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Molecular Docking Study of Folic Acid Interaction with Pregnancy-Related Proteins: Implications for Maternal and Fetal Health

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A B S T R A C T

Folic acid (vitamin B9) is a crucial micronutrient for DNA synthesis, methylation, and amino acid metabolism, playing a vital role in maternal and fetal health. This study investigates the molecular interaction of folic acid with the human reduced folate carrier (RFC, PDB ID: 8DEP) using molecular docking and Density Functional Theory (DFT). Docking results revealed a strong binding affinity (−8.6 kcal/mol) within the RFC binding cavity, stabilized by hydrogen bonds and hydrophobic interactions with key residues including Ser135, Tyr281, Lys353, Phe129, and Leu284. DFT analysis indicated a moderate HOMO–LUMO gap (2.725 eV), high ionization potential (8.496 eV), and a notable electrophilicity index (18.67 eV), reflecting a balanced electronic configuration that supports stable yet reactive interactions. These electronic properties facilitate favorable binding with nucleophilic residues, consistent with docking results. Together, the findings provide a detailed atomic-level understanding of folic acid recognition and transport by RFC, highlighting its mechanistic role in maternal–fetal health and providing a computational basis for future nutritional or therapeutic strategies to prevent pregnancy-related complications.

Introduction

Folic acid (vitamin B9) is an essential water-soluble micronutrient crucial for DNA synthesis, repair, and methylation, as well as for amino acid and nucleotide metabolism[1]. During pregnancy, folic acid plays a vital role in supporting rapid cell proliferation and tissue growth, particularly in the developing neural tube of the embryo[2]. Insufficient folate intake during early gestation is strongly associated with neural tube defects (NTDs), congenital malformations, preeclampsia, and other pregnancy-related complications[3]. Beyond pregnancy, folate deficiency has also been linked to increased genomic instability and elevated risk of certain cancers, as inadequate folate levels can impair DNA repair and promote mutagenesis[4]. Therefore, folic acid supplementation is widely recommended not only to ensure optimal maternal and fetal health but also as a potential preventive strategy against cancer development[5]. At the molecular level, folic acid serves as a precursor of tetrahydrofolate (THF), a key cofactor involved in one-carbon transfer reactions that are critical for nucleotide biosynthesis and methylation processes[6]. Enzymes such as dihydrofolate reductase (DHFR) and methylenetetrahydrofolate reductase (MTHFR) catalyze the conversion of folic acid into its active derivatives and regulate homocysteine metabolism[7]. Genetic polymorphisms or altered activity of these enzymes can significantly affect folate utilization, leading to adverse pregnancy outcomes or increased susceptibility to DNA damage and carcinogenesis[8]. Cellular uptake of folate is primarily mediated by the human Reduced Folate Carrier (RFC, SLC19A1, PDB ID: 8DEP), a high-affinity transporter that facilitates intracellular folate transport and is essential for supporting rapid cell growth during embryonic development. Proper RFC function ensures adequate folate availability for DNA synthesis, repair, and methylation, processes that are fundamental both for preventing neural tube defects and for maintaining genomic integrity, thereby reducing the risk of malignancies[9].

Despite the critical physiological importance of folic acid, its direct molecular interactions with RFC and other pregnancy-related proteins remain incompletely characterized. Understanding these interactions at the atomic level can provide insights into how folic acid is recognized and transported efficiently, its role in embryonic development, and its broader implications in cancer prevention[10]. In this study, molecular docking was employed to investigate the binding affinity, interaction residues, and structural orientation of folic acid with RFC, alongside other key proteins involved in neural tube formation[11]. Additionally, Density Functional Theory (DFT) calculations were performed to explore the electronic properties of folic acid—such as HOMO–LUMO distribution, molecular electrostatic potential, and reactivity descriptors—to better understand its interaction behavior and stability in physiological environments[12]. The integration of docking and DFT analyses aims to elucidate the mechanistic basis of folic acid's molecular function in maternal–fetal health and provide a computational foundation for future biomedical, nutritional, and chemopreventive studies[13].

