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MOLECULAR DOCKING OF HUMAN SERUM ALBUMIN WITH PENICILLIN G DETERMINANTS

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Background: Human serum albumin (HSA) is the main pharmacokinetic effector of many medications, including penicillin G and its metabolites. An urgent problem of practical medicine is immediate hypersensitivity reactions caused by penicillin toxicity (about 8 % compared to other medications), accompanied by skin pathology, anaphylaxis, and fatality.

Objectives: The aim of this study is to describe the structures of penicillin G determinants-HSA complexes and to identify favorable binding sites and the amino acid residues involved in the interaction.

Material and Methods: The crystal structure of HSA (ID: 1AO6 from Protein Data Bank) (www.rcsb.org) was chosen as a docking target. Molecular docking methods (AutoDock Tools 1.5.7, AutoDock Vina 1.1.2) were used to gain insight into the interaction of HSA with the major (benzyl penicilloyl G, penicillanic acid) and minor (penicillamine, penicilloic acid, penilloic acid) determinants of penicillin G. Visualization of docking results was implemented in PyMol 2.5. The Protein Plus server (https://proteins.plus) was used to evaluate potential binding pockets. The PLIP tool (https://plip-tool.biotec.tu-dresden.de) was used to identify non-covalent interactions between HSA and its ligands.

Results: The molecular docking data indicate that the major determinants of penicillin G are involved in the formation of hydrogen bonds with such HSA residues as Trp214, Arg218, His242, and Asn295; for the minor determinants — Asp108, His146, Tyr148, Ser193, Arg197, Gln204. Both types of determinants are located in the hydrophobic cavity of subdomains IIA and IB. Hydrophobic interactions are present mainly between penicillin G determinants and amino acid residues of subdomain IIIA, such as Ala350, Asp451, Tyr452, and Gln459.

Conclusion: The study of penicillin G determinants-HSA complexes is important in the pathogenesis of antibiotic allergy. Identification of specific binding sites can be useful for the development and synthesis of new immunogenic antigens (complexes of major and minor determinants of penicillin G with HSA) that can stimulate the immune system and produce specific antibodies to prevent allergic reactions.

KEY WORDS: human serum albumin; penicillin G determinants; immediate hypersensitivity reactions; molecular docking.

Penicillins are the first-line medications for the treatment of various types of bacterial infections that can affect the throat, lungs, skin, intestines, and many other organs. In addition, surgical site infections are the most common infections in surgical patients. In this case, there is a need for pre- and postoperative antibiotic therapy for patients [1, 2]. At the same time,

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clinical trials have shown a high percentage of immediate hypersensitivity reactions to penicillins [3]. In general, medication hypersensitivity reactions include symptoms and manifestations caused by medication at a dose that is perceived by normal people. Immediate hypersensitivity reactions occur within the first hour after medication intake and are manifested by complete anaphylaxis, with damage to the skin, gastrointestinal tract, respiratory and cardiovascular systems [4]. Approximately 8 % of the population is allergic to penicillin. It has been reported that 35 % of anaphylaxis cases accompanied by angioedema, urticaria, bronchospasm, and hypotension are associated with immediate hypersensitive reactions to penicillins [5]. Penicillin G is an inactive antigen, a chemical hapten that does not exhibit immunogenic properties. The immune response can be induced by metabolically biotransformed penicillin molecules into immunologically reactive major and minor antigenic determinants [6].

Human serum albumin (HSA) is a protein present in large quantities in blood plasma that provides important physiological functions, such as the regulation of colloidal osmotic pressure, transport of many endogenous (fatty acids, hormones, amino acids, toxic metabolites) and exogenous (drugs, nutrients) compounds [7, 8]. HSA is a globular single-chain protein consisting of 585 amino acids and has three homologous domains with amino acid residues: domain I (1–195), domain II (196–383), and domain III (384–585). Each domain is divided into two subdomains: IA (1–107), IB (108–196); IIA (197–297), IIB (298–383), IIIA (384–497), IIIB (498–585). The molecular weight of HSA is 66,5 kDa [9]. 35 cysteine residues, except for one (Cys34 in domain I), are involved in 17 disulfide bonds that stabilize the structure of HSA [10].

The concentration of HSA in plasma is high, so the binding affinity of HSA to drugs is the key factor determining their pharmacokinetics and pharmacodynamics, especially when creating new dosage forms and vaccines [11, 12]. For several decades, the interaction of antibiotics with HSA has been studied by fluorescence spectroscopy, visible spectroscopy, Fourier transform infrared spectroscopy, and protein-ligand docking methods [13, 14]. For example, fluorescence studies show that displacement of fluorescents probes such as 1-amilinonaphthalene-8-sulfonate, and dansylsarcosine piperidinium salt from the binding site on albumin leads to the fact that subdomain II is involved in the binding of isoxazolylpenicillins [15]. In addition, fluorescence spectroscopy has been used to directly determine the binding sites of other antibiotics to HSA, and the results were similar: subdomain II of HSA was responsible for ligand binding [16, 17]. However, a detailed and substantiated mechanism for the formation of the HSA complex with possible molecular metabolites of penicillins has not yet been established, although there is evidence of haptenization of penicillin determinants by conjugation with HSA, which leads to the formation of IgE antibodies.

