Determining Mechanisms of Ionic Liquid-Induced Activity Loss of Enzymes with Molecular Simulation

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Abstract
The purpose of this research was to visualize and analyze simulation trajectories of an endocellulase E1 enzyme from Acidothermus cellulolyticus (1ECE) in the ionic liquid (IL) 1-butyl-3-methylinidazolium chloride ([BMIM][CHL]). The primary goal, which has applications in general biocatalysis, was to provide the Karr Lab at the University of Colorado-Boulder with molecular level insight into the origins of enzymatic activity loss in IL, as seen in their experiments. This was undertaken by analyzing the enzyme’s evolution in time with Visual Molecular Dynamics (VMD) and using tools provided with the program to visualize enzyme-IL interactions. The results suggest the reason for loss of activity of the enzyme was IL interacting with key residues in the enzyme’s active site.

Introduction
Three hypotheses initially existed to explain the activity loss of 1ECE seen to occur in experiments with the IL [BMIM][CHL], proposed by experimentalists at the University of Colorado-Boulder:

1) Denaturation of the enzyme by the IL, causing loss of secondary structure to the alpha-helical regions;
2) Precipitation or “salting out” of the enzyme out of solution due to IL-induced enzyme aggregation;
3) Loss of enzyme activity due to IL entering into the active site of the enzyme.

Advanced visualization tools were used to post-analyze simulation trajectories to identify the main hypothesis responsible for the observed behavior of the enzyme.

Methods
- Visual Molecular Dynamics (VMD) was used to analyze, in a post-processing manner, molecular simulation trajectories of 1ECE in [BMIM][CHL].
- The trajectories were analyzed over specific regions in time where the root mean squared deviation (RMSD) of the enzyme from its crystal structure was seen to be at a maximum, to determine the primary reason for the observed loss of activity in IL as seen in experiments.
- Various tools in VMD were used to visualize the data, such as changing the colors of certain residues and/or secondary structure motifs and watching them change over time, as well as visualizing the interactions between certain atoms using graphing tools.
- After finding evidence to support one of the three aforementioned hypotheses, VMD analysis tools will be used to produce short clips of the data to communicate the results to the experimentalists.

Results
- IL was found to be in the enzyme’s active site throughout a large portion of the simulation and seemed to be interacting strongly with multiple residues in the active site, lending support to the third hypothesis (see Introduction).
- Specifically, IL was found to interact most often with arginine, glutamic acid, and histidine residues.
- Analysis of enzyme-IL behavior led to the conclusion that IL interacts through strong electrostatic and hydrophobic interactions with active site residues in the enzyme.
- The observed enzyme-IL active site interactions help us to understand the origin of activity loss of 1ECE in [BMIM][CHL], as seen in experiments.
- Upon reporting these findings back to the Karr lab, it will then be possible for them to mutate the active site residues we identified to other residues that interact less strongly with IL. This should ultimately stabilize the enzyme against IL-induced denaturation.

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References