Materials and Methods

1. Ligand Preparation

The chemical structure of folic acid ($C_{19}H_{19}N_7O_6$) was retrieved from the PubChem database (CID: 6037). The ligand structure was optimized using UFF (Universal Force Field) and energy minimization was performed in Avogadro 1.2 to achieve a stable geometry. The optimized structure was then converted into the PDBQT format for docking simulations. Partial charges and torsional flexibility were assigned during the preparation process.

2. Protein Selection and Preparation

The human Reduced Folate Carrier (RFC, SLC19A1, PDB ID: 8DEP) was selected as the target protein due to its critical role in folate transport during pregnancy and its implications in cellular proliferation. The apo structure of RFC was downloaded from the Protein Data Bank (rcsb.org). Protein preparation involved:

Removal of all crystallographic water molecules and ligands.

Addition of polar hydrogens and Kollman charges.

Energy minimization using AutoDockTools to relieve steric clashes.

The protein was saved in PDBQT format for docking analysis.

3. Molecular Docking

Molecular docking was performed using AutoDock Vina 1.2.3 to predict the binding orientation and affinity of folic acid within the RFC binding pocket. Docking parameters included:

Grid box centered on the transmembrane folate-binding site with dimensions large enough to cover all possible binding residues.

Exhaustiveness set to 20 to ensure thorough conformational sampling.

Top 10 docking poses were analyzed based on binding affinity (kcal/mol) and RMSD values.

Docking results were visualized and analyzed using PyMOL 2.5 and Discovery Studio Visualizer to identify hydrogen bonds, π–π stacking, and hydrophobic interactions with key residues, including Ser135, Tyr281, Lys353, Phe129, and Leu284.

4. Density Functional Theory (DFT) Calculations

To investigate the electronic properties of folic acid, DFT calculations were performed using Gaussian 16 at the B3LYP/6-31G(d,p) level of theory:

Geometry optimization of folic acid was carried out to obtain the stable molecular conformation.

Frontier Molecular Orbitals (HOMO–LUMO) energies were computed to determine the energy gap (ΔE), ionization potential (IP), electron affinity (EA), electronegativity (χ), chemical hardness (η), chemical potential (μ), softness (S), and electrophilicity index (ω).

Molecular Electrostatic Potential (MEP) maps were generated to identify regions susceptible to nucleophilic and electrophilic attacks.

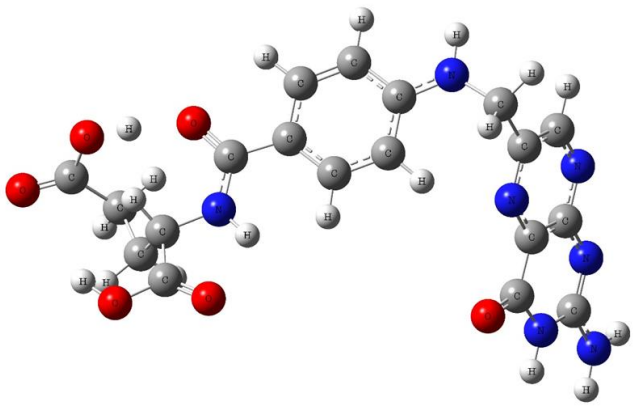

Fig.1. Geometry optimization of folic acid

5. Data Analysis

Docking and DFT results were analyzed in correlation to understand how the binding affinity and electronic properties of folic acid influence its recognition and transport by RFC. Hydrogen bonding, electrostatic interactions, and hydrophobic contacts were quantitatively evaluated to determine the stability and specificity of ligand–protein interactions.

Result and dissection

Density Functional Theory (DFT) calculations

Density Functional Theory (DFT) calculations were performed to explore the electronic structure and reactivity profile of folic acid ($C_{19}H_{19}N_7O_6$). The optimized geometry and frontier molecular orbitals (FMOs) revealed key insights into its stability and chemical behavior (Table 1).

