Abstract:

Effects of cell-like quinary interactions over enzyme catalysis

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Experiments to characterize parameters relevant for enzyme catalysis, like catalytic rates and Michaelis constants, are usually performed in vitro. However, most enzymes perform their function inside cells, which is an environment with a high concentration of nucleic acids, proteins and other macromolecules. Such environment, characterized by macromolecular crowding, can affect catalysis and enzyme-substrate binding through excluded volume effects and quinary, weak interactions with other macromolecules. The aim of this project is to understand how the cell and its individual components affect the Michaelis constants and catalytic rates of the reactions catalyzed by the human aldehyde oxidase. Experiments to measure kinetics with cell lysates and cell components will be performed in the group of Prof. Dr. Silke Leimkühler, and modeling of the quinary interactions between aldehyde oxidase, its substrates and cell components will be performed in the group of Dr. Ariane Nunes Alves. This project will provide mechanistic insights about how cell-like quinary interactions may affect the activity of enzymes.

Extended description version of the project:

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1. Overall goal of the project

The aim of this project is to understand how the cell environment affects the Michaelis constants and catalytic rates of the reactions performed by the human aldehyde oxidase (hAOX1). In order to achieve this goal, kinetic characterization of overexpressed hAOX1 in human cell lines in addition to purified hAOX1 in the presence of defined cellular components will be performed. Later, modeling of the quinary interactions between hAOX1, substrates and cell components will be performed to obtain mechanistic insights about the effects of cell components over enzyme catalysis.

2. State of the art

Experiments to characterize parameters relevant for enzyme catalysis, like catalytic rates (k_{cat}) and Michaelis constants (K_M), are usually performed in vitro, using low concentration of enzyme and substrate. Such condition does not reflect the cell environment, which is crowded with macromolecules such as proteins and nucleic acids. Macromolecules can achieve a concentration of up to 300 g/L (tens of millimolar) inside cells (1). Macromolecular crowding may affect the binding of substrate-enzyme complexes through excluded volume effects and quinary, weak interactions. A crowded environment may reduce substrate diffusion and hinder enzyme-substrate binding, leading to a K_M value higher than the one determined in vitro. For instance, the catalytic efficiency (k_{cat}/K_M) of TEM1-β-lactamase was shown to be reduced inside cells (2).

However, quantifying k_{cat} and K_M values inside cells is challenging. Other strategies to probe the effects of the cell environment include kinetic characterization using cell lysates, protein crowders (3), or individual cell components, such as ribosomes (4). A recent study (3) showed that the catalytic efficiency of dihydrofolate reductase is reduced in the presence of protein crowders, such as lysozyme and casein. Another study (5) showed that changes in the ^{1}H-^{15}N heteronuclear single quantum coherence (HSQC) nuclear magnetic resonance (NMR) spectrum for γD-crystallin inside cells are lost when ribosomes are destabilized, suggesting that ribosomes are the main partner for quinary interactions of γD-crystallin inside cells. Moreover, a low concentration of ribosomes also increased the K_M values of the reactions catalyzed by dihydrofolate reductase and thymidylate synthase by 10 and 20 fold, respectively (4). In these studies, it is not clear whether the effects over K_M were caused by the interactions of ribosomes and proteins crowders with the enzymes or with the substrates. Studies with one promiscuous enzyme, which can metabolize multiple substrates, can help to provide mechanistic insights of how enzyme-substrate binding changes inside cells and elucidate whether these changes are due to interactions with the enzyme or with the substrate.

hAOX1 in general accepts a wide range of unrelated substrates, such as aldehydes and N-heterocycles. hAOX1 is a multifunctional molybdo-flavoenzyme present in the cytosol and expressed in different tissues. It catalyses diverse reactions, such as oxidation and amide hydrolysis. The importance of hAOX1 is related to its prominent role as a drug and xenobiotic metabolizing enzyme, being responsible for unexpected drug metabolism, for promoting drug interactions, for affecting drug's efficacy and ultimately, for leading to failures in clinical trials. One of the enigmas on hAOX1 relates to its true physiological function that is still unknown. There are some hypotheses based on known endogenous substrates, and the fact that hAOX1 is an important source of reactive oxygen species suggests a role in signaling and cellular redox stress (6). Kinetic characterization of hAOX1 using different substrates has been done before in vitro, but not yet inside cells or using cell lysates.

3. Specific aims and how they may be reached

Work package 1 (WP1): kinetic characterization of hAOX1 activity in human cell lines

The aim of this WP is to establish an expression system for the overexpression of hAOX1 in human cell lines (e.g. HEK293 cells) to measure k_{cat} and K_M values for hAOX1 and a few substrates in the natural environment. The overexpression is necessary, since the concentration of hAOX1 in the cytosol of human cells is very low and below the detection limit. With the overexpression, activity assays in human cell lines can be performed which will allow the determination of K_M and k_{cat} values with different substrates under more physiologic conditions in cell lysates of human cell lines. The non codon-optimized hAOX1 gene will be cloned into a vector which will allow for its transfection into human cell lines (e.g. HEK293 cells). The substrates used will be selected to form a group with a diverse set of molecular features, with different sizes and degrees of hydrophobicity. The substrates with the largest changes in K_M values (compared to control, in vitro measurements) will be used in WP2.

WP2: kinetic characterization of hAOX1 activity in the presence of selected cellular components

The aim of this WP is to further understand which components of the cell are responsible for the changes in K_M values observed in WP1. k_{cat} and K_M values for hAOX1 will be measured in the presence of cell metabolites and ribosomes, using the same concentrations as observed inside cells. The experiments with results that resemble the most the K_M values with cell lysates will serve as the basis for modeling in WP3. This approach will allow to analyze hAOX1 activity of the recombinant enzyme expressed in E. coli with more defined cellular components which will allow to determine components that inhibit or activate hAOX1 activity.

WP3: modeling of the interactions of hAOX1 and substrates with cell components

The aim of this WP is to model the interactions of hAOX1 and the substrates with the cell components tested in WP2 to obtain mechanistic insights about how the cell components affect enzyme-substrate binding. Docking of the cell metabolites to hAOX1, and of hAOX1 to the ribosome, will be performed. Then, molecular dynamics simulations will be performed to test the stability of the complexes and to characterize the interactions of hAOX1 with the cell components. Docking of the substrates to the ribosome will be performed, and molecular dynamics simulations will be done to test the stability of the complexes and to characterize ribosome-substrate interactions. Finally, simulations of cell metabolites and substrates will be performed to test whether the metabolites can aggregate and sequester the substrates, hindering substrate binding.

This project will provide mechanistic insights about how cell-like quinary interactions affect the activity of hAOX1.

References:

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