EAST EUROPEAN JOURNAL OF PHYSICS. **2.** 133-140 (2022)
DOI:10.26565/2312-4334-2022-2-17 ISSN 2312-4334

INTERACTIONS OF FIBRILLAR INSULIN WITH PROTEINS: A MOLECULAR DOCKING STUDY[†]

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Received April 15, 2022, revised May 25, 2022; accepted May 30, 2022

During the last decades growing attention has been paid to ascertaining the factors responsible for the toxic potential of particular protein aggregates, amyloid fibrils, whose formation is associated with a range of human pathologies, including the neurodegenerative diseases, systemic amyloidosis, type II diabetes, etc. Despite significant progress in elucidating the mechanisms of cytotoxic action of amyloid fibrils, the role of fibril-protein interactions in determining the amyloid toxicity remains poorly understood. In view of this, in the present study the molecular docking techniques has been employed to investigate the interactions between the insulin amyloid fibrils (InsF) and three biologically important multifunctional proteins, *viz.* serum albumin, lysozyme and insulin in their native globular state. Using the ClusPro, HDOCK, PatchDock and COCOMAPS web servers, along with BIOVIA Discovery Studio software, the structural characteristics of fibril-protein complexes such as the number of interacting amino acid residues, the amount of residues at fibril and protein interfaces, the contributions of various kinds of interactions, buried area upon the complex formation, etc. It was found that i) hydrophilic-hydrophilic and hydrophilic-hydrophobic interactions play dominating role in the formation of fibril-protein complexes; ii) there is no significant differences between the investigated proteins in the number of fibrillar interacting residues; iii) the dominating hydrogen bond forming residues are represented by glutamine and asparagine in fibrillar insulin, lysine in serum albumin and arginine in lysozyme; iv) polar buried area exceeds the nonpolar one upon the protein complexation with the insulin fibrils. The molecular docking evidence for the localization of phosphonium fluorescent dye TDV at the fibril-protein interface was obtained.

Key words: insulin amyloid fibrils; serum albumin; lysozyme; fibril-protein complex; phosphonium probe.
PACS: 87.14.C++c, 87.16.Dg

Over the past decades biomedical research has been revolutionized by the emergence of powerful computational methods among which one of the most widespread is the molecular docking technique allowing to gain atomic-level insights into the mechanisms of various types of biomolecular interactions and high-throughput drug screening [1]. Prediction of three-dimensional structure of the complexes formed by biological macromolecules is a key to better understanding of their functioning. This is a multidimensional optimization problem that is practically unsolvable in an exact way because of complex energy landscapes with many local minima. A multitude of energy functions and optimization algorithms have been developed and the quality of molecular docking predictions is continuously improving [2, 3]. One area where molecular docking tool has found numerous applications concerns exploring the protein-protein interactions (PPIs). The interactions of this kind are involved in a wide variety of fundamental biological processes, such as signal transduction [4], cell growth, differentiation and apoptosis [5, 6], protein synthesis and transport [7], DNA replication and RNA transcription, host-pathogen interactions [8], immune response [9], the assembly of cellular components, regulation of enzymatic activity, etc. Cell functioning is controlled by a complex PPI network termed "interactome" [10], and up to 200,000 PPIs are thought to be involved in the human interactome [11]. Moreover, the aberrant PPIs are associated with a number of human pathologies and are regarded as potential drug targets for a broad range of therapeutic areas, such as cancer therapy [12], infectious diseases [13], heart failure [14], inflammation and oxidative stress [15], neurological disorders [16], etc. A special class of PPIs involves protein self-association into amyloid fibrils, the ordered aggregates with a core β-sheet structure. Amyloid transformation of about 50 disease-specific proteins and accumulation of fibrillar aggregates in various organs and tissues are associated with multiple human disorders, including type-II diabetes [17], cancer [18], neurodegenerative diseases [19], systemic amyloidosis, etc. For instance, extracellular amyloid beta plaques and intra-cellular Tau tangles are formed in Alzheimer's disease, α-synuclein positive Lewy bodies are found in Parkinson's disease and human islet polypeptide deposits are characteristic of type 2 diabetes [20]. Accumulating evidence lends support to hypothesis that multiple species formed along the aggregation pathway (oligomers, protofibrils and polymorphs of mature fibrils), can coherently account for amyloid-induced cellular dysfunction [21]. The suggested mechanisms of amyloid cytotoxicity lie in disruption of plasma and intracellular cell membranes [22-25], suppression of proteasomal degradation [25], impairment of of mitochondrial function [26], generation of reactive oxygen species [27] and sequestration of other proteins [23]. It appeared that amyloid fibrils can interact with endogenous proteins, as was demonstrated, in particular, for Aβ peptide and human serum albumin [28]. The

