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COMPUTER SIMULATION OF COMPLEXATION OF BOVINE SERUM ALBUMIN WITH THIOHROME DYE

Introduction. ***Most groups of drugs contain cyclic π-conjugated fragments in their structure, which provide them with the ability to enter into various types of interactions. That is why the study of complexation of proteins with sensors with π-conjugated fragments, which are sensitive to the characteristics of the environment and are characterized by good spectral properties. Such enzymes can be markers of a particular type of interaction, and as a result, they can be functional elements that provide a particular binding of a medicinal agent.***

Methods. ***The parameters of complex formation of thiochrome dye with a protein molecule were studied using the methods of molecular dynamics and quantum chemistry.***

Results. ***The natural dye thiochrome was chosen as a sensor in the research process. It has a branched aromatic π-conjugated structure, absorption and luminescence in the range of active protein elements, and accordingly, can be a marker of a particular type of interaction and indicate the possibility of being used to create application drugs.***

Conclusions. ***Possible binding sites of the components were determined and changes in the electronic structure that can manifest in the optical spectra are predicted.***

Keywords: ***bovine serum albumin, tryptophan, thiochrome, conformations, electronic structure.***

Introduction

Biomolecular nanomaterials based on proteins are intensively studied due to their properties of forming ordered self-organized nanostructures, binding and recognizing the specific molecules and therefore could be used as functional materials for bionanotechnologies, the creation of application drugs, ecological

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biocatalytic boards, tissue engineering (Capezza, & Mezzenga, 2024; Pino et.al., 2024). A key fundamental question is how the functional agents are distributed in proteins pores and channels and what is the interaction. Most groups of drugs in their structure contain cyclic π-conjugated fragments, which often provide them with the ability to enter into various types of interactions. Therefore, the study of complex formation with proteins of sensors with π-conjugated fragments is interesting and important. In this work, the natural dye thiochrome, which has a branched aromatic π-conjugated structure, absorption and luminescence in the range of active elements of the protein, can be a marker of one or another type of interaction. In turn, bovine serum albumin is a well-studied protein that forms films with a crystalline structure, can be an effective prototype for creating the indicated doping matrices. The purpose of the work is to study the peculiarities of the formation of complexes based on bovine serum albumin with thiochrome and the electronic structure to establish the mechanisms of their interaction.

Methods

Molecular dynamics simulations for the biomolecules BSA and thiochrome dye were performed in NAMD programme package, molecular docking in Autodock 4.0. PyMOL and DoGSiteScorer were used for molecular visualization. Quantum chemichal calculations for the dye and spectral active aminoacid complexes were performed in Gaussian 09, (TD SCF, DFT 6-31G).

Results

In our experimental study of the the electronic structure of films based on BSA and thiochrome, we have identified a probable binding site of thiochrome within the protein and have assumed formation of a complex involving parallel π-π* stacking between tryptophan and thiochrome (Gaponov et al., 2024a; 2024b). The absorption maxima of composite BSA protein films with thiochrome shift relative to the maxima typical for films of individual components. Subsequent analysis of the vibrational structure of the film showed minor shifts in the amide I (2 cm^{-1}) and amide II (8 cm^{-1}) bands towards lower frequencies upon the addition of thiochrome to BSA.

We have performed molecular docking simulations to complement the experimental data, providing possible conformations of the ligand within the protein. Let consider the affinity of different conformations of the thiochrome ligand to the BSA protein target using molecular docking. This will help to understand the mechanisms of their interaction and indicate the most likely conformations for quantum chemical research. As a result of the simulations, eight variants of ligand placement were predicted. We identified one conformation in subdomain IB, near tryptophan 134 and another one is in subdomain IIIB (Fig. 1).

Table 1. provides the data of 8 conformational states for the BSA-thiochrome system: the binding energy, charachteristics of non-covalent interactions: namely hydrophobic interactions and hydrogen bonds of amino acid residues with the thiochrome dye.

Molecular dynamics simulations (NAMD) over 3 ns revealed that thiochrome's position significantly affects complex stability in subdomain IB, near the tryptophan residue 134 (Fig. 2). In contrast, thiochrome's position in subdomain IIIB was less stable due to its placement in a more hydrophobic pocket.