The aim of this study is to describe the structures of penicillin G determinants-HSA complexes and to identify the favorable binding sites involving certain amino acid residues.

MATERIALS AND METHODS

The 3D model of HSA was downloaded from the Protein Data Bank (www.rcsb.org) (ID: 1AO6); the 3D crystal structure is the result of X-ray diffraction at 2,5 Å resolution and was used as a docking target. The ligands — benzyl penicilloyl G, penicillanic acid (major determinants), penicillamine, penicilloic acid, penilloic acid (minor determinants) from (www.pubchem.ncbi.nlm.nih.gov) were used to identify the energetically most favorable binding sites on the HSA molecule.

Molecular docking methods (AutoDock 1.5.7, AutoDock Vina 1.1.2) were used to study the interaction of penicillin G determinants with HAS, which helps to find the space for the

most energetically favorable conformation of the protein-ligand complex and allows to estimate its geometric dimensions with the lowest binding energy [21]. The AutoDock scoring function is a subset of the Amber force field that processes molecules using the United Atom model [22]. The grid spacing, i.e., the distance between the grid points, was 0,503 Å. The search space is a 50 Å × 50 Å × 50 Å square; the exhaustiveness parameter was 8. This block covers amino acid residues of subdomain IIA and part of subdomain IB. Since the PDB files were missing hydrogen atoms, Coleman charges were added to fit the electrostatic potentials, and hydrogen atoms were added using AutoDock Tools to properly optimize the proteins. Protonation of amino acid residues at pH=7 was checked using Propka 3.1 [23].

Visualization of docking results was done in PyMol 2.5 [24]. To convert PyMol files to pdb format, the interactive converter MichelaNGLo was used (https://michelanglo.sgc.ox.ac.uk/pymol).

DoGSiteScorer from Proteins Plus was used to detect potential binding pockets and to compare with docking results. The surface area, the volume and depth of pockets, and chemical features were calculated using DoGSiteScorer [25, 26]. The relative hydrophobicity of amino acid residues was evaluated to know the general properties of HSA. Two-dimensional diagrams of complexes with known 3D structures, which describe the directed hydrogen bonds between the protein residues and the ligand were created for some complexes with PoseView [27, 28].

For the identification of non-covalent interactions between HSA and its ligands, the protein-ligand interaction profiler (PLIP) web tool was applied (https://plip-tool.biotec.tu-dresden.de) [29].

RESULTS AND DISCUSSION

Our molecular docking study was performed on the crystal structure of HSA, which was downloaded from the Protein Data Bank (ID: 1AO6), to identify possible binding sites, which are major and minor determinants of penicillin G. The five ligands are listed in Tabl. 1. Most ligands have an open β-lactam ring and a closed thiazolidine ring. The maximum molecular weight of the ligand was 465.6 g/mol, and the lowest was – 149.21 g/mol. The range of free binding energies predicted by AutoDock Vina was from -7.9 kcal/mol to -4.7 kcal/mol. The best energy results of -7.9 kcal/mol and -7.2 kcal/mol showed that both major and minor determinants can be located within subdomain IIA.

It is known that under physiological conditions, 95 % of penicillin spontaneously degrades to major and minor antigen determinants. Immediate hypersensitivity reactions, and allergic reactions in particular, occur less to the main β-lactam part of the penicillin molecule, and mostly form IgE antibodies directed against a certain side chain R (variable side chain of penicillins) [4, 5]. The structure of the penicillin core is shown in Fig. 1.

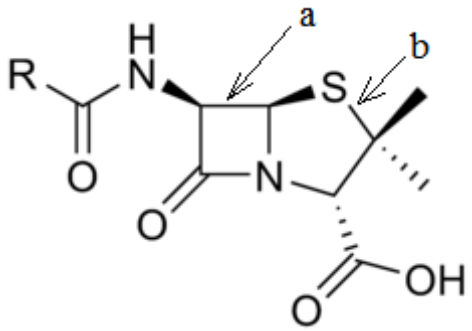
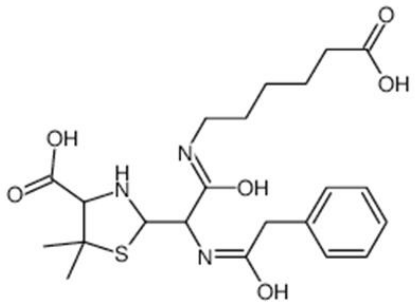
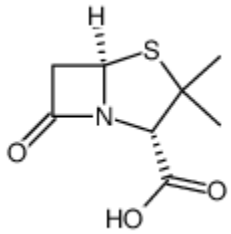
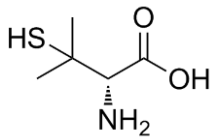
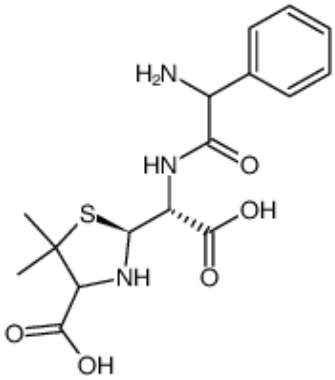
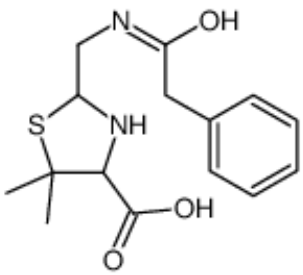