[†] ***Cite as***: V. Trusova, O. Zhytniakivska, U. Tarabara, K. Vus, and G. Gorbenko, East Eur. J. Phys. **2**, 133 (2022), https://doi.org/10.26565/2312-4334-2022-2-17

amyloid-related aspects of PPIs encompass a range of issues: i) protein self-assembly into amyloid fibrils [20]; ii) inhibition of amyloid growth by proteinaceous compounds [29]; iii) amyloid cross-seeding aggregation [30]; iv) interaction of oligomers and fibrils with endogenous proteins. The last aspect has been addressed in our previous work focused on investigating the complexation of the insulin amyloid fibrils (InsF) with one of three globular proteins, *viz*. serum albumin (SA) lysozyme (Lz) and insulin (Ins) using the fluorescent phosphonium probe TDV. To create a basis for a more comprehensive interpretation of the obtained fluorescence data, the aim of the present study was to elucidate the atomistic details of the interactions between InsF and SA/Lz/Ins utilizing the molecular docking approach.

METHODS

To predict the most favorable modes of interactions between the insulin amyloid fibrils and proteins, the molecular docking studies were conducted using the ClusPro (https://cluspro.bu.edu/login.php) [31, 32] and HDOCK (http://hdock.phys.hust.edu.cn/) web servers [33, 34]. The ClusPro was developed to perform the rigid body docking using a Fast Fourier Transform correlation approach. The docking procedure involves filtering the generated complexes according to their desolvation and electrostatic energies, followed by clustering the retained structures with lowest energy and energy minimization for a limited number of structures. A distinguishing feature of ClusPro lies in the scoring of docking solutions on a basis of the cluster size rather than the energy values. This approach assumes that the energy range of the lowest energy docked complexes is comparable to the error in the energy calculation so that further discrimination between the docking structures becomes impossible [31]. The HDOCK implements an FFT-based hierarchical algorithm of rigid-body docking through mapping the receptor and ligand molecules onto grids and global sampling of the possible binding modes with an improved shape complementarity scoring method in which one molecule is fixed, while the second one adopts evenly distributed orientations in rotational Euler space and translational space within a grid. The resulting docking solutions are ranked according to their binding energy and clustered [33]. The PatchDock algorithm involves a geometry-based shape complementarity principles and consists of three main steps: i) molecular shape segmentation into concave, convex and flat patches; ii) surface patch matching and iii) filtering and ranking of the docking positions through their evaluation by geometric shape complementarity fit and atomic desolvation energy scoring function [35]. The web server COCOMAPS (bioCOmplexes COntact MAPS) was used to analyze the properties of interfacial region in the fibril-protein complexes (https://www.molnac.unisa.it/BioTools/cocomaps/) by setting a distance cut-off of 5 Å. In the COCOMAPS analyses two residues are considered to be in contact if they present at least two heavy atoms separated by a distance ≤ 5 Å. The TDV structure was built in MarvinSketch (version 18.10.0) and the dye geometry was further optimized in Avogadro (version 1.1.0). The dye was docked with the complexes of 50-monomer fragment of insulin fibril model provided by M. Sawaya (http://people.mbi.ucla.edu/sawaya/jmol/fibrilmodels/) with one of three proteins, viz. bovine serum albumin (PDB code 4f5s), hen egg white lysozyme (PDB code 1aki) and bovine insulin (PDB code 2zp6, chains A, B). The selected docking poses were visualized with the UCSF Chimera software (version 1.14) and analyzed with BIOVIA Discovery Studio Visualizer, v21.1.0.20298, San Diego: Dassault Systemes; 2021.

RESULTS AND DISCUSSION

As illustrated in Fig. 1, both employed docking tools, ClusPro and HDOCK, provide evidence for the ability of fibrillar insulin to form complexes with serum albumin (Fig.1, A, D), lysozyme (Fig.1, B, E) and insulin (Fig.1, C, F).

The analysis of the selected highest-score docking structures in the web application COCOMAPS revealed the following main tendencies (Table 1): i) the number of interacting residues of fibrillar insulin is comparable for the examined proteins despite the differences in their size, amino acid sequence and physicochemical properties, while the number of interacting residues in protein seems to reflect these differences; ii) the lysozyme-fibril complexes are distinguished by the highest numbers of hydrophilic-hydrophobic and hydrophilic-hydrophilic interactions; iii) the number of hydrophobic-hydrophobic contacts is significantly lower than the number of hydrophilic ones, following the order: SA > Ins > Lz; iv) the size of total interface area is greatest for the complex InsF-SA and is comparable for the complexes InsF-Lz and InsF-Ins; v) the fraction of interface area in fibrillar insulin insignificantly differs for the examined systems; vi) the fraction of the protein interface area follows the order: Ins > Lz > SA; vii) the polar interface (buried) area exceeds the nonpolar one by a factor of ~ 3 for InsF complexes with SA and Ins, and by a factor ~ 1.7 for the system InsF-Lz.

