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ANALYSIS OF THE INTERACTION BETWEEN HYDROGEN SULFIDE AND RECEPTORS OF HYPOXIA-INDUCABLE FACTORS THROUGH MOLECULAR DOCKING: AN IN-SILICO EXPERIMENT

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Abstract: Hydrogen sulfide (H2S) is a small gaseous molecule that, despite being known as a toxic and smelly gas derived from decomposition processes, has physiological properties similar to other gases in the human body, such as nitric oxide, acting as a gasotransmitter. Animal studies have demonstrated that H2S has an anti-inflammatory role during systemic infections and periods of physiological stress. Hypoxia-inducible factors (HIFs) are critical regulators of human homeostasis, managing oxygen supply and demand to cells. This system is frequently exploited by cancer cells, as its hyperactivation promotes gene transcription, cell division and angiogenesis, facilitating nutrition and cancer dissemination. Molecular docking is a technique that positions two individual structures and simulates their interactions, classifying different possibilities of connection. This experimental tool directs in vitro and in vivo studies, minimizing the risk of failure. The present work consists of an in silico experimental study that investigated the interaction between H2S and HIF receptors through molecular docking simulations. The PDB codes 1h2k, 1h2l, 1h2m, 30d4, 2ilm, 5tbm, 3f1n, 6x28, 4wn5 and 7v7w were used to simulate HIFs receivers. Hydrogens were added separately to receptors and ligands, and Gasteiger charges were calculated using ADT software, including nonpolar hydrogens. The dimensions of the grid box were set to 30 Å for each axis, positioned at the coordinates of the atoms in the active site region and at the interface. In the experiments, a minimum binding energy of 0.9 kcal/mol was observed between H2S and the tested HIF receptors, indicating a reduced interaction of the gas with the receptor, in contrast to the existing literature. Possible explanations include limitations of computational models and the simplification of molecular docking mechanisms, which may not capture all

interactions between H2S and HIFs. The choice of predefined coordinates in a specific active site of the proteins and variations in the three-dimensional structure of the receptors and H2S can also influence the binding affinity results. Therefore, there is a need for more studies to understand the metabolic pathway of HIF receptors and the influence of H2S in inhibiting this pathway and combating oxidative stress. Despite the low affinity observed between H2S and HIF in the molecular docking study, these results are significant and offer valuable insights, contributing to knowledge about the interaction between H2S and HIF. They indicate that, under the conditions tested, there is no strong connection between the two entities, suggesting the need for additional investigations to explore other possible interactions between H2S and HIF in different experimental conditions or considering other factors, such as post-translational modifications of the proteins.
Keywords: Molecular Docking. Computational Simulation. Oxidative stress

INTRODUCTION

Hydrogen sulfide (H2S) is a small gaseous molecule that, although popularly known as a toxic gas, producing bad odors and derived from decomposer processes, has physiological properties similar to other gases present in the human body, such as oxide. nitric acid, giving it the function of a gasotransmitter, since they are produced endogenously, they can cross cell membranes freely, due to their size, and act as signaling metabolic responses. Animal studies have proven an anti-inflammatory role for H2S during systemic infections and times of physiological stress. Another benefit of this compound is the ability to reduce damage to brain tissue from reactive oxygen species (ROS) as well as generating a vasodilatory effect on the cardiovascular system. (POWELL; DILLON; MATSON, 2018).

Hypoxia-inducible factors (HIFs) are important regulators of human homeostasis, as they act in the management, supply and demand of oxygen to each of the 50 trillion cells in the human body. Because of this, this system is often targeted by cancer cells as its hyperactivation leads to increased gene transcription, cell division and angiogenesis, which are essential for nutrition and the spread of cancer. Furthermore, HIFs influence intestinal motility, remodeling of the extracellular matrix, skin regeneration, glucose and lipid metabolism, as well as control of the immune system, among other functions (WICKS; SEMENZA, 2022).

Molecular docking is a technique that involves positioning two individual structures, followed by a series of simulations. Its objective is to look for conformations that vary the bonding modes between these structures, providing a classification system that punctuates the different possibilities. During this process, small changes occur in the center of gravity, orientation and rotation of the structures. This approach aims to find the system with the lowest energy expenditure, thus distinguishing the compounds with greater and lesser affinity to the selected receptors. It is also noteworthy that this tool is a valuable experimental alternative, as it can more precisely direct in vitro and in vivo studies, reducing the risk of failures. Furthermore, it requires a significantly lower initial investment compared to the operating costs of conventional experiments. The computational tools used in docking have a high degree of precision, which can accelerate both the development of new drugs and the understanding of the physiology of certain experiments. (AFONSO, 2019; BARBOSA, 2013).