Fig. 1. The structure of penicillin: a) four-membered β-lactam ring, b) five-membered thiazolidine ring. The molecular formula is R-$C_9H_{11}N_2O_4S$, where R is a side chain, in the case of penicillin G, R is a phenyl ring.

Table 1. List of ligands

PubChem ID	ΔG, kcal/mol	Name	M, g/mol	2D structure of hapten determinants
Compound ID: 119212	-7.9	Benzyl penicilloyl G	465,6	
Compound ID: 6891	-6.0	Penicillanic acid	201.25	
Compound ID: 5852	-4.7	Penicillamine	149.21	
Compound ID: 52921568	-6.3	Penicilloic acid	262.29	
Compound ID: 255293	-7.2	Penilloic acid	308.4	

The optimal recognition of penicillin determinants by specific IgE antibodies is considered to be specific binding to a carrier molecule such as HSA. The parameters of binding of penicillin G determinants to HSA are the main factors in the ability of each complex to bind to the antigen-presenting cells to initiate subsequent hypersensitivity reactions.

Therefore, in this study we used molecular docking and a detailed analysis of its results to identify energetically favorable binding sites of penicillin G determinants-HSA complexes. The calculation of the hydrophobicity of HSA upon binding to different ligands was evaluated using Protein Plus. Our results confirmed the data of molecular dynamic modeling that the properties of HSA in the studied subdomains are more hydrophobic, but some amino acids are hydrophilic [30]. The hydrophobicity values of the binding pockets were in the range of 0.65–0.76.

The binding site volume and the binding site depth were estimated to predict the topology and geometry of the active site in complexes of HSA-penicillin G determinants to visualize the depth of penetration and placement of ligands based on their structure. Identification and characterization of the size of binding pockets based on these parameters was evaluated in Protein Plus using DoGSiteScore. This is a method based on predicting active sites in proteins based on the difference in Gaussian (DoG) approach [25]. With this approach, spatial and topological molecular descriptors help in finding the best surface area, binding site volume, and binding site depth. In the study, the volume of the ligand-binding pocket varied widely in the range of 155.65 $Å^3$ – 801.28 $Å^3$, the depth of the ligand-binding pocket also varied and ranged from 13.69 Å to 32.19 Å. The surface area also ranged from 445.09 $Å^2$ to 2151.78 $Å^2$.

The binding of the benzyl penicilloyl G molecule involves weak interactions, in particular, between the β-lactam ring and the thiazolidine ring and amino acid residues, which are capable of forming hydrogen bonds and hydrophobic interactions at the level of the hydrophobic pocket located in subdomain IIA and in subdomain IIIA (Fig. 2, Fig. 3). Lys195, Asp451, Tyr452, and Lys444 are involved in the formation of hydrophobic interactions, and Trp214, Arg218, Asn295, Pro339, and Pro447 form hydrogen bonds between the nitrogen atom of the open β-lactam ring and the thiazolidine ring and the oxygen atom of the benzyl penicilloyl G molecule.

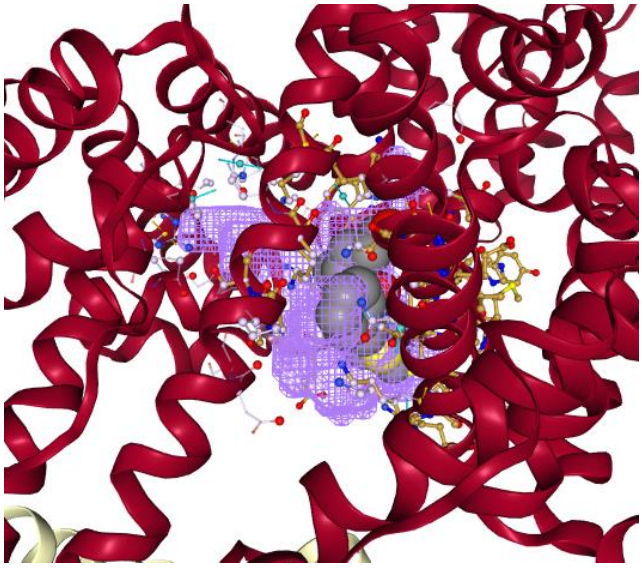


Fig. 2. The HSA-benzyl penicilloyl G complex (constructed in Protein Plus tool). The structure of HSA is represented by ribbons; the ligand benzyl penicilloyl G is represented as balls. The ligand binding pocket of HSA is marked in pale purple and has a depth of 28.74 Å. The surface area and volume of the binding site are 1617.97 $Å^2$ and 801.28 $Å^3$, correspondingly. The hydrophobicity of the binding pocket is 0.69.