Table 1. The general parameters of fibril-protein complexes predicted by ClusPro

Parameter	InsF+SA	InsF+Lz	InsF+Ins	Parameter	InsF+SA	InsF+Lz	InsF+Ins
Number of interacting residues in InsF	40	39	32	Interface area (Å^2)	1620.5	1383.7	1251.8
Number of interacting residues in protein	43	35	20	Interface area InsF (%)	5.67	7.29	5.30
Number of hydrophilic-hydrophobic interactions	35	57	48	Interface area Protein (%)	2.69	10.98	12.88
Number of hydrophilic-hydrophilic interactions	47	60	32	Polar interface area (Å^2)	1204.6	863.7	933.45
Number of hydrophobic-hydrophobic interactions	16	6	13	Nonpolar interface area (Å^2)	415.95	520.0	318.4

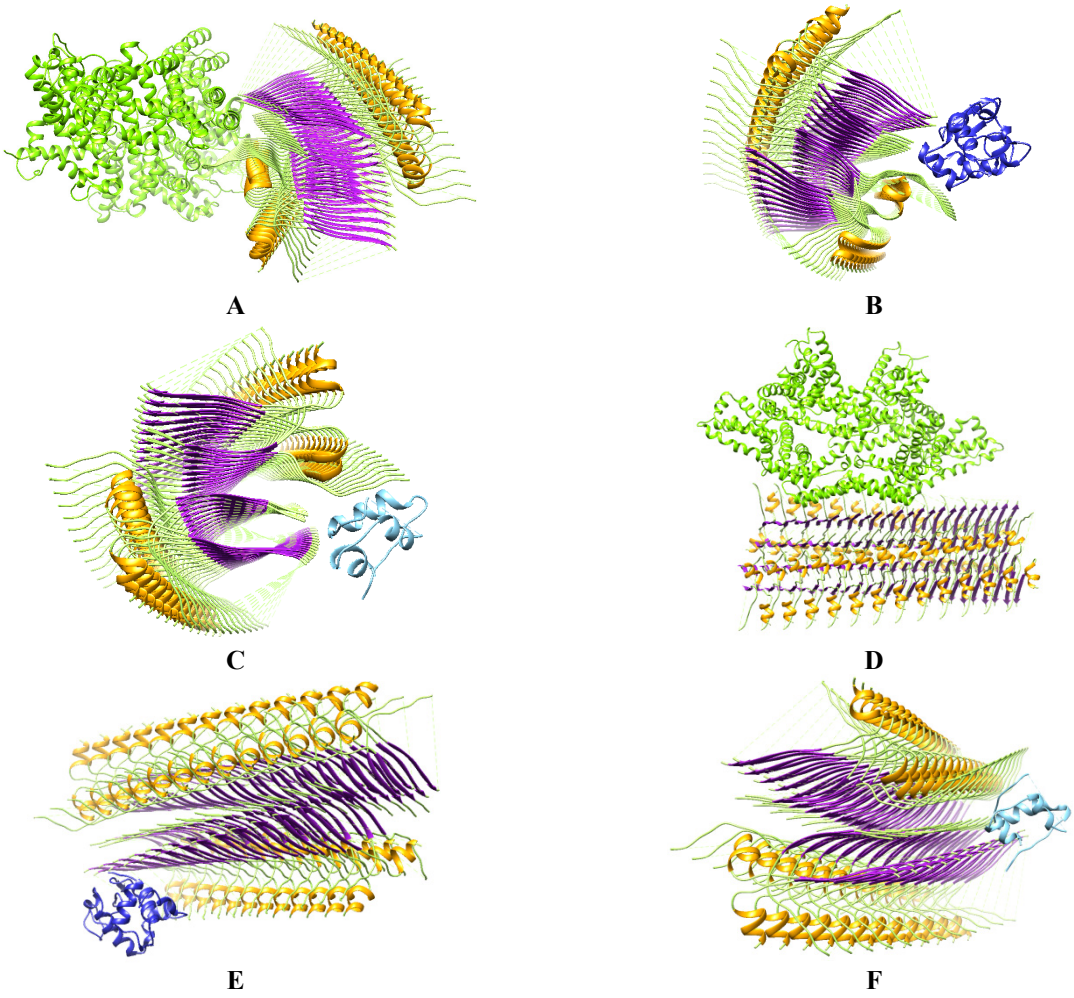


Figure 1. The highest-score non-end docking positions obtained from ClusPro (A, B, C) and HDOCK (D, E, F) for the complexes of fibrillar insulin with serum albumin, PDB code 4f5s (A), lysozyme, PDB code 1aki (B) and insulin, PDB code 2zp6, chains A and B (C).