In this sense, this research aims to increase the understanding of the action of H2S in the

human body, through simulation tools, as well as to investigate a possible inhibitory activity of H2S in HIFs.

METHODOLOGY

This is an experimental study based on an in-silico model. In this study, the PubChem website was used as a source to obtain the chemical structures of H2S ligands in SDF format. The three-dimensional conformation of the HIF 1 receptor was extracted from the online protein database Protein Data Bank (PDB), using the PDB identification codes 1h2k, 1h2l, 1h2m, 30d4, 2ilm, 5tbm, 3f1n, 6x28, 4wn5 and 7v7w. This data was analyzed with the help of Discovery Studio Visualizer V21.1.0.20298.

During the analysis, the binding coordinates of the HIF receptor were identified, and then the Chimera program was used to purify the protein. To carry out the simulations, the Vina AutoDock program was used. The preparation of ligands and proteins for calculations was conducted using AutoDock Tools (ADT) 1.5.6. (TROTT et al., 2010). Hydrogen atoms were added separately to the receptors and ligands. Gasteiger charges were further calculated using ADT software, and nonpolar hydrogens were included. The dimensions of the grid box were established as 30 Å for each axis, being positioned at the coordinates of the remaining atoms present in both the active site region and the interface region. The parameter for the number of modes was set to a value of 50, while the parameter for exhaustivity was set to 24. A series of twenty molecular docking calculations were performed, since establishing more accurate values for the energy of binding (ΔGbind) is recognized as a crucial factor in evaluating the efficiency of interactions. (MORRIS et al., 2009). Regarding the literature review to complement the discussion, the Virtual Health Library (VHL) and Pubmed databases were used.

RESULTS

Ligand-Protein Complex (CLP)	ΔG bind[a] ($kcal.mol^{-1}$)
H2S-1h2k	-0,9
H2S- 1h2l	-0,8
H2S – 1h2m	-0,8
H2S- 3od4	-0,7
H2S – 2ilm	-0,8
H2S – 5tbm	-0,6
H2S – 3f1n	-0,9
H2S – 6x28	-0,7
H2S – 4wn5	-0,8
H2S – 7v7w	-0,8

Subtitle: ΔG bind[a] ($kcal.mol^{-1}$) = Lowest binding energy within the 20 simulations performed on each ligand – protein complex.

It is noteworthy that 10 protein complexes linked to hydrogen sulfide gas were analyzed, which were extracted from the protein data bank website. HIF activity is regulated by oxygen-dependent hydroxylation. Under normoxic conditions, hydroxylation of proline residues triggers the destruction of its alpha subunit, while hydroxylation of Asn (803) in the C-terminal transactivation domain of HIF-1 alpha (CAD) prevents its interaction with p300. (ELKINS et al., 2003). It is known that the HIF binding complex with the p300 protein contributes to the nutritional angiogenesis of tumors such as breast cancer, and therefore its inhibition is the target of possible therapies for neoplasms (VLEUGEL et al., 2006). The first protein to be selected for docking with H2S was 1h2k, the second was 1h2l and the third 1h2m, both consist of crystalline structures of asparagine hydroxylase (HIF factor inhibitor, FIH) complexed with Fe ((II)), 2-oxoglutarate cosubstrate and CAD fragments (C-terminal of HIF 1 alpha), therefore, are examples of a HIF inhibition pathway and when applying them to an anchorage, the affinity of H2S with these pathways is verified. The fourth protein encoded by the nickname 3od4 is a human