Penicillanic acid is also the major determinant of penicillin allergy. Among other ligands, only penicillanic acid has closed rings both β-lactam and thiazolidine (Fig. 4). In the case of penicillanic acid, the surface area of the ligand binding pocket was 13.69 Å.

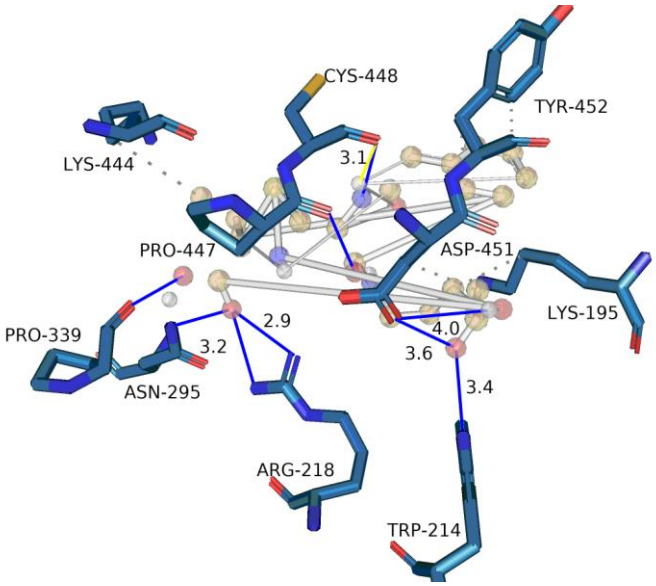


Fig. 3. The 3D contact map of benzyl penicilloyl G and HSA binding domain (constructed in PLIP tool). Hydrogen bonds are shown as thin blue solid lines, hydrophobic interactions are shown as gray dotted lines.

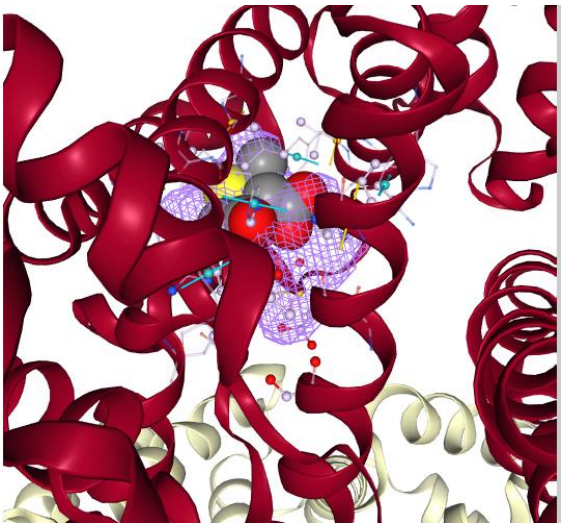


Fig. 4. The HSA-penicillanic acid complex (constructed in Protein Plus tool). The structure of HSA is represented by ribbons; the ligand penicillanic acid is represented as balls. The ligand binding pocket of HSA is marked in pale purple and has a depth of 13.69 Å. The surface area and volume of the binding site are 445.09 $Å^2$ and 155.65 $Å^3$, correspondingly. The hydrophobicity of the binding pocket is 0.76.

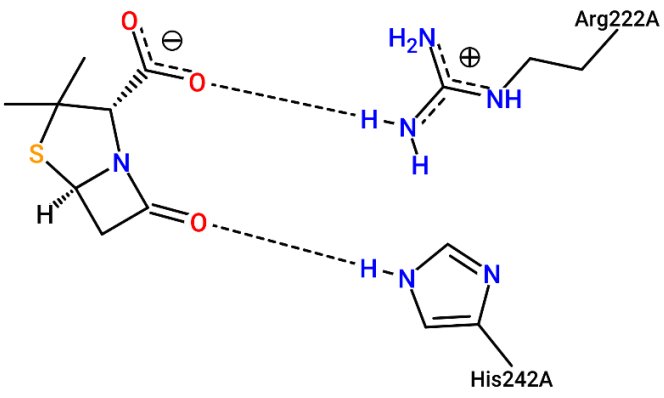


Fig. 5. Two-dimensional diagram of HSA-penicillanic acid complex with hydrogen bonds between the protein residues and the ligand; hydrogen bonds are shown as black dot lines.

As shown in Fig. 5, the oxygen atoms of the thiazolidine ring and the β-lactam ring formed hydrogen bonds with Arg222 and His242, respectively. At the same time, hydrogen bonds are not exclusively one type of interaction between HSA and penicillanic acid: the hydrophobic interactions were observed at the contact sites with Ile290, Leu219, and Leu238 (Fig. 6).

