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In vitro and Computational Modeling of Hemin and 5-ALA Combination in Cancer Photodynamic Therapy: PpIX and ROS efficacy through the analysis of Density Functional Theory

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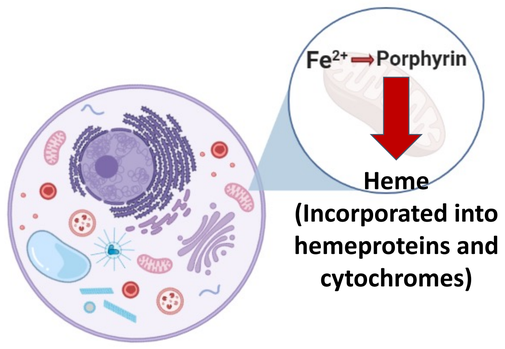
1. Introduction

5-aminolevulinic Acid (ALA) is formed from glycine and succinyl CoA in mitochondria.

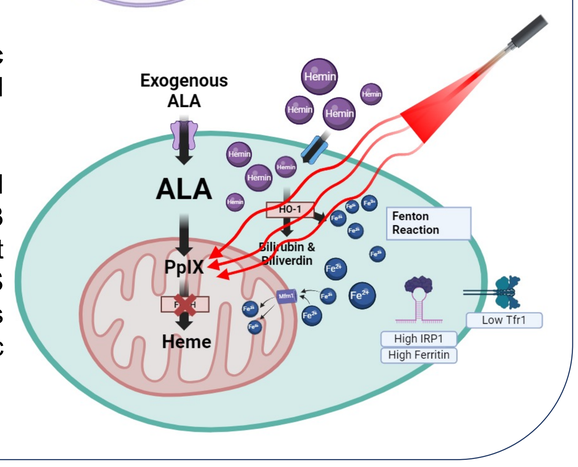
Normal Cells: 5-aminolevulinic acid exogenous addition will be converted into protoporphyrin IX. Addition ferrous iron ⟶ **generates heme.**

Cancer Cells: FECH is inactivated, and metabolic changes related to heme generation occur → **PpIX accumulates.**



Hemin: Protoporphyrin IX containing a ferric iron (Fe^{3+}) ion with a coordinating chloride ligand forming a covalent bond.

The efficacy of 5-Aminolevulinic acid photodynamic therapy (ALA-PDT) in PC3 prostate carcinoma is often limited by insufficient protoporphyrin IX (PpIX) accumulation and ROS production. While co-administering hemin is known to enhance cytotoxicity, the specific molecular mechanism remains unclear.



2. Hypothesis

This study investigates the combined effect of ALA and hemin on PpIX accumulation, evaluates their therapeutic efficacy in mouse models, and presents a computational analysis of the underlying photodynamic interactions.

3. Materials and Methods

In vitro Studies

- Materials: PC3 prostate cancer cell line.
- Treatment: ALA 1 mM, Hemin 0.2 mM
- Experimental studies:
  - Confocal Microscopy (Keyence BZ-X1000)

Computational Studies

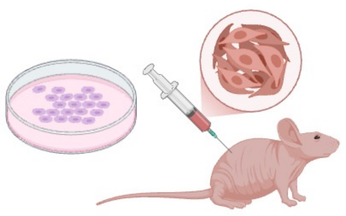
- Density Functional Theory (DFT) is being carried out using ORCA. Analysis included:
  - Structure Geometric Optimization
  - Determination of the electronic structure of the ALA-Hemin complex.

ORCA

In Vivo Analysis

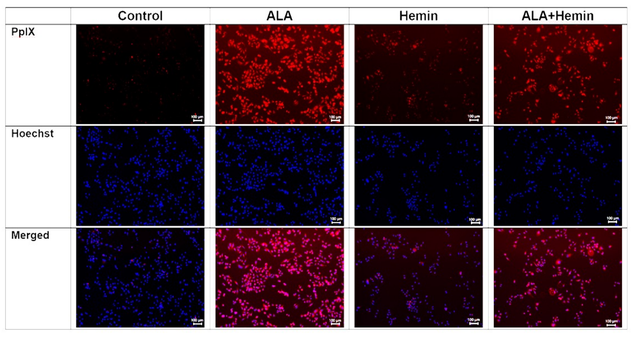
- Nude mouse Balb c nu/nu 20 mouse.
- PC3 cancer cells seeding with $1.9x10^6$ cell each mouse (subcutane)
  - ALA injection 100 mg/kg mouse
  - Hemin injection 26 mg/kg mouse

All 4 hours incubation, with no drinks and no lights.



