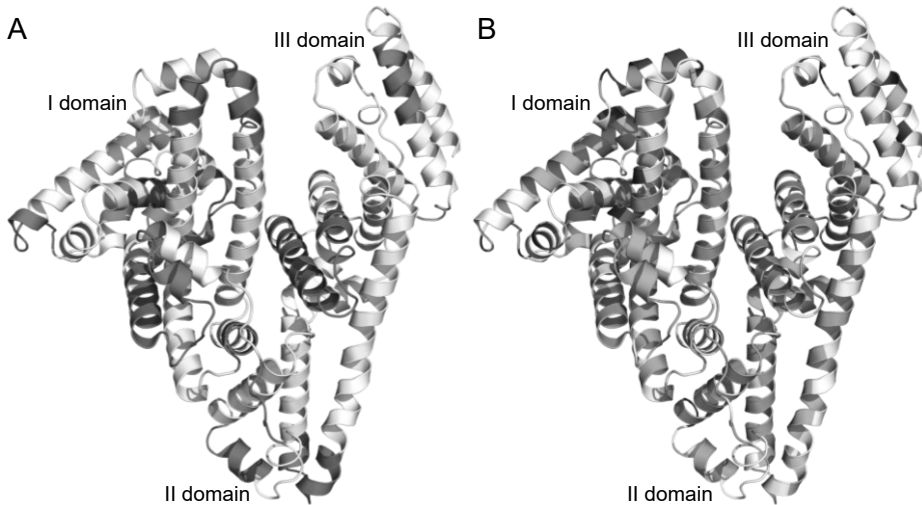


Fig. 2. A. HSA structure (PDB code 1AO6) with highlighted areas most “sensitive” to mutations. B. Alanine scanning of HSA based on I-mutant. Dark gray indicates amino acids/regions corresponding to $\Delta\Delta G > 0.5$, light gray: $\Delta\Delta G < -0.5$.

Most of the identified regions (Fig. 2A) correspond to surface and/or disordered regions that play a key role in HSA interactions with low weight, peptide and protein ligands [33]. In addition, some of these regions are located in regions between I and III domains, as well as between II and III structural domains, which corresponds to the localization of probable binding sites with monomeric and oligomeric forms of A β , according to the results of experimental and molecular dynamics studies [25], as well as bioinformatics studies [26].

4 Conclusion

Thus, in this work, we realized an algorithm for virtual screening of all possible mutant forms of HSA based on the data of the I-Mutant service. Regions which are “sensitive” to substitutions were identified. The data obtained will be further used to develop a strategy for directed mutagenesis of HSA in order to obtain HSA forms with increased affinity to A β and to study the effect of mutations on changes in HSA affinity to A β under the action of various low molecular weight ligands, as well as peptide and protein ligands.

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