As seen in Table 2, the buried area upon the formation of fibril-protein complexes is rather high, ranging between 3241 for SA and 2504 for Ins. The surface area buried at a protein–protein interface is usually calculated as the sum of the solvent accessible surface areas of the interacting molecules minus the solvent accessible surface area of the complex, not taking into account the possibility of conformational changes of the proteins upon complex formation. The number of residues at the fibrillar interface varies from 36 (InsF-Lz, InsF-Ins) to 47 (InsF-SA), while the amount of residues at the protein interface appeared to be considerably higher for SA (205), compared to Lz (60) and Ins (41).

Table 2. Buried and interface areas in the fibril-protein complexes

Parameter	InsF+SA	InsF+Lz	InsF+Ins	Parameter	InsF+SA	InsF+Lz	InsF+Ins
Buried area upon the complex formation (Å^2)	3241.0	2767.4	2503.6	Polar Interface (%)	74.34	62.42	74.57
Buried area upon the complex formation (%)	3.65	8.76	7.51	Nonpolar interface (%)	25.67	37.58	25.44
Polar buried area upon the complex formation (Å^2)	2409.2	1727.4	1866.9	Residues at the interface	252	96	77
Nonpolar buried area upon the complex formation (Å^2)	831.9	1040.0	636.8	Residues at the interface (InsF)	47	36	36
				Residues at the interface (Protein)	205	60	41

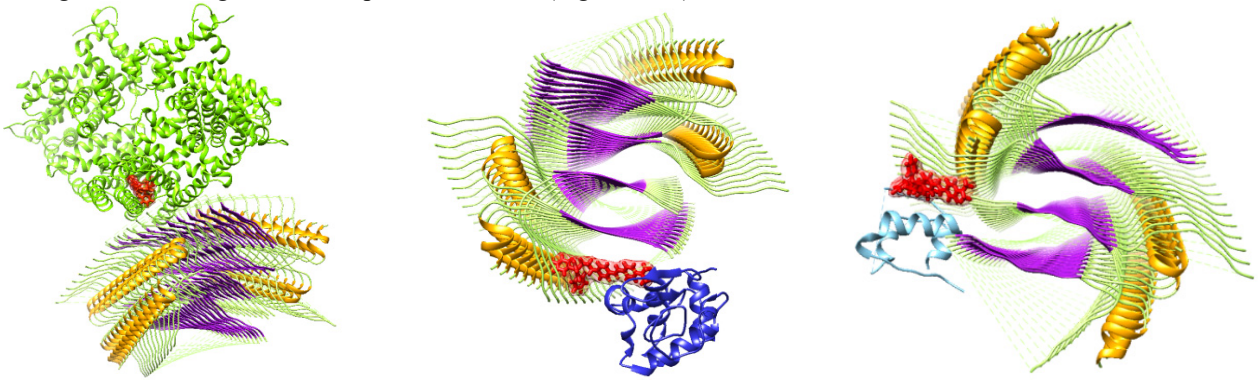
Notably, the COCOMAPS data are indicative of a marked role of hydrogen bonding in stabilizing the investigated fibril-protein complexes. As shown in Table 3, the most abundant amino acid residues forming hydrogen bonds are glutamine and asparagine in fibrillar insulin, lysine in serum albumin and arginine in lysozyme. A more detailed analysis of amino acid composition of the contact region in the examined complexes showed that the interface of serum albumin consists of 37% of nonpolar (Leu_{177}, Leu_{397}, Leu_{574}, Ala_{500}, Ala_{552}, Ala_{175}, Ala_{581}, Ala_{583}, Ala_{568}, Val_{569}, Val_{575}, Val_{576}, Pro_{179}, Pro_{498}, Pro_{572}, Phe_{501}) residues, 21% of negatively charged (Asp_{561}, Asp_{172}, Asp_{561}, Asp_{562}, Glu_{171}, Glu_{395}, Glu_{503}, Glu_{548}, Glu_{570}), 16% of positively charged (Lys_{180}, Lys_{396}, Lys_{499}, Lys_{504}, Lys_{535}, Lys_{556}, Lys_{573}) and 26% of polar (Gln_{542}, Gln_{542}, Ser_{579}, His_{534}, His_{509}, Thr_{507}, Thr_{545}, Thr_{580}, Asn_{549}, Cys_{176}, Cys_{566}) residues. The interface of lysozyme in its complexes with insulin fibril contains 37% of nonpolar (Val_2, Phe_3, Phe_{38}, Ile_{88}, Ile_{124}, Ala_{122}, Ala_{10}, Ala_{11}, Leu_8, Leu_{129}, Gly_4, Gly_{16}, Gly_{126}), 6% of amphipathic (Met_{12}, Trp_{123}), 9% of negatively charged (Asp_{87}, Asp_{119}, Glu_7), 23% of positively charged (Arg_5, Arg_{14}, Arg_{125}, Arg_{128}, Lys_1, Lys_{13}, Lys_{33}, Lys_{96}) and 25% of polar (Asn_{37}, Asn_{39}, Asn_{93}, Thr_{89}, Ser_{86}, Gln_{121}, Cys_6, Cys_{127}, His_{15}) residues. The interface residues of insulin are represented by 40% of nonpolar (Leu_6, Leu_{13}, Leu_{15}, Leu_{16}, Leu_{17}, Val_{10}, Val_{12}, Val_{18}), 10% of amphipathic (Tyr_{14}, Tyr_{16}), 15% of negatively charged (Glu_{13}, Glu_{17}, Glu_{21}), and 35% of polar (Ser_9, Ser_{12}, Cys_7, Cys_{19}, His_5, His_{10}, Gln_4) residues. The interface area of the insulin fibrils for all complexes under study includes hydrophobic (Leu, Val, Phe), polar (Asn, Gln, His, Cys) and negatively charged (Glu) residues. The above analyses show that the contribution of hydrophobic amino acids in the fibril-protein complexation is comparable for all examined proteins (~40%), positively charged residues (lysine in SA, arginine and lysine in lysozyme) can interact electrostatically with negatively charged glutamic acid of InsF. Nevertheless, electrostatics is unlikely to play a critical role in the complex formation, since the Ins interface does not contain any positive charge.