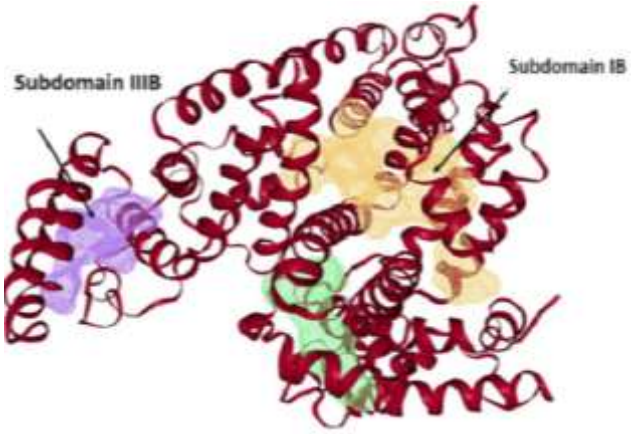

Fig. 1. Visualization of the three ligand binding sites in the BSA protein (coloured with yellow, green and purple) predicted by the DoGSiteScorer algorithm and visualization of the location of thiochrome conformations in PyMol in these subdomains

Table 1

Binding energy E_b, hydrophobic interactions and hydrogen bonds of amino acid residues with the thiochrome dye for BSA-thiochrome system obtained by molecular docking

Stable conformer	E_b, kcal/mol	Hydrogen bonds	Hydrophobic interactions with amino acid residues:
1	-7.27	Phe-506	Phe-506, Lys-524, Leu-528, Val-546, Phe-550, Leu-574, Val-575, Thr-578
2	-7.18	Val-575	Phe-506, Phe-508, Ala-527, Leu-528, Leu-754, Val-575, Thr-578
3	-6.92	Tyr-400	Tyr-400, Lys-524, Leu-528, Val-546
4	-6.54	Pro-113, Leu-115, Tyr-137, Arg-144	Lys-114, Ile-141, Tyr-160, Ile-181, Met-184, Arg-185
5	-6.14	Lys-132, Tyr-160	Asp-129, Lys-132, Lys-136
6	-6.11	Lys-350, Leu-480	Ala-212, Asp-323, Leu-326, Leu-346, Lys-350, Leu-480,
7	-6.09	Pro-298, Leu-301, Leu-304, Arg-336	Ile-297, Leu-301, Leu-304, Arg-336
8	-6.02	Phe-133, Tyr-160	Tyr-137, Glu-140, Ile-181, Arg-185

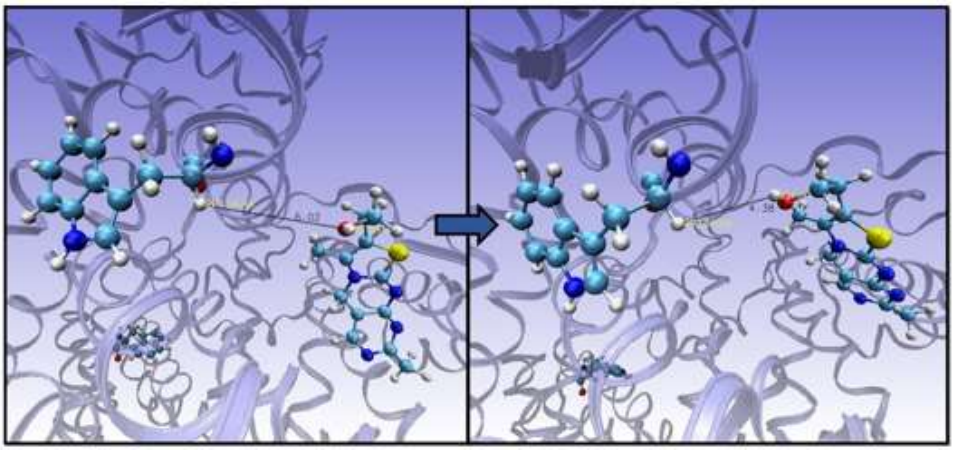

Fig. 2. One of the simulations of changes in the location of thiochrome in the complex with BSA during molecular dynamics

Since the absorption of the BSA protein is mainly caused by tryptophan, let analyze electron transitions with this amino acid. The main electronic transition for thiochrome, tryptophan and the complex is determined by the HOMO-LUMO transition (Gaponov et al., 2024a). Quantum chemical modeling (TD SCF, DFT 6-31G) of the interaction between tryptophan and thiochrome indicated a correlation between the absorption maximum shift and the intermolecular distance. For π-π* stacking at a distance of 3.5 Å, a red shift of 9 nm was observed, which decreased to 1 nm at a distance of 4.5 Å and disappeared at greater distances.