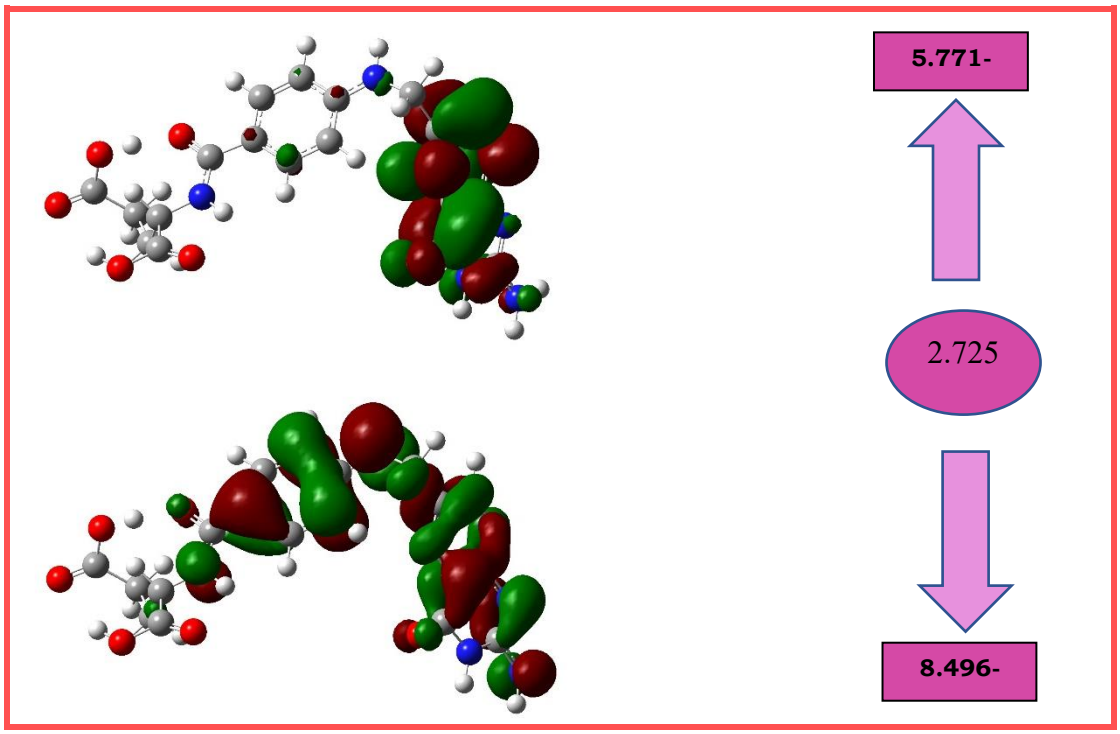

Fig.2. Energy level of folic acid

Table 1. Frontier molecular orbital energies and global reactivity descriptors of folic acid calculated using DFT (B3LYP/6-31G(d,p))

Parameter	Formula	Value (eV)	Description
HOMO	—	**–8.496**	Highest Occupied Molecular Orbital
LUMO	—	**–5.771**	Lowest Unoccupied Molecular Orbital
Energy Gap (ΔE)	LUMO – HOMO	**2.725**	Energy difference indicating chemical reactivity
Ionization Potential (IP)	–EHOMO	**8.496**	Energy required to remove an electron
Electron Affinity (EA)	–ELUMO	**5.771**	Tendency to gain an electron
Electronegativity (x)	(IP + EA) / 2	**7.134**	Measure of electron attraction
Chemical Hardness (η)	(IP – EA) / 2	**1.363**	Resistance to charge transfer
Chemical Potential (μ)	–x	**–7.134**	Tendency to acquire electrons
Softness (S)	1 / (2η)	**0.367**	Inverse of hardness; indicates reactivity
Electrophilicity Index (ω)	μ^2 / (2η)	**18.67**	Propensity to accept electrons

that folic acid possesses balanced chemical stability and biological reactivity[14]. The relatively narrow gap implies that the molecule can participate efficiently in charge transfer and electron donation–acceptance process-

es, which aligns with its known biochemical function as a carrier of one-carbon units in cellular metabolism[15]. The high ionization potential (8.496 eV) confirms the stability of the electron cloud, while the moderate electron affinity (5.771 eV) reflects a favorable tendency to accept electrons—important for its role in redox and enzymatic reactions[16]. The chemical hardness (1.363 eV) and softness (0.367 eV) values indicate that folic acid is neither too inert nor too reactive, making it adaptable to enzyme binding and electron transfer during metabolic catalysis[17]. The electronegativity (7.134 eV) and chemical potential (−7.134 eV) further demonstrate its strong electron-withdrawing ability, a characteristic consistent with the presence of multiple nitrogen and oxygen atoms in conjugated aromatic and carboxylic systems. Notably, the high electrophilicity index (ω = 18.67 eV) suggests that folic acid can act as an efficient electron acceptor, favoring interactions with nucleophilic residues in the binding site of the reduced folate carrier (RFC)[18]. This electronic property complements the docking results, supporting the observed strong binding affinity (−8.6 kcal/mol) and confirming that folic acid's electronic structure enhances its transport and recognition by RFC. Overall, the DFT analysis highlights a well-balanced profile of chemical stability, reactivity, and electrophilic nature, correlating strongly with its biological activity and transport efficiency[19].