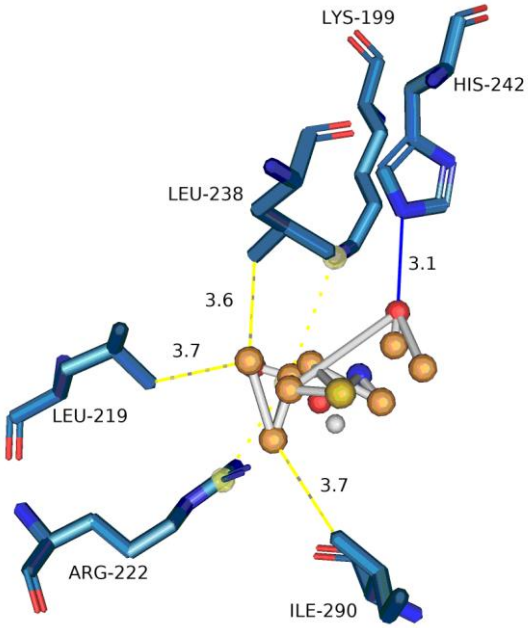
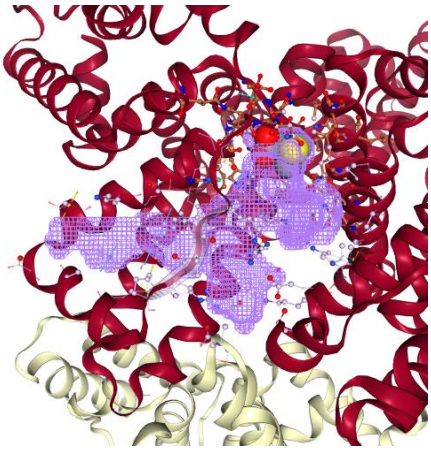
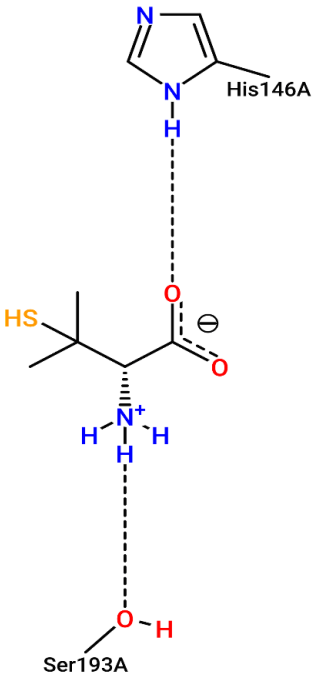


Fig. 6. The 3D contact map of penicillanic acid and HSA binding domain (constructed in PLIP tool). Hydrogen bonds are shown as thin blue solid lines, and hydrophobic interactions are shown as yellow dotted lines.





a) b)

Fig. 7. The structure of HSA-penicillamine complex (constructed in Protein Plus). a) The structure of HSA is represented by ribbons; ligand penicillamine is represented as balls. The ligand binding pocket of HSA is marked in pale purple and has a depth of 32.19 Å. The surface area, and volume of the binding site are 2151.78Å^2 and 709.12 Å^3, correspondingly. The hydrophobicity of the binding pocket is 0.71; b) The two-dimensional diagram of HSA-penicillamine complex with hydrogen bonds between the protein residues and the ligand.

As seen from Tabl. 1 the free energy change is the lowest for minor determinant with the lowest molecular weight that has both open β-lactam and thiazolidine ring (penicillamine) compared to the other determinants. The penicillamine binding site is located inside the hydrophobic pocket formed by several subdomains – IA, IIA, and IIIA. In this case, the depth of the ligand binding pocket is the largest and is 32.19 Å (Fig. 7a). Positively charged Arg197 and neutral Gln459 established hydrophobic bonds with the ligand (Fig. 8). Fig. 7b shows that polar amino acid residue Ser193 and positively charged residue His146 also form hydrogen bonds with penicillamine ligand.

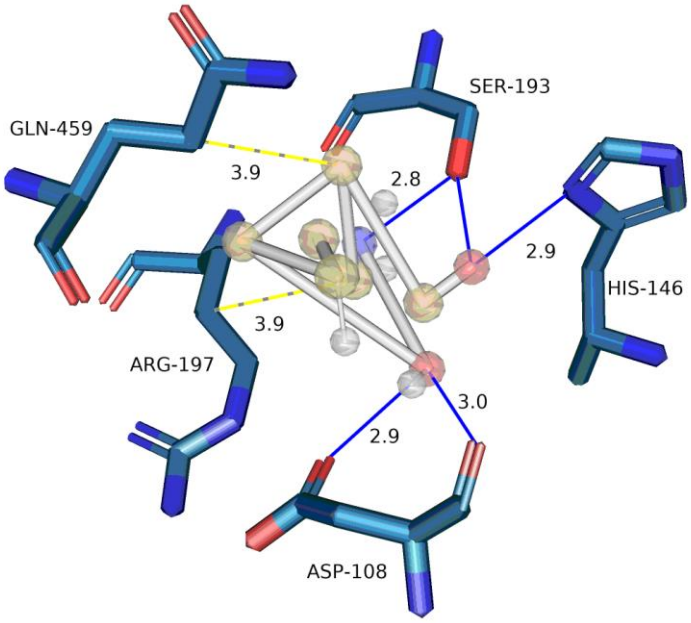


Fig. 8. The 3D contact map of penicillamine and HSA binding domain (constructed in PLIP). Hydrogen bonds are shown as thin blue solid lines, and hydrophobic interactions are shown as yellow dotted lines.

