Understanding the Mechanism of Salt Activation of Enzymes in Nonaqueous Media

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ABSTRACT

The goal of this research is to gain a more complete understanding of excipient-induced enzyme activation for nonaqueous biocatalysis. Within the past decade, several techniques have been developed that accelerate enzymatic reactions in a wide range of organic solvents. One of the more intriguing approaches is to lyophilize enzymes from an aqueous solution containing salts and other additives, which are often called excipients. An example of dramatic activation is the nonbuffer salt activation of subtilisin (a typical serine protease) for transesterification reactions in different solvents. In the case of cesium acetate as the excipient, activations of >35,000-fold over that of the non-salt activated preparations are obtained. Despite this tremendous activation, the mechanism of salt activation remains largely unknown. The motivation for this thesis research is based on the likelihood that formulating a better understanding of the mechanism of enzyme activation in nonaqueous media will lead to a set of design principles to activate a wide range of enzymes for use in nonaqueous media.

Hypothesis: Strongly activated enzyme formulations in nonaqueous media will yield a transition state that is closer to that obtained in aqueous buffer than for less active formulations.

This work investigates the structure of activated enzyme preparations in nonaqueous media and compares it to unactivated enzyme formulations. Correlations are made between the retention of native-like secondary structure and increased activity with a range of salt types. In addition, evidence is presented which suggests that the mechanism of salt activation is distinct from lyoprotection.

Kinetic studies are reported which provide information on the transition state characteristics of both salt-free and salt-activated enzyme formulations. Data is presented which shows the direct effects of salt type, solvent medium and water content on the enzymatic transition state, allowing for predictive measures of salt activation.

Finally, preliminary studies are introduced that examine the transition state characteristics and high activation of solubilized enzyme systems. Various parameters, such as substrates, water content and solvent medium, are examined to correlate changes in activity and specificity induced by direct solubilization methods.