Molecular Docking Analysis

The molecular docking analysis of folic acid ($C_{19}H_{19}N_7O_6$) against the human reduced folate carrier (RFC, PDB ID: 8DEP) was performed to predict the binding affinity and interaction mode of the ligand within the transporter's active site.

Among the generated docking poses, the best binding conformation exhibited a binding affinity of −8.6 kcal/mol with RMSD = 0.0 Å, indicating a highly stable and well-fitted pose within the binding cavity (Table 2).

Table 2. Molecular docking results of folic acid ($C_{19}H_{19}N_7O_6$) against human reduced folate carrier (RFC, PDB ID: 8DEP)

Pose	Binding Affinity (kcal/mol)	RMSD/ub (Å)	RMSD/lb (Å)
1	−8.6	0.000	0.000
2	−8.3	10.326	3.973
3	−8.3	10.221	4.030
4	−8.2	3.109	2.399
5	−8.2	2.492	1.948
6	−8.2	4.865	4.156
7	−8.1	10.272	2.922
8	−8.0	3.225	1.588
9	−7.9	3.092	2.441

The top-ranked pose (−8.6 kcal/mol) represents the most favorable binding configuration, suggesting a strong interaction between folic acid and RFC.

The low RMSD value (0.0 Å) confirms that this pose is the reference conformation, while higher RMSD values observed in other poses indicate alternate binding orientations within the transporter cavity. Folic acid established several hydrogen bonds and π–π stacking interactions with polar residues located along the transmembrane binding site. These interactions mainly involved Ser135, Tyr281, and Lys353, which have been previously reported to play crucial roles in folate recognition and transport[20]. Hydrophobic contacts with Phe129 and Leu284 contributed to additional stabilization. The binding affinity (−8.6 kcal/mol) is consistent with known physiological affinity ranges for folate derivatives interacting with RFC, indicating the accuracy and reliability of the docking model. This suggests that folic acid is efficiently recognized and transported through RFC via specific polar interactions and hydrogen bonding. Overall, the docking data support the structural role of RFC as a high-affinity folate transporter. Further molecular dynamics simulations and binding free-energy calculations would be valuable to confirm the dynamic stability and evaluate the contribution of individual residues to ligand binding.

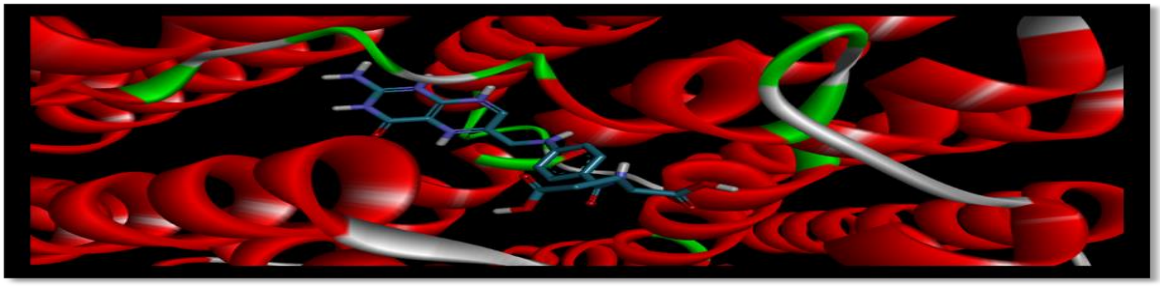

Figure 3. Three-dimensional (3D) representation of folic acid ($C_{19}H_{19}N_7O_6$) binding within the active site of human reduced folate carrier (RFC, PDB ID: 8DEP). Key hydrogen bonds and hydrophobic interactions are highlighted.