Table 3. Amino acid residues forming hydrogen bonds in the fibril-protein complexes

Serum albumin	Insulin fibrils	Distance, Å	Lysozyme	Insulin fibrils	Distance, Å
Lys535	Gln304	2.66	Asn39	Asn21	2.84
Lys573	Cys120	3.09	Asn39	Asn121	2.83
Lys573	Asn121	2.94	Arg14	Glu104	2.80
Lys556	Asn21	2.69	Arg14	Asn303	2.77
His534	Gln304	2.85	Arg5	Asn21	3.24
Thr507	Gln304	3.24	Asn37	Asn21	2.83
			Lys33	Asn121	2.57
Insulin			Arg5	Cys319	2.94
Ala14	Gln304	3.30	Arg128	Glu104	2.89
Gln4	His305	2.91	Arg14	Gln304	2.94
Phe1	Gln304	2.81	Arg125	Asn121	3.13
Glu13	His305	2.89	Leu129	Gln304	2.98
			Glu7	Leu306	3.02

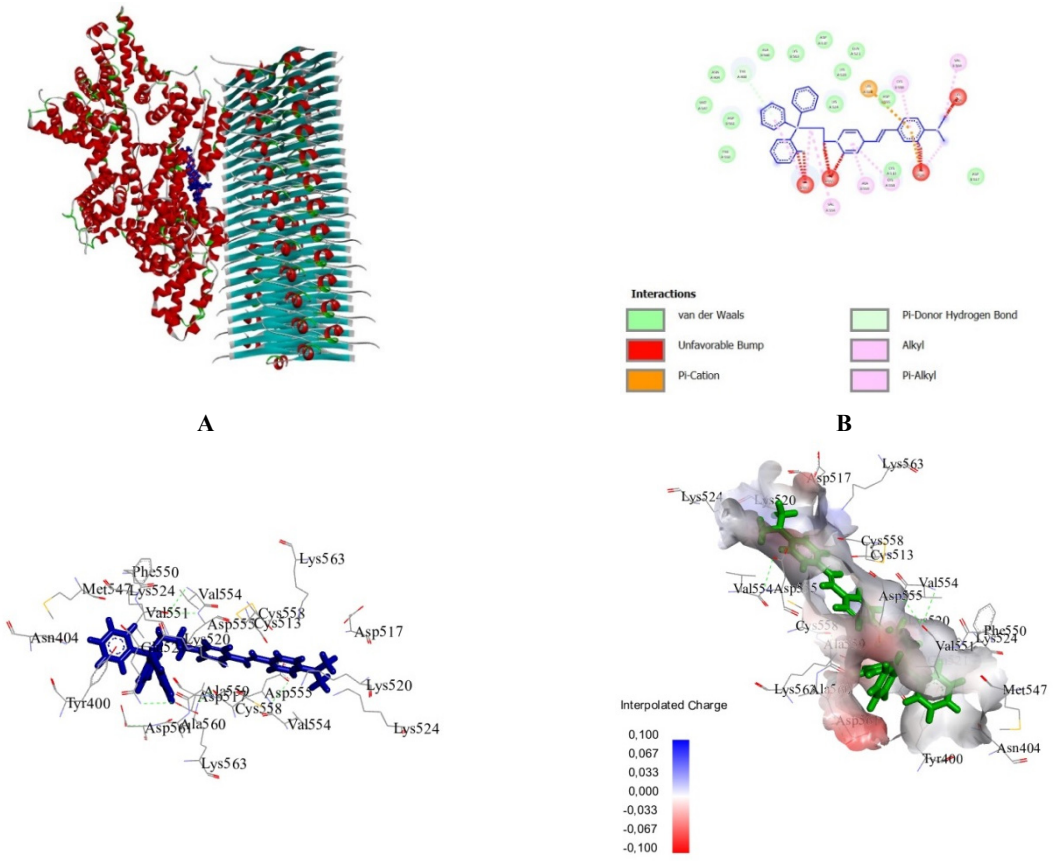
At the next step of the study we made an attempt to gain molecular docking insights into the binding behavior of the phosphonium dye TDV in the ternary systems InsF + protein + TDV. This dye has been used in our recent fluorescence study of the interactions between the insulin fibrils and serum albumin / lysozyme / insulin [36]. It has been hypothesized that TDV responsiveness to fibril-protein complexation is associated with its location in the interfacial region. To verify this hypothesis, the molecular docking between InsF-protein structures and TDV was performed using the PatchDock server. As illustrated in Fig. 2 and 3-5, TDV indeed tends to reside at the fibril-protein interface.

