Novel chemical probes to modulate polyamine metabolism

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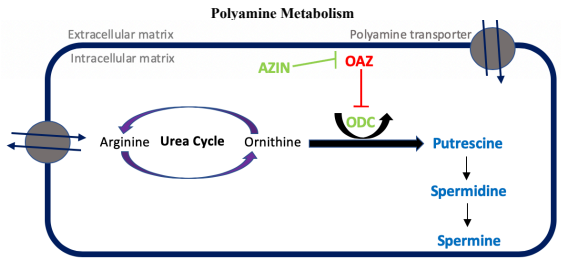
Introduction

Polyamines are small aliphatic polycations that play a crucial role in key cellular processes such as cell growth, cell proliferation, apoptosis, and gene regulation. Aberrant accumulation of polyamines is associated with various pathological states including cancers. Polyamine homeostasis within the cell is regulated by three proteins, Ornithine decarboxylase (ODC), its antizyme (OAZ) and the antizyme inhibitor protein (AZIN). ODC functions as the rate limiting enzyme by converting ornithine to putrescine. OAZ binds the carboxylic end of ODC and targets it for proteasomal degradation. ODC is also regulated by its non-catalytic homolog AZIN, which forms a tighter complex with OAZ thereby disrupting the ODC-OAZ interaction. This results in the rescue of ODC activity and prevents its degradation by proteasome. ODC is overexpressed in various cancers, and more recently a resurgence of interest in targeting ODC has emerged as a treatment of neuroblastoma and esophageal cancer. More importantly, polyamine uptake by immune cells leads to immune suppression by increasing myeloid-derived suppressor cells (MDSCs), which attenuates T-cell function in the tumor-microenvironment. Targeting polyamine biosynthesis and transport using combination drugs have shown promising results in depriving polyamines in the tumor microenvironment leading to the activation of tumoricidal T-cells. The current study proposes the development of novel chemical probes that disrupts ODC function. The study also proposes a novel mechanism of inhibition of ODC *in vitro*.



In silico identification of a peptide binder

- In search of the best peptide sequence, which would disrupt ODC dimer-interface thereby inhibiting ODC enzymatic activity, four different software were utilized
- Rosie peptiderive identifies the best peptide sequence based on the structure of ODC
- Based on the results from Rosie, an additional sequence was derived based on the crystal structure of OAZ
- Pepattract utilized the peptide sequence to dock the peptide onto the ODC monomer
- Hdock and Frodock utilize the PDB file of the peptide sequence, which allows them to dock the secondary structures of the peptide onto ODC

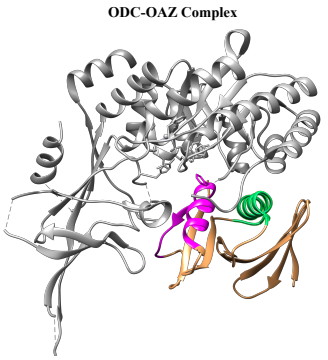
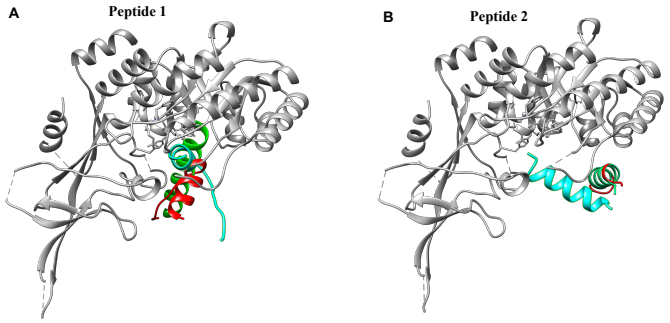


Figure 1. ODC-OAZ complex (PDB ID: 4ZGY), ODC is shown in grey & OAZ is in beige. The magenta and lime green colored peptides seen in the crystal structure of OAZ are thought to have the strongest interactions with ODC and are the basis of the hypothesized peptide inhibitors of ODC.

Proposed docking sites



- Docking results suggests that the OAZ-mimetic Peptide-1 can bind in a similar region where the C-terminal α-helix in OAZ is located
- Frodock and pepattract predicted similar peptide orientations similar to the OAZ helix in the crystal structure, whereas Hdock predicted a peptide with a slightly different orientation
- Docking results suggests that the OAZ-mimetic Peptide-2can bind in a similar region where the N-terminal α-helix in OAZ is located
- While Frodock predicted a peptide nearly identical to that of the OAZ structure, Hdock predicted a peptide with a different orientation, as well as different protein-protein interactions

Figure 2. The ODC complex is grey, the peptides derived from Frodock are red, and the peptides derived from Hdock are cyan. (A) Docking sites proposed for peptide 1 using pepattract, Frodock, and Hdock. (B) Docking sites proposed for peptide 2 using the crystal structure of OAZ, Frodock, and Hdock.