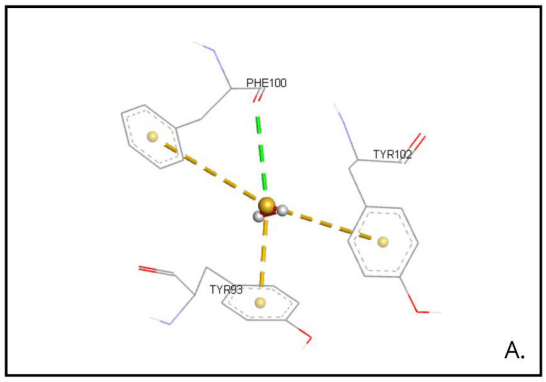
peptidylprolyl isomerase FKBP51 (FK506-binding protein 51). FKBP51 is a protein belonging to the family of peptidylprolyl isomerases that plays a role in modulating several cellular pathways, including the response to cellular stress and steroid hormone signaling. It is noteworthy that it has inhibitory activity in relation to HIF-1 alpha and its pathway has 8-hydroxyquinoline-5-carboxylic acid as its protagonist. The fifth protein to be complexed with H2S was 2ilm, the protagonists of which are 2-oxoglutaric acid, glycerol, sulfate ion, bicarbonate ion and Fe II ion. Such a protein from its iron-using pathway has the potential to affect HIF alpha in an inhibitory manner. A structure of dimeric HIF in complex with Fe(II), 2OG and HIF-1α revealed that HIF's side chain carboxylate Asp-201 not only coordinates iron through one oxygen, but that its other oxygen is positioned to accept a bond of hydrogen from a water molecule linked to Fe(II); Upon substrate binding, the water interaction is apparently weakened because Asp-201 is repositioned to form a hydrogen bond with the amide nitrogen of the HIF-1α Asn-803 backbone, as observed in the crystal structures. (HEWITSON et al., 2008).

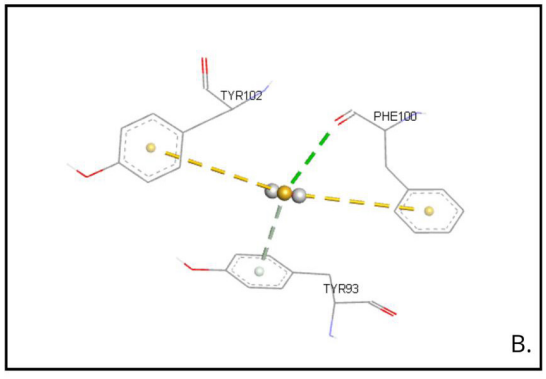
It is known that HIF2α is involved in the emergence of clear cell renal cell carcinoma (CCRCC), around 90% of this type of carcinoma exhibit inactivation of the Von Hippel-Lindau tumor suppressor gene (PVHL). Inactivation of pVHL results in the stabilization of the hypoxia-inducible transcription factors HIF1α and HIF2α, leading to the expression of a genetic program essential for the initiation and progression of CCRCC. Thus, the sixth protein to be used as a receptor in docking was 5tbm, which contains the compound PT2385 in its composition, which is a potent and selective molecule inhibitor for HIF2α. Its specific HIF2α antagonist is based on the allosteric blockade of HIF2α dimerization. This protein present in the 5tbm complex inhibited the expression of HIF2α-dependent genes, including VEGF-A, PAI-1 and cyclin D1 in CCRCC cell lines and tumor xenografts. Treatment of tumor-bearing mice with PT2385 caused dramatic tumor regressions, validating HIF2α as an essential oncogenic factor in CCRCC. (WALLACE et al., 2016). This pathway is proven to be effective in combating a type of renal cell carcinoma, which is why we tested the interaction of H2S with it.

The seventh protein complex to be used was 3f1n, which has the ability to inhibit transcriptions of the HIF 2 pathway, reducing its action. This protein complex has 1-2-ethanediol as its main ligand. (SCHEUERMANN et al., 2009). Similar to the seventh, the eighth selected protein, 6x28, has the ability to destabilize the HIF pathway, it consists of a protein complex based on the combination of PT2243 with the HIF2α complex, its main ligand is the polymer (1R)-4-(3,5-difluorophenoxy)-7-(trifluoromethyl)-2,3-dihydro-1H-inden-1-ol. (MADEJ et al., 2014).