The analysis of the docking data via BIOVIA Discovery Studio revealed that different types of binding contacts are involved in the dye-protein complexation, such as van der Waals, alkyl/pi-alkyl, pi-cation interactions, pi-donor and carbon hydrogen bonds (Figs. 3-5, B). Likewise, long-range electrostatic interactions may contribute to the TDV orienting and anchoring at the fibril-protein interface (Figs. 3-5, D).



A **B** **C**

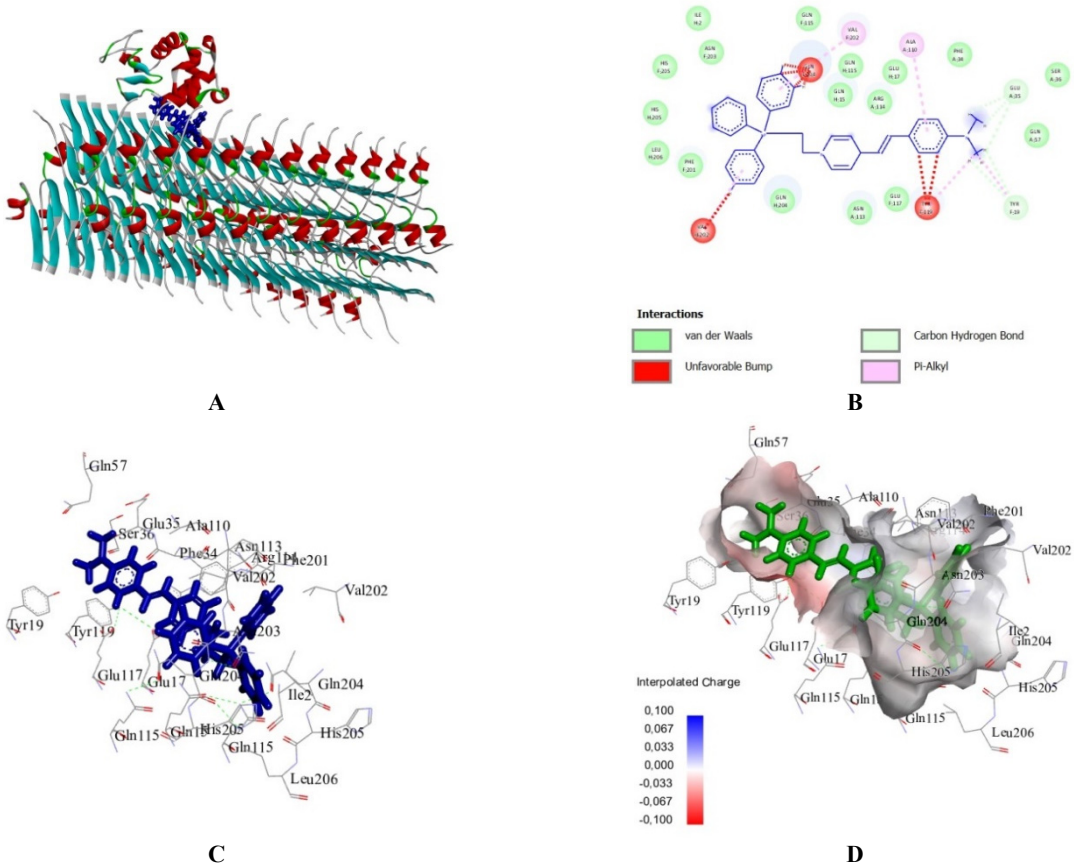
Figure 2. Highest-score docking solutions for the ternary complexes insulin fibrils – serum albumin (A)/lysozyme (B)/ insulin (C) - TDV predicted by PatchDock. The TDV molecule is colored in red



