



## Characteristics of Catalysis in Non-Aqueous Solvents

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### DESCRIPTION

Non-aqueous enzymology is now an active field, having matured to the point where, in addition to its large implications in basic protein science, it has a significant impact in biotechnology, where it grew its roots. Many studies over the last two decades have contributed to this, and many of the factors influencing catalysis in non-aqueous solvents (mostly organic solvents) are now known. Despite this wealth of knowledge, little is known about the precise molecular events that occur in proteins when they catalyze under these non-physiologic conditions. Water concentration has been identified as a major controlling factor of enzyme activity in non-aqueous media.

It was early recognized that the presence of water was required for catalysis, and that water was controlled by the number of molecules bound to the enzyme rather than its percentage in the organic solvent. Furthermore, the effect of water was not straight forward. Adding water to the dry enzyme increased activity up to a point, but then activity decreased when the amount of bound water was increased further; the maximum activity was for 10-15% (w/w) of bound water. This bell-shaped dependence was also observed for subtilisin in compressed propane, with the maximum activity occurring at 10-12% (w/w).

Increased hydration was linked to increased enzyme flexibility, which was interpreted as if water could act as a lubricant for the protein in contact with the organic solvent. Dry enzymes are very rigid, but when they bind water, they become flexible, allowing catalysis. The decrease in enzyme activity at higher levels of hydration was proposed to be explained by conformational changes that could alter the active site, which is consistent with the observation that enzymes have much more native structure in pure organic solvents than in aqueous-organic mixtures. Proteins denature in organic solvents, but not in dry conditions,

due to their low flexibility and inability to cross the barrier from the folded to the unfolded state. The effect of pH on enzyme activity is related to the effect of counterions. Enzymes in aqueous solutions have strong pH dependence, with a maximum of activity at a given value. Because of the lack of free protons in non-aqueous solutions, the concept of pH is difficult to apply, leaving the ionization equilibrium of protein groups somewhat undefined. However, enzyme suspensions exhibited catalytical profiles that were dependent on the pH of the solution from which they were prepared, and this profile was similar to that found in aqueous solution. As a result, the enzyme retained "pH memory" from the media it was prepared in.

This happens in small model compounds containing protein functional groups as well. Kinetics provides an explanation for this strange behaviour. Ionisable groups would be more stable in neutral states in non-aqueous solvents, but they can't get there because there's no way to exchange or transfer protons. A protein's structure in a non-aqueous solvent is difficult to predict. However, there are now a sufficient number of protein structures made with crystals grown in aqueous conditions (some cross-linked later) and placed in organic. When compared to the corresponding structures in water, no significant conformational changes were observed.

Small side chain rearrangements, as well as the binding of organic solvent molecules, were observed in a few places on the protein. It should be noted that these crystals always contained a certain amount of water bound to the protein, the structure which could be determined. Full-detail Molecular Dynamics (MD) simulations have previously been used to gain insight into the structure and behaviour of enzymes in organic solvents. When compared to corresponding water simulations, these studies revealed overall structural conservation, increased electrostatic interactions, and decreased protein flexibility.

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