Penicilloic acid is the minor determinant that is formed from penicillins by hydrolytic opening of the β-lactam ring. In the case of penicilloic acid (Fig. 9), the ligand binding sites are located in subdomain IA (Leu103), IB (Asp108), and in the hydrophobic cavity in subdomain IIA (Tyr148).

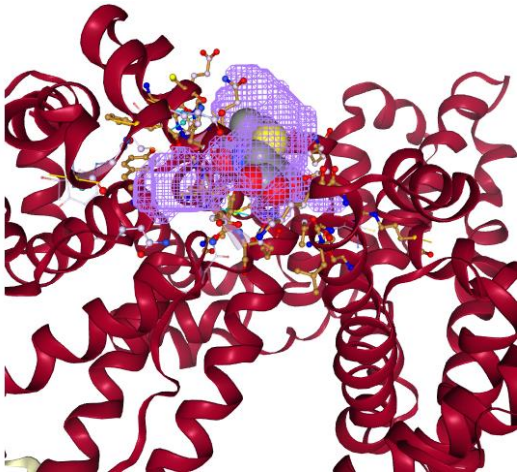


Fig. 9. The complex HSA-penicilloic acid (constructed in Protein Plus). The structure of HSA is represented by ribbons; ligand penicilloic acid is represented as balls. The ligand binding pocket of HSA is marked in pale purple and has a depth of 22.37 Å. The surface area and volume of the binding site are 1260.67 $Å^2$ and 538.11 $Å^3$, correspondingly. The hydrophobicity of the binding pocket is 0.65.

The penicilloic acid-HSA complex is stabilized by hydrogen bonds between Tyr148 and Arg197 residues of subdomains IB and IIA and the nitrogen of the thiazolidine ring and the oxygen of the open β-lactam ring, respectively (Fig. 10). The hydrophobic interaction is also

involved in the binding through Gln204. In addition to hydrophobic and hydrogen bonds, salt bridges are involved in the ligand-protein complexation (Fig. 11).

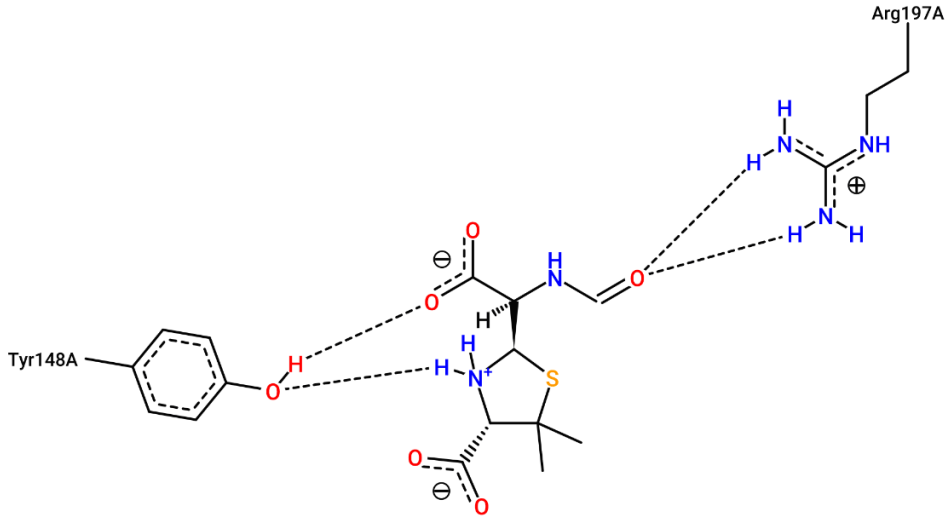


Fig. 10. Two-dimensional diagram of HSA-penicilloic acid complex with hydrogen bonds between the protein residues and the ligand.