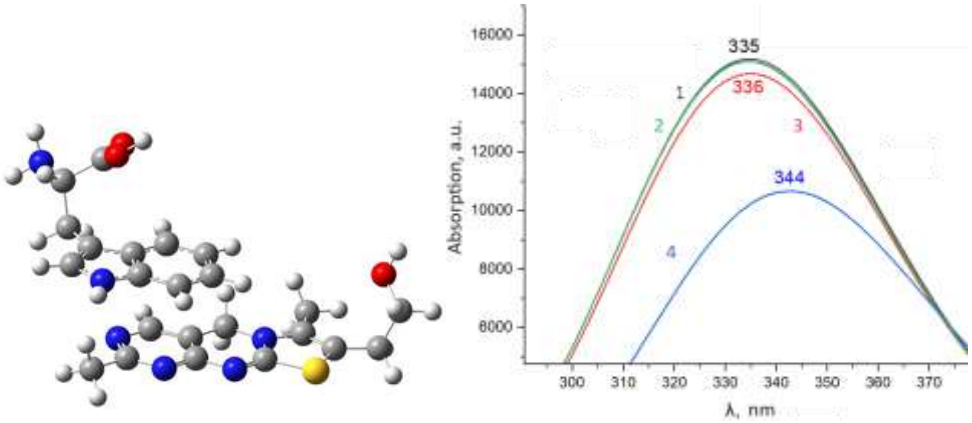

Fig. 3. Calculated spectra of tryptophan-thiochrome complex (on the left side of the fig.3) at different distances *d* between the components:
1 – thiochrome, 2 – *d* = 9 nm, 3 – *d* = 4,5 nm, 4 – *d* = 3,5 nm,
method of calculation TD SCF (DFT, 6-31G)

The obtained data are in good agreement with our experimental spectra (Gaponov et al., 2024a; 2024b), therefore we can conclude that thiochrome is located near tryptophan, according to molecular dynamics simulations, and affects the optical spectra of the protein. These findings, together with experimental data and results from molecular docking and dynamics, suggest that the most probable location of thiochrome is the pocket in BSA subdomain IB.

Discussion and conclusions

Molecular docking results show on two thiochrome binding sites in the protein. The position of the thiochrome in the protein significantly affects the stability of the complex: the higher stability is observed for position, where the thiochrome is located in less hydrophobic region of the protein. Quantum-chemical modeling of the tryptophan-thiochrome complex confirmes their interaction. The studies are important for the use of thiochrome in biosensing of proteins.

Authors' contribution: Anastasia Ryzhkova – calculations, analysis, revision and editing; Andriy Momot, Anton Gaponov – data validation, writing, editing; Olena Pavlenko, Oksana Dmytrenko – conceptualization, methodology, revision and editing.

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References

Capezza, A.J., & Mezzenga, R. (2024). Proteins for Applied and Functional Materials. *Biomacromolecules*, 25, 4615–4618. https://doi.org/10.1021/acs.biomac.4c00884

Gaponov, A.M., Pavlenko, O.L., Dmytrenko, O.P., Kulish, M.P., Ryzhkova, A.S., Lesiuk, A.I., Obernikhina, N.V., Łuszczyńska, B., & Kachkovsky, O.D. (2024a). Molecular heteroassociation in films of thiochrome and tryptophan. *Molecular Crystals and Liquid Crystals*, 768(3), 71–77. https://doi.org/10.1080/15421406.2023.2257515

Gaponov, A.M., Ryzhkova, A.S., Pavlenko, O.L., Dmytrenko, O.P., Kulish, M.P., Lesiuk, A.I., Kolomys, O.F., Obernikhina, N.V., & Kachkovsky, O.D. (2024b). Spectral features of films of bovine serum albumin with thiochrome. *Molecular Crystals and Liquid Crystals*, 1–6. https://doi.org/10.1080/15421406.2024.2355394

Pino, P., Vigani, B., Valentino, C., Ianev, D., Ruggeri, M., Boselli, C., ... & Rossi, S. (2024). Sustainable whey proteins-nanostructured zinc oxide-based films for the treatment of chronic wounds: New insights from biopharmaceutical studies. *International Journal of Biological Macromolecules*, 263, 130655. https://doi.org/10.1016/j.ijbiomac.2024.130655

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КОМП'ЮТЕРНЕ МОДЕЛЮВАННЯ КОМПЛЕКСОУТВОРЕННЯ БИЧАЧОГО СИРОВАТКОВОГО АЛЬБУМІНУ З БАРВНИКОМ ТІОХРОМОМ

Вступ. ***Більшість груп лікарських препаратів у своїй структурі містять циклічні π-спряжені фрагменти, які забезпечують їх можливість вступати в різні типи взаємодій. Саме тому дослідження комплексоутворення білків з сенсорами з π-спряженими фрагментами, які чутливі до особливостей навколишнього оточення і володіють гарними спектральними властивостями, можуть бути маркерами того чи іншого виду взаємодії і, як наслідок, функціональними елементами, що забезпечують те чи інше зв'язування лікарського агента.***

Методи. ***У цій роботі дослідження проводилися за допомогою декількох типів комп'ютерних моделювань, зокрема таких, як молекулярний докінг, молекулярна динаміка та квантова хімія.***

Результати. ***Як сенсор у процесі дослідження було обрано природний барвник тіохром, що має розгалужену ароматичну π-спряжену будову, поглинання та люмінесценцію в діапазоні активних елементів білка, відповідно, може бути маркером того чи іншого виду взаємодії і свідчити про можливість бути використаним для створення аплікаційних ліків.***

Висновки. ***Отримані дані відкривають шлях для подальших досліджень механізмів взаємодії π-спряжених молекул з білками.***

Ключові слова: ***бичачий сироватковий альбумін, триптофан, тіохром, конформації, електронна структура.***

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