A **B**

C **D**

Figure 3. Binding residues and types of interactions between TDV and InsF + SA complexes



A **B**

C **D**

Figure 4. Binding residues and types of interactions between TDV and InsF + Lys complexes.

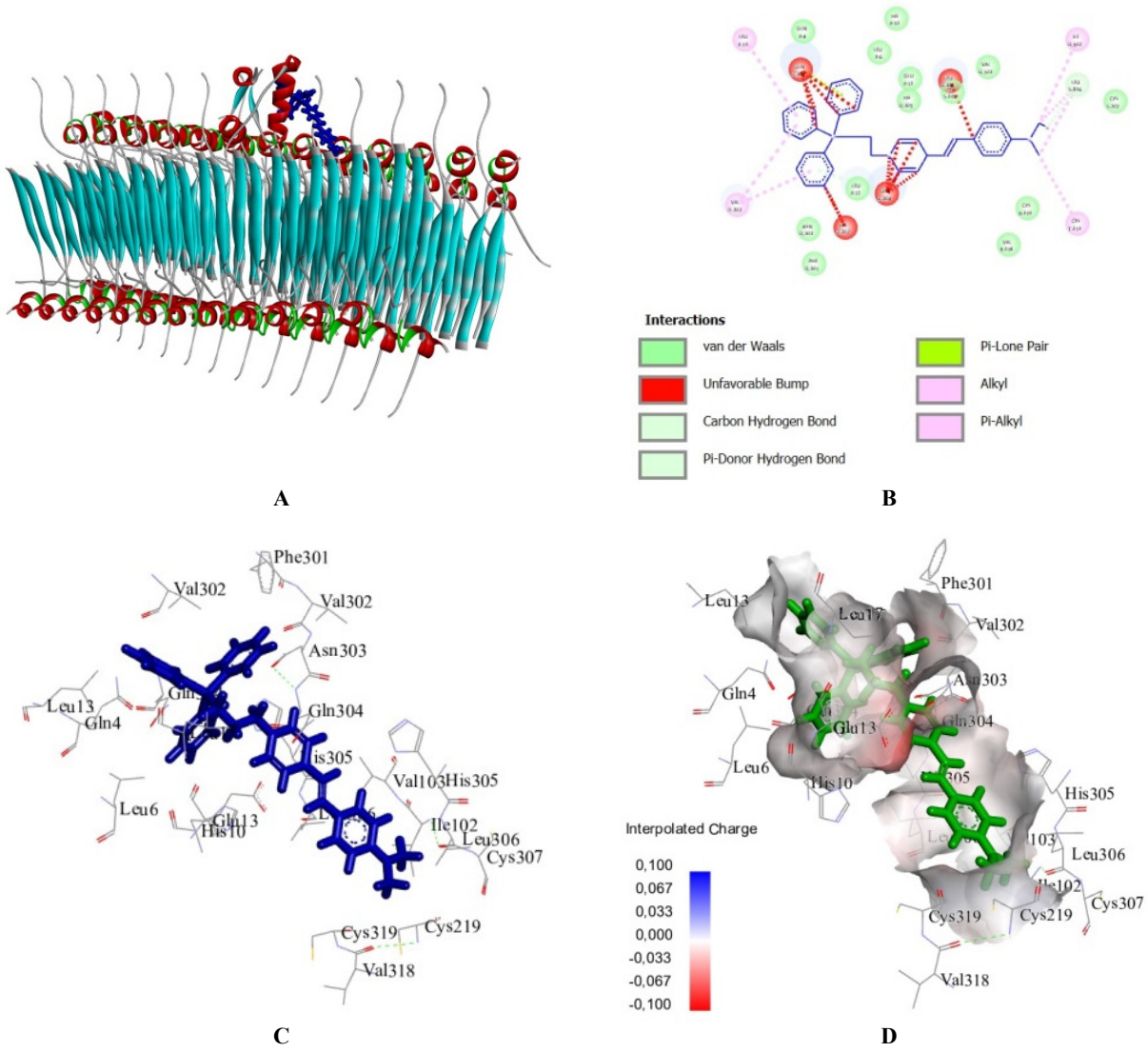


Figure 5. Binding residues and types of interactions between TDV and InsF + Ins complexes.