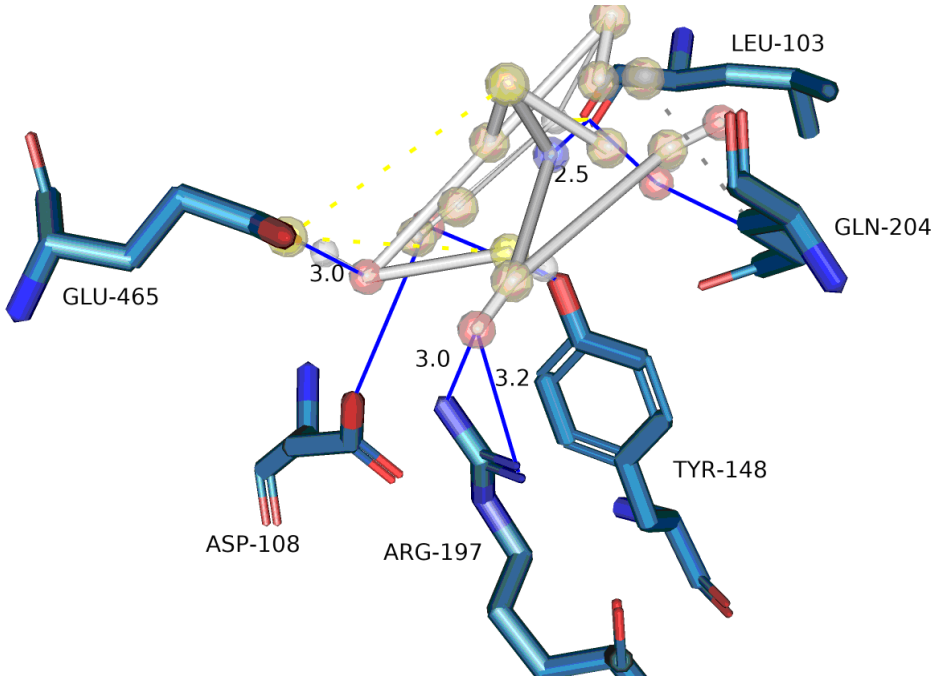


Fig. 11. The 3D contact map of penicilloic acid and HSA binding domain (constructed in PLIP tool). Hydrogen bonds are shown as thin blue solid lines, hydrophobic interactions are shown as gray dotted lines, and salt bridges are shown as yellow dotted lines.

Residues Arg209, Lys212, Val216, and Ala350 of HSA have been implicated in the binding process with penilloic acid and they are all located mainly in subdomain IIA, and in part in subdomain IIIA (Fig. 12, Fig. 13). In the case of penilloic acid, hydrogen bonds and hydrophobic interactions are available. Hydrophobic interactions are involved in the binding of Lys212 and Val216 to the minor determinant. Hydrogen bond is formed between the nitrogen of Arg209 and the hydrogen of the thiazolidine ring of the ligand.

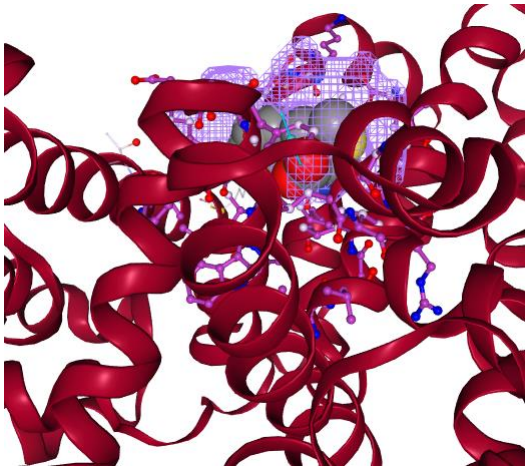


Fig. 12. The HSA-penilloic acid complex (constructed in Protein Plus). The structure of HSA is represented by ribbons; the ligand penilloic acid is represented as balls. The ligand binding pocket of HSA is marked in pale purple and has a depth of 20.46 Å. The surface area and volume of the binding site are 792.03 $Å^2$ and 357.38 $Å^3$, correspondingly. The hydrophobicity of the binding pocket is 0.74.

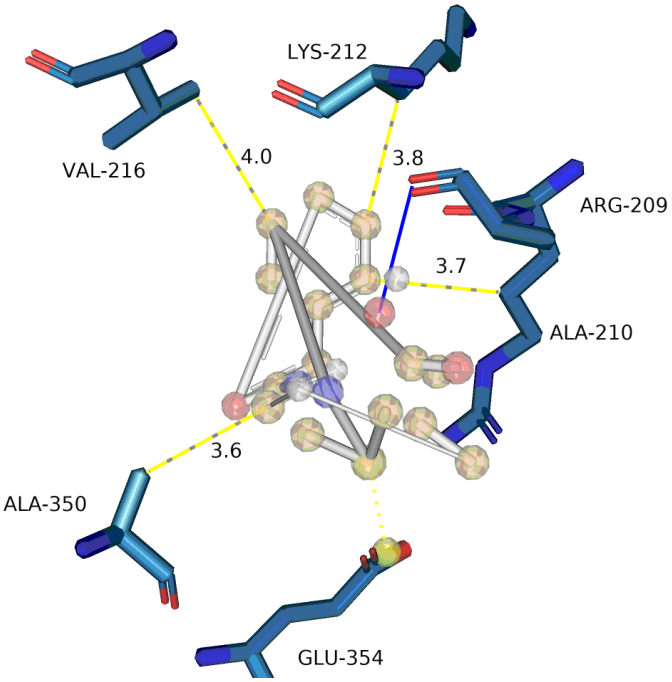


Fig. 13. The 3D contact map of penilloic acid and HSA binding domain (constructed in PLIP tool). Hydrogen bonds are shown as thin blue solid lines, and hydrophobic interactions are shown as yellow dotted lines.

HSA has structural homology (about 80 %) with bovine serum albumin (BSA) [9]. It has been reported that the β-lactam antibiotics have very similar binding properties to HSA and BSA [31]. The binding sites of the penicillin G to BSA are located in the subdomain IIA. In our study, all five penicillin G determinants were mainly inserted into the subdomain IIA by hydrogen bonds and hydrophobic interaction, while in the case of BSA, the same binding site exists but is stabilized by hydrogen bonds and Van der Waals forces. Similar to the penicillin G molecule, which binds to BSA in the subdomain IIA through hydrogen bonds (Arg194, Arg198, Arg256), benzyl penicilloyl G also binds to HSA at the subdomain IIA with the participation of Arg197, and Arg218 [32].

