Kinetics of Oxygen Transport in Monomeric Sarcosine Oxidase

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Flavin-containing oxidases are a class of proteins which use oxygen to regenerate the oxidized state of the isoalloxazine ring in flavin adenine dinucleotide (FAD) or flavin mononucleotide (FMN) after it has been reduced by substrate oxidation. Though well characterized experimentally, many questions linger regarding how small molecules access the active site as well as where oxygen activation occurs in flavin-containing oxidases. A prototypical member of this family is monomeric sarcosine oxidase (MSOX), and it is perhaps the most well studied. Despite knowing which features are essential for catalysis as well as the location of the substrate binding site, it is unclear how oxygen accesses the site since the only clear entryway can be partially blocked by the larger substrate sarcosine. As such, two competing mechanisms have gained attention centering around the order by which ligands enter the binding site.

In this thesis, we detail the use of all atom molecular dynamics (MD) studies to identify how oxygen accesses the MSOX active site, as well as characterize the resulting kinetic network. We use the single sweep method to identify four potential routes for oxygen to travel from the surface of MSOX to the active site. Then, using Markovian milestoning with Voronoi tessellations (MMVT), we refine the pathways identified in single sweep and develop a Markov state model describing the kinetics of oxygen entry and exit. We calculate entry and exit mean first passage times (MFPT) for oxygen from this model, which are used to compute second order rate constants for entry and first order rate constants for exit. Our calculated rate constants and mechanisms show that the presence of a substrate-mimicking inhibitor markedly influences the kinetics of oxygen entry and exit. The bound competitive inhibitor changes the protein structure sufficiently to shut down almost all major oxygen channels save one, which it opens, speeding entry but greatly slowing down oxygen exit, relative to the substrate-free enzyme. This means that our kinetic analysis predicts oxygen exhibits a longer residence time within MSOX when a substrate-like ligand is present. This supports the so-called “modified ping-pong” mechanism, in agreement with

previous experimental results, thus lending validity to our approach. Furthermore, our computed second-order entry rate constants are larger by about an order of magnitude than are experimentally determined oxygen consumption rate constants. Since oxygen consumption combines the processes of entry and electron transfer, we conclude that of these two, entry is not rate-limiting in the overall catalytic cycle, regardless of whether or not a substrate-like ligand is bound. Finally, because this work represents the first test of the MMVT approach for comparing kinetics of ligand entry for an enzyme in two distinct states, we not surprisingly uncovered inefficiencies in the approach. We tested one idea for gaining efficiency based on the “finite-temperature” string method, in which transport channels can be determined and kinetically characterized on-the-fly, rather than sequentially. Our results indicate that more research in that area is needed.