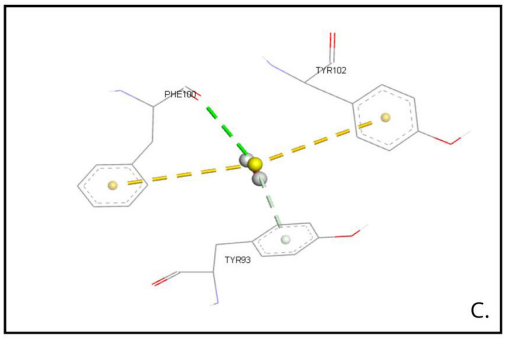
The ninth protein selected to form an anchoring complex with H2S was 4wn5, unlike the others, it represents the HIF3α receptor, which, although insensitive to intracellular oxygen levels and incapable of transactivation, is a dominant negative regulator of HIF-1α. Due to the use of different transcription initiation sites and a combination of splicing events in HIF3α, such a protein complex is based on the C-terminal Per-Arnt-Sim (PASb) sensor domain. The PASb domains on HIF-α subunits play a crucial role in the formation of active HIF heterodimers and the recruitment of coregulators. A link with fatty acids and lipid compounds that can influence this regulatory agent was found in the 4wn5 protein. The main lipid agent of this protein is Monovaccenin. (FALA et al., 2015).

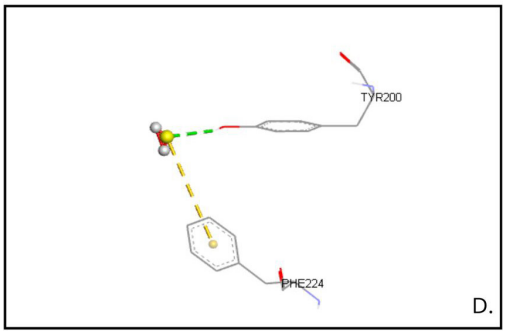
Furthermore, the last docking carried out was based on the 7v7w protein complex, this complex has an endogenous lipid oleolethanolamide (OEA) as a ligand capable of selectively connecting to HIF-3α. OEA is a natural ethanolamide produced in the small intestine after eating and regulates satiety and body weight. Thus, studies reveal that HIF-3α acts as a selective lipid sensor that may be related to obesity, as well as that endogenous metabolites can interact directly with HIF-α proteins to modulate their activities. In this sense, although less studied, HIF-3α exerts a strong influence on other HIF receptor pathways. (DIAO et al., 2022).



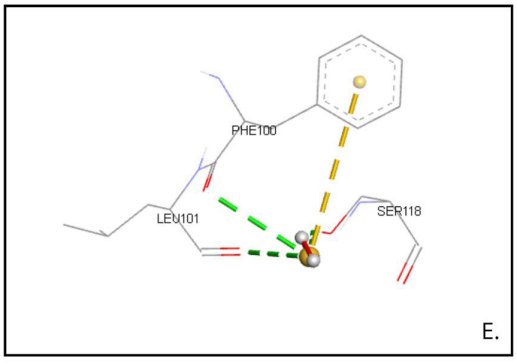


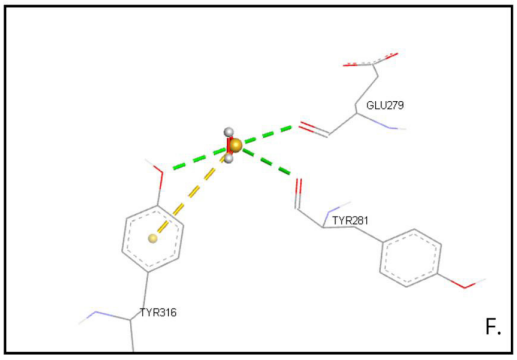
Subtitle: A represents the ligand complex – H2S-1h2k protein; B represents H2S- 1h2l. TYR102, PHE100 and TYR93 represent the amino acids involved in anchoring.



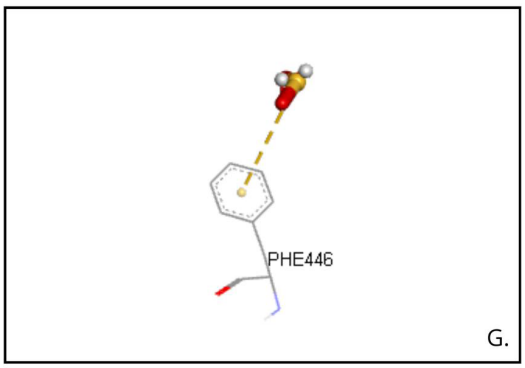


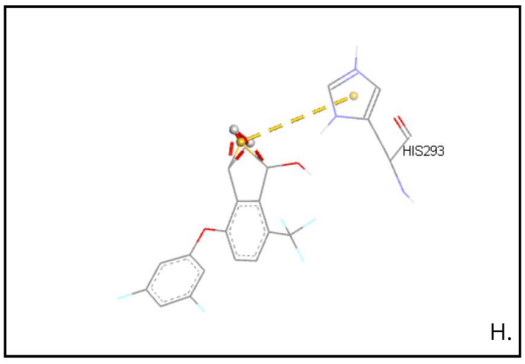
Subtitle: C represents the PLC H2S – 1h2m; D is equivalent to CLP H2S- 3od4; TYR102, PHE100, TYR93, TYR 200 and PHE 224 represent the amino acids involved in anchoring.