Yvon M., et al. has reported that benzyl penicilloyl G can also form stronger bonds between the carbonyl group and ε-amino groups of the HSA molecule, especially with Lys190, Lys195, Lys199, and Ser193, forming conjugates that have the immunogenic potential [33].

Our results have been confirmed by other studies. The fluorescence spectroscopy experiments of Seedher N., et al. [15] revealed that β-lactam antibiotics bind to HSA, and the

binding site is located in the subdomain IIA of HSA. This result confirms our molecular docking results. Similar to other studies, our results showed that hydrogen bonds and hydrophobic interaction were the driving forces for the spontaneous placement of penicillin G determinants in the HSA binding site, since the Gibbs free energy of complexes has minimal values -7.9 kcal/mol is our result of molecular docking of HSA-benzyl penicilloyl G vs. -6.4 kcal/mol is the result of molecular docking of HSA-sulfadimethoxine obtained by Zhang Y. and co-authors. [34]).

CONCLUSIONS

Today there is little published information about HSA-penicillin G determinants complexes. Therefore, in the presented study we elucidated the binding sites using molecular docking. The obtained results and their analysis allow us to make the following conclusions.

Penicillin G determinants binding to HSA occur with the participation of a few subdomains. The amino acid residues of subdomain IIA and subdomain IB, which are involved in hydrogen bonds and hydrophobic interactions have the main role in ligand binding; the amino acid residues of subdomain IIIA take part in binding partially.

The study of HSA-penicillin G determinants complexes is important in the pathogenesis of antibiotic allergy. Identification of specific binding sites can be useful for the development and synthesis of new immunogenic antigens (complexes of major and minor determinants of penicillin G with HSA) that can stimulate the immune system and produce specific antibodies to prevent allergic reactions.

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CONFLICT OF INTEREST

The authors report that there is no conflict of interest.

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МОЛЕКУЛЯРНИЙ ДОКІНГ СИРОВАТКОВОГО АЛЬБУМІНУ ЛЮДИНИ З ДЕТЕРМІНАНТАМИ ПЕНІЦИЛІНУ G

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Актуальність. Сироватковий альбумін людини (САЛ) є основним фармакокінетичним ефектором багатьох ліків, в тому числі пеніциліну G та його метаболітів. Гострою проблемою практичної медицини є реакції гіперчутливості негайного типу, які зумовлені токсичністю пеніцилінів (близько 8% проти інших препаратів), що супроводжуються патологією шкіри, анафілаксією та летальністю.

Мета роботи. Метою цього дослідження є опис структур комплексів САЛ-детермінанти пеніциліну G і виявлення сприятливих сайтів зв'язування та амінокислотних залишків, які залучені до взаємодії.

Матеріали та методи. Кристалічна структура САЛ (ID:1AO6 з Protein Data Bank) (www.rcsb.org) була вибрана як мішень для докінгу. Для отримання уявлення про взаємодію САЛ з основними (бензіл пеніцилоїл G, пеніциланова кислота) і другорядними (пеніциламін, пеніцилоєва кислота, пенілоєва кислота) детермінантами пеніциліну G були застосовані методи молекулярного докінгу (AutoDock Tools 1.5.7, AutoDock Vina 1.1.2). Візуалізація результатів докінгу була реалізована в PyMol 2.5. Для оцінки потенційних сайтів зв'язування був використаний Protein Plus сервер (https://proteins.plus). Для ідентифікації нековалентних взаємодій між САЛ та його лігандами був застосований засіб PLIP (https://plip-tool.biotec.tu-dresden.de).

Результати. Дані молекулярного моделювання свідчать, що основні детермінанти пеніциліну G беруть участь в утворенні водневих зв'язків з такими залишками САЛ, як Trp214, Arg218, His242 та Asn295; для другорядних детермінант — Asp108, His146, Tyr148, Ser193, Arg197, Gln204. Обидва типи детермінант розташовуються в гідрофобній порожнині субдоменів IIA та IB. Гідрофобні взаємодії присутні переважно між детермінантами пеніциліну G і амінокислотними залишками субдомену IIIA, такими як Ala350, Asp451, Tyr452 і Gln459.

Висновки. Вивчення комплексів САЛ-детермінанти пеніциліну G має важливе значення в патогенезі алергії на антибіотики. Виявлення специфічних сайтів зв'язування може бути корисним для розробки та синтезу нових імуногенних антигенів (комплексів основних і другорядних детермінант пеніциліну G із САЛ), які зможуть стимулювати імунну систему та виробляти специфічні антитіла для запобігання алергічної реакції.

КЛЮЧОВІ СЛОВА: сироватковий альбумін людини; детермінанти пеніциліну G; реакції гіперчутливості негайного типу; молекулярний докінг.