Proximity-dependent covalent ligation

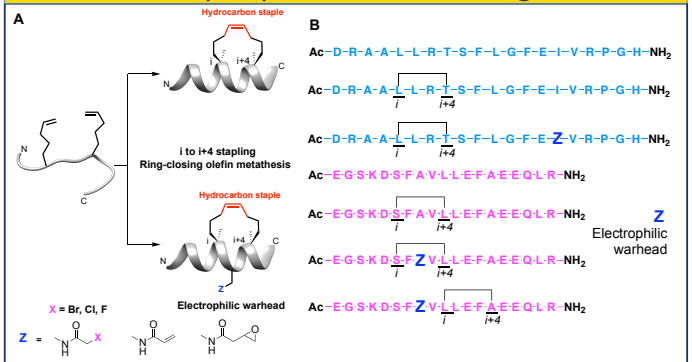


Figure 3. (**A**) Helical stapling is known to increase the helical content of peptides that would otherwise have a low helical content. The chemistry involves a ring closing metastasis using Grubbs catalyst to introduce a staple. (B) An electrophilic warhead consisting of functional groups would be introduced to react with cysteine residues. This improves the binding of the peptide to the target protein and results in a higher half life.

Proximity-dependent covalent ligation relies on the reactivity of nucleophilic amino acids such as cysteines to covalently attach an interacting molecule via electrophilic functionalities. When a molecule (ligand) and a protein (receptor) is within close proximity, an irreversible thioester linkage is produced, which increases the potency of the ligand.

Preliminary Experiments to Verify Enzymatic Activity

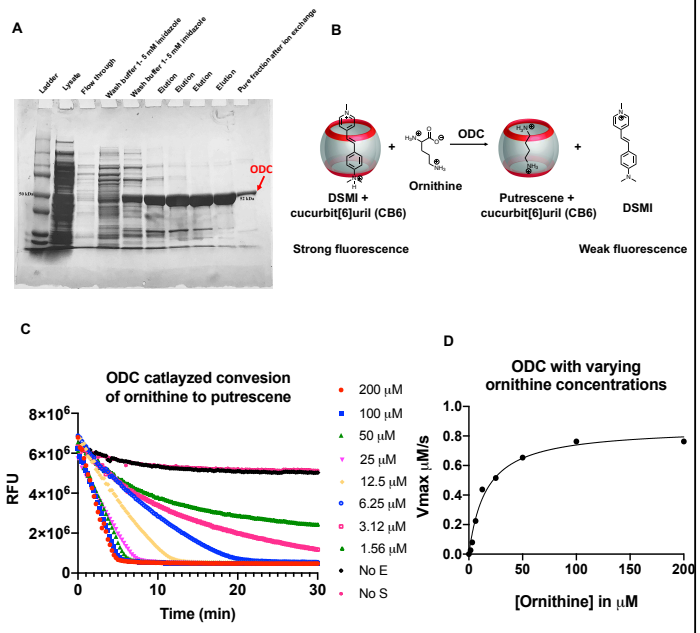


Figure 4. **(A)** Recombinant ODC was expressed in BL21DE3 cell line. The expressed protein was then purified using a nickel affinity column, followed by anionic exchange. Protein purity was confirmed using SDS page gel with a molecular weight of 52 kDa. Protein concentration was subsequently determined using Bradford Assay. (**B**) The illustration depicts the details of the fluorescent assay performed to determine the activity of ODC. DSMI is more fluorescent when bound to CB6. When ODC is converted to putrescine, putrescine and DSMI compete, resulting in a decrease in fluorescence. (**C**) The graph depicts a plot showing the conversion of various concentrations of ornithine to putrescine by measuring the decrease in fluorescence over time. (**D**) The graph shows a Michaelis-Menten plot depicting the activity of ODC against various substrate concentrations.

Future perspectives

- All peptides will be synthesized and assayed against ODC
- The Tandem Fluorescent assay system will be used to identify and screen peptide inhibitors
- Peptide inhibitors will also be confirmed using alternate assay systems, such as LC-MS
- Circular dichroism spectroscopy will be used to identify and confirm the secondary structures of the identified peptide inhibitors
- Proximity-dependent covalent ligation will be confirmed and evidenced via mass spectroscopy and SDS page gel experiments