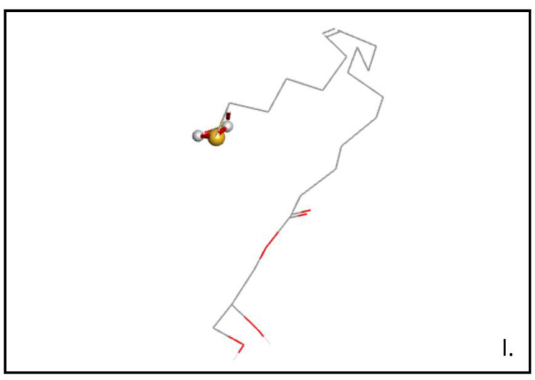


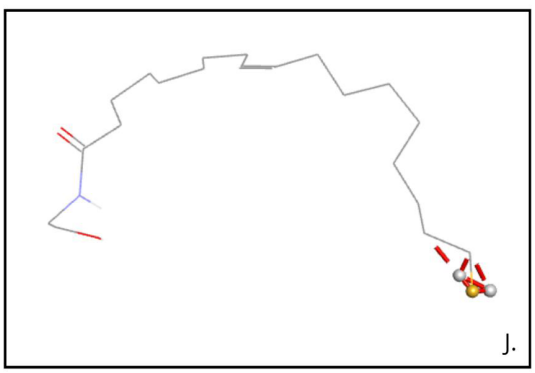
Subtitle: And represents the PLC H2S – 2ilm; F represents the PLC H2S – 5tbm; PHE100, LEU 101, SER 118, GLU 279, TYR 316, TYR 281 represent the amino acids involved in anchoring.





Subtitle: G consists of the PLC H2S – 3f1n; H is equivalent to CLP H2S – 6x28; PHE 446 and HIS 293 were the amino acids covered by the CPL.





Subtitle: 1 is equivalent to CLP H2S – 4wn5; J is equivalent to CLP H2S – 7v7w.

From these results it can be seen that in the experiment obtained the lowest binding energy of H2S with the HIF receptor experimented was 0.9 kcal/mol obtained from the protein complexes 1h2k and 3f1n. This result revealed a low interaction of the gas studied with this receptor, which differs from the literature studied. This contrast can be explained by some situations such as: limitation of the applied computational models, the molecular docking models are simplifications of reality and may not have captured all the interactive nuances between H2S and HIFs since in the experiment a simulation was performed with pre-defined coordinates in an active site of a certain HIF inhibitory protein such as 3od4, there is the possibility that the HIF interaction is based on another mechanism. Another possibility is that docking models depend on the availability of accurate three-dimensional structures for both the ligand and receptor, so if the files used do not accurately reflect the actual structure of HIF or H2S there may be low affinity as a result.

Furthermore, there is the possibility that the effect of H2S depends on a cytokine-based environment, since its antioxidant effect was visualized through the attenuation of the release of pro-inflammatory cytokines such as TNF and interleukin 6 in macrophages that expressed HIF 1 (LOHNINGER et al., 2015).

CONCLUSION

It is important to highlight the need for further studies to understand the metabolic pathway of HIF receptors, as well as the influence of H2S in inhibiting this pathway and combating oxidative stress. Although the results of the molecular docking study indicated a low affinity between H2S and HIF, it is important to highlight the significance of these results in concluding the study. These findings offer valuable insights and contribute to the growing body of knowledge

in the area of the interaction between H2S and HIF. Furthermore, even though low affinity was observed, this result provides crucial information about the interaction between H2S and HIF. It clarifies that, at least under the conditions tested, there is no strong connection between these two entities. The results also suggest areas for further investigation, as additional studies may be needed to explore other possible interactions between H2S and HIF under different experimental conditions or considering other factors, such as post-translational modifications of proteins.

It is important to recognize the limitations of the molecular docking study, such as the simplifications inherent to the computational model and the specific experimental conditions used. This helps to contextualize the results and provide insights into possible directions for future research.

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