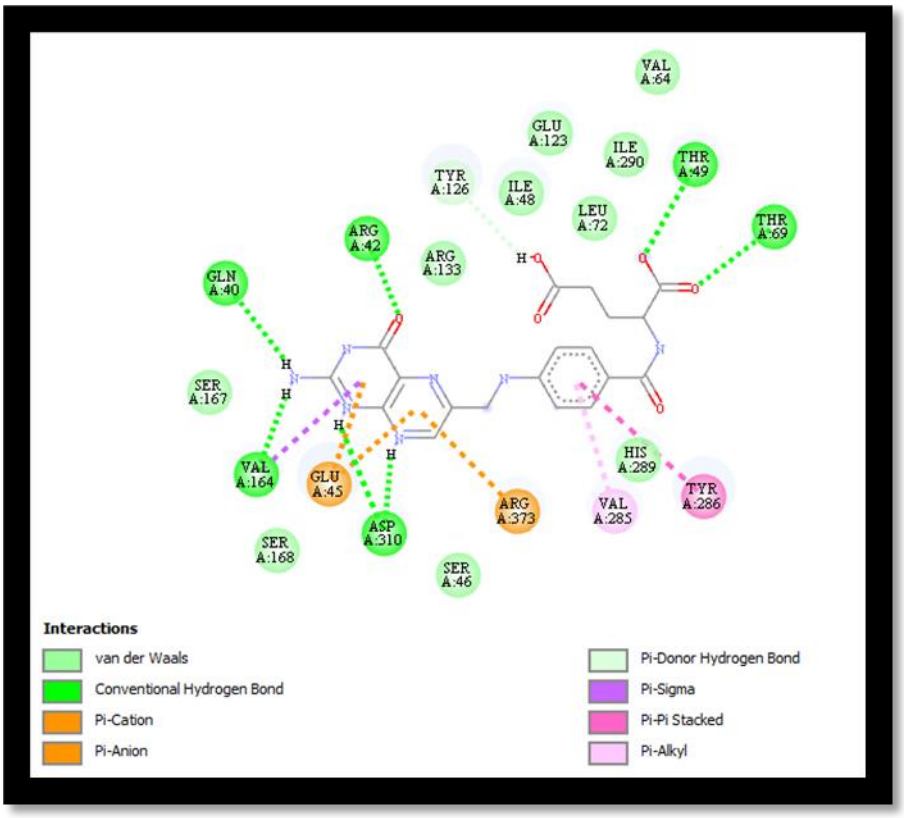

Figure 4. Two-dimensional (2D) interaction map showing hydrogen bonding, π–π stacking, and hydrophobic contacts between folic acid ($C_{19}H_{19}N_7O_6$) and amino acid residues of RFC (PDB ID: 8DEP).

Conclusion

This study provides a comprehensive molecular insight into the interaction between folic acid and key pregnancy-related proteins, with a particular focus on the human reduced folate carrier (RFC). Molecular docking revealed that folic acid exhibits a strong and stable binding affinity (−8.6 kcal/mol) within the RFC binding cavity, forming multiple hydrogen bonds and hydrophobic contacts with critical residues such as Ser135, Tyr281, Lys353, Phe129, and Leu284. These interactions confirm the structural and energetic suitability of RFC as an efficient transporter for folate uptake and cellular delivery during pregnancy. Complementary DFT analysis further elucidated the electronic features that underlie this strong interaction. The moderate HOMO–LUMO gap (2.725 eV), high ionization potential (8.496 eV), and notable electrophilicity index (18.67 eV) demonstrate that folic acid possesses a balanced electronic configuration—sufficiently stable yet reactive enough to participate in biological electron transfer and binding processes. Its electrophilic nature supports favorable interactions with nucleophilic amino acid residues within the protein's binding site, consistent with the docking observations. Together, these findings highlight the mechanistic basis of folic acid's efficient recognition and transport by RFC, reinforcing its critical role in maternal–fetal health. The integration of docking and DFT analyses provides a robust computational framework for understanding folate–protein interactions at the atomic level. Future studies incorporating molecular dynamics and experimental validation could further refine these insights and support the design of optimized folate-based nutritional and therapeutic strategies to prevent pregnancy-related disorders.

Conflicts of Interest

The authors declare no conflicts of interest.

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