The most abundant residues in the TDV binding sites are represented by Lys (5), Asp (4), Cys (4), Val (3) for InsF + SA system, Gln (6), Glu (3) for InsF + Lz, and Val (4), Gln (4), His (3), Cys (3) for InsF + Ins. All the above findings, taken together with the results of the previous fluorescence study of analogous fibril-protein and fibril-protein-dye systems, support the idea that amyloid fibrils can form complexes with endogenous proteins, thereby affecting their structural and functional properties.

CONCLUSIONS

To summarize, the present molecular docking investigation has been undertaken to elucidate the nature of interactions between the insulin amyloid fibrils and biologically important proteins, *viz.* serum albumin, lysozyme and insulin. Using the ClusPro, HDOCK and COCOMAPS web servers, a range of fibril-protein complexation parameters including the number of interacting amino acid residues, the number of different types of interactions, buried area upon the complex formation, the amount of residues at fibril and protein interfaces, etc. Based on the presented results, the following main conclusions can be drawn: i) hydrophilic fibril-protein interactions dominate over the hydrophobic ones in all examined complexes; ii) the amounts of insulin fibril residues interacting with globular proteins are similar for serum albumin, lysozyme and insulin; iii) the hydrophobic leucine, valine and phenylalanine, polar asparagine, glutamine, histidine, cystein and negatively charged glutamic acid prevail among the interacting residues of fibrillar insulin; iv) glutamine and asparagine in fibrillar insulin, lysine in serum albumin and arginine in lysozyme represent the most abundant hydrogen bond forming residues. The analysis of the binding behavior of fluorescent phosphonium dye TDV using the PatchDock and Discovery Studio tools confirmed the assumption that this dye is located at the fibril-protein interface and can be employed for probing the protein-protein interactions involving the polypeptide chains in amyloid state.

Acknowledgements

This work was supported by the Ministry of Education and Science of Ukraine (the Young Scientist projects № 0120U101064 "Novel nanomaterials based on the lyophilic self-assembled systems: theoretical prediction, experimental investigation and biomedical applications" and the project "Development of novel means of medical diagnostics by biomedical nanotechnologies and modern ultrasonic and fluorescence methods").

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ВЗАЄМОДІЯ ФІБРИЛЯРНОГО ІНСУЛІНУ З БІЛКАМИ: ДОСЛІДЖЕННЯ МЕТОДОМ МОЛЕКУЛЯРНОГО ДОКІНГУ

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Протягом останніх десятиріч зростаюча увага приділяється з'ясуванню факторів, відповідальних за токсичний потенціал специфічних білкових агрегатів, аміллоїдних фібрил, утворення яких пов'язане із низкою патологій людини, включаючи нейродегенеративні захворювання, системний амілоїдоз, діабет II-го типу, тощо. Незважаючи на значний прогрес у встановленні механізмів цитотоксичної дії амілоїдних фібрил, роль фібрил-білкових взаємодій у визначенні амілоїдної токсичності залишається маловивченою. З огляду на це, у даній роботі методом молекулярного докінгу було проведено дослідження взаємодії між амілоїдними фібрилами інсуліну (InsF) та трьома біологічно важливими мультифункціональними білками, сироватковим альбуміном, лізоцимом та інсуліном в нативному глобулярному стані. З використанням web-серверів ClusPro, HDOCK, PatchDock, COCOMAPS та програмного пакету BIOVIA Discovery Studio, були визначені структурні характеристики фібрил-білкових комплексів, а саме: число взаємодіючих амінокислотних залишків, кількість залишків на інтерфейсі фібрили та білків, внески різних типів взаємодій, занурена площа при утворенні комплексу, тощо. Було встановлено, що: i) гідрофільно-гідрофільні та гідрофільно-гідрофобні взаємодії відіграють головну роль в утворенні фібрил-білкових комплексів; ii) число фібрилярних взаємодіючих залишків незначно відрізняється для досліджуваних білків; iii) водневі зв'язки утворюються, головним чином, між глутаміном та аспарагіном фібрилярного інсуліну, лізином сироваткового альбуміну та аргініном лізоциму; iv) полярна занурена площа перевищує неполярну при комплексоутворенні білків з фібрилами інсуліну. Методом молекулярного докінгу були отримані докази локалізації фосфонієвого флуоресцентного барвника TDV на фібрил-білковому інтерфейсі.

Ключові слова: амілоїдні фібрили інсуліну; сироватковий альбумін; лізоцим; комплекс фібрила-білок; фосфонієвий зонд.