Computational studies of novel inhibitors of aldolase A via molecular dynamic simulations

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Introduction: Aldolases are enzymes that convert fructose-1,6-biphosphate into glyceraldehyde 3-phosphate and dihydroxyacetone phosphate as part of the glycolytic pathway. Aldolase A, a homotetrameric class I aldolase found in muscle cells has been identified as a potential chemotherapeutic target, by targeting hypoxia inducible transcription factor (HIF-1).[1] HIF-1 is necessary for the proliferation of hypoxic tumor cells commonly found in solid tumors. Aldolase A has been co-crystallized with naphthalene-2,6-diyl bisphosphate (ND1), an inhibitor with an experimental K_i of 0.8929 μM. The phosphate (P_i) groups on ND1 are prone to cleavage by intestinal phosphatases. We explore a series of ND1 analogues by inserting or substituting difluoromethylene (CF_2). We conduct an in-depth analysis of protein-inhibitor binding and showcase the potential of MD simulations and polarizable force fields in screening chemotherapeutic drug leads and developing therapeutic strategies.

Materials and Methods: Molecular dynamics (MD) simulations of the ND series binding to aldolase were performed using the AMOEBA polarizable force field.[2] The MD Trajectory Python library was used to compute solvent accessible surface area (SASA) using the Shrake-Rupley algorithm.[3] For interaction energy calculation, the dimer structures were optimized using MP2/cc-pVTZ with pcm in Gaussian[4] and the interaction energies were calculated using MP2/aug-cc-pVT/QZ in Psi4[5] and extrapolated to complete basis set. Lab-written python scripts were used to track distances between protein and ligand and count water molecules within the binding pocket.

Results and Discussion: Free energy of binding was computed and results in energies of -0.22, -10.29, 0.97, -16.35, and 2.75 kcal/mol for ND1, NDA1, NDA2, NDB, and ND5, respectively. Experimentally it is known that ND1 is a strong inhibitor (K_i = 0.8929 μm) while ND5 is a weak inhibitor (11.22 μm), ΔΔG ~1.49 kcal/mol relative to ND1. NDB and ND1 have negative (-6.33, -24.37 kcal/mol) and NDA1, NDA2 and ND5 have positive (43.47, 15.61, 16.66 kcal/mol) ΔH_{bind}. Distance tracking found CF_2 to displace P_i in key interactions in ND5 (2.9 Å, 5.6 Å) but not in NDB (4.4 Å, 3.6 Å). Interaction energy calculations found that P_i-H_2O interactions and CF_2-H_2O interactions have approximate mean energies of -30 and -5 kcal/mol, respectively. SASAs of the binding pocket values are 6.341, 6.052, 6.935, 5.14, and 5.18 $Å^2$ for ND1, NDA1, NDA2, NDB, and ND5, respectively. Water counts are ~17 for all inhibitors.

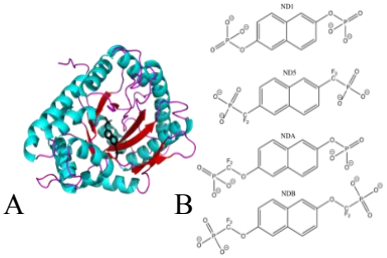

Figure 1. A. Human aldolase A crystal structure in complex with ND1 inhibitor.
B. ND series of aldolase inhibitors. Note that NDA is asymmetric, NDA1 refers to an orientation with the end containing CF_2 oriented in the binding pocket while NDA2 refers to an orientation with the regular phosphate end oriented in the binding pocket.

Conclusions: The ND series can be ordered by decreasing binding affinity as follows: NDB, NDA1, ND1, NDA2, ND5. To understand the differences, NDB and ND1 can be tentatively characterized as enthalpically driven and NDA1, NDA2 and ND5 as entropically driven. Substitution of CF2 for the connecting oxygen alters positioning and displaces phosphate interactions but insertion of CF2 does not negatively affect phosphate interactions. As fluorine interactions are weaker than phosphate interactions, the displacement contributes to free energy differences. Larger SASAs generally correlate with higher ΔG (lower affinity), suggesting that binding pocket conformation plays a role. Preliminary water counting suggests that water displacement is not particularly significant. This work suggests that the inhibitors containing insertions of CF_2 have potential as chemotherapeutic drug leads, pending synthesis.

Acknowledgements: NSF Research Experience for Undergraduates Award #1461192 and NIH R01GM114237.

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