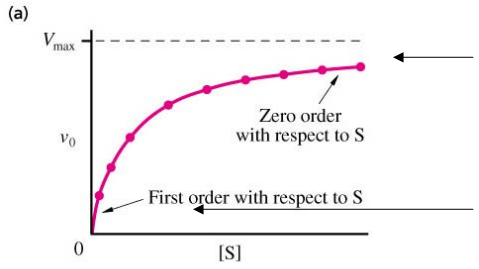
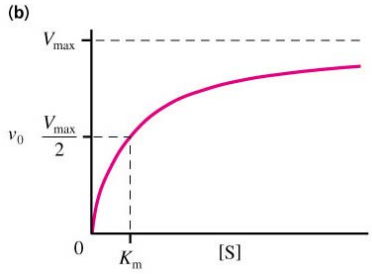
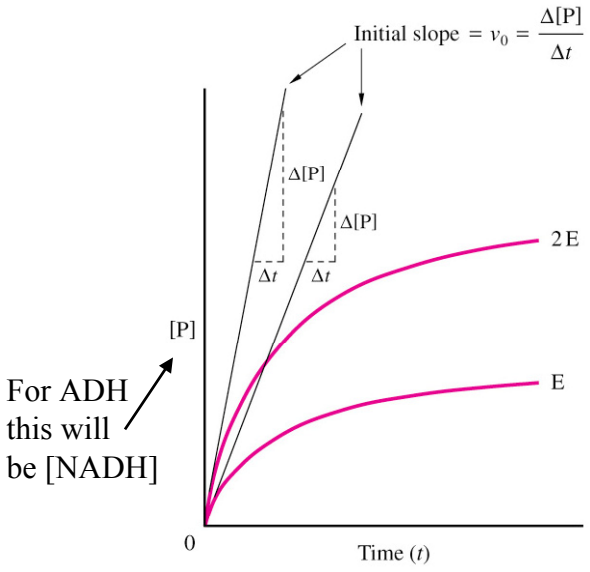
Catalytic Strategies of Enzymes - ADH as an example 10/4/07



$v = k_{cat}$ [ES]

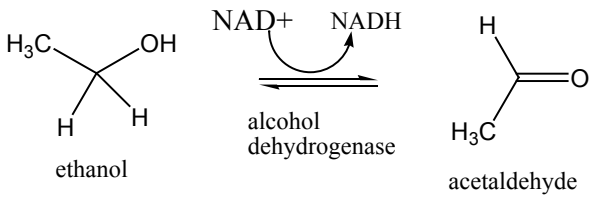
$v = \frac{k_{cat}}{K_M}$ [Et] [S]





For ADH this will be [NADH]

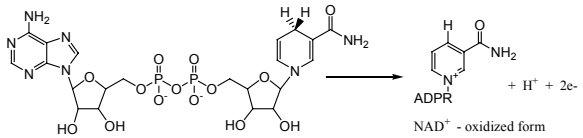
Follow reaction by appearance of NADH ε340 = 6,220 M^{-1} cm^{-1}



The ADH kinetic scheme

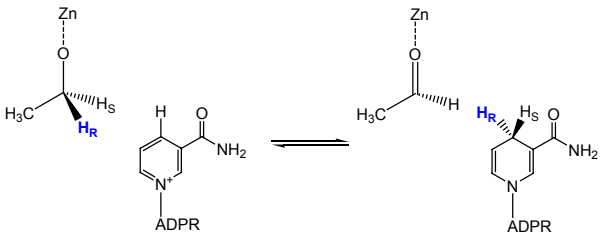
$$E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E.NAD^+ \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E.NAD^+.EtOH \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} E.NADH.Acet. \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} E.NADH \underset{k_{-5}}{\overset{k_5}{\rightleftharpoons}} E$$

The Structures of NADH and NAD+

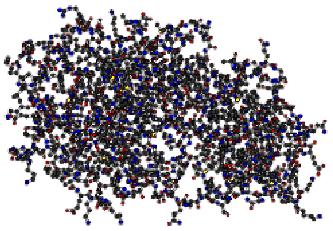


NADH - nicotinamide adenine dinucleotide
high energy reduced form

The ADH Reaction



I. Conformational Change (induced fit)



This transition has a large interdomain conformational change of 5-6 Å, which has the effect of the tight packing of side chains, NAD^+ and alcohol ultimately.