4. Results and Discussions

In vitro Data

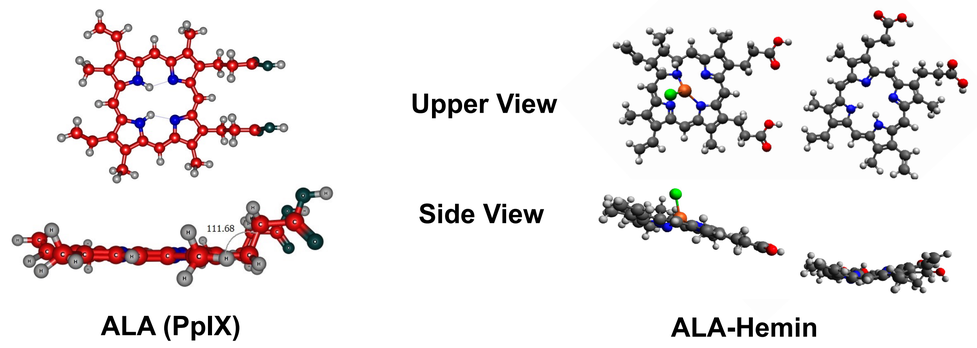


Red: PpIX accumulation that is utilized for PDT.
ALA and ALA+Hemin show significant amount compared to Hemin only or Control.

Blue: Hoechst binds to DNA and can show the amount of "alive cells". Control and ALA only show brighter results compared to cells with hemin addition.

Using Hoechst staining, less DNA was stained properly in hemin samples → **might indicate cell death or apoptosis.**

Computational Data



In the ALA-hemin combination, **the addition of hemin induces a planar structure, caused by distortions of bonds from hemin addition → more effective light absorbance and ROS production.**

ALA-only dipole moment: 7.55 Debye, ALA-hemin: 12.43 Debye.

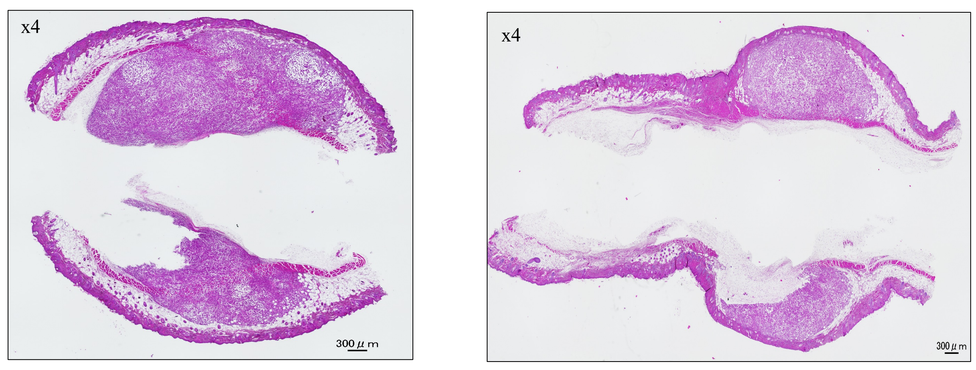
The higher the dipole moment, the more soluble the molecule is in polar solvents.

Structures	ΔE_{S-T}
Vacuum condition	
ALA	-35.928
ALA-Hemin	-55.306
Solvent condition	
ALA	-37.045
ALA-Hemin	-59.81857

In PDT, energy gap is needed as the reaction of photosynthesizer + photon from light. The calculated negative values = **more stable in their normal (singlet) state than in their excited (triplet) state.**

- The molecule ALA-Hemin shows **a smaller energy gap (ΔE_{s-t}) compared to ALA in both vacuum condition and solvent condition.**

In vivo Data



Control Tissue (No treatment)

ALA-Hemin Treatment

Preliminary studies show that compared to the control (saline only), **ALA-hemin treatment reduced the size of PC3 cancer tissues by almost half in mouse models.** In the control, the tumor mass was densely stained with hematoxylin (deep purple). This high density of nuclei suggests high viability and rapid proliferation. In the ALA-Hemin treatment, while a tumor mass was still present, the tissue appeared more disrupted compared to the control.

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5. Conclussions

This study provides additional evidence that **PpIX accumulation occurs in cancer cells after ALA and ALA+Hemin addition,** and **reduces cell viability,** resulting from less DNA staining. H&E staining of **ALA+Hemin shows a reduction of cancer size compared to the control.**

Computational data indicate that **the addition of hemin modifies the molecular geometry, affinity, and reactivity of the molecules.** This supports the greater ROS production observed with the ALA-hemin combination compared to ALA alone.

Future considerations: Investigate protein expression related to apoptosis or cell death and conduct additional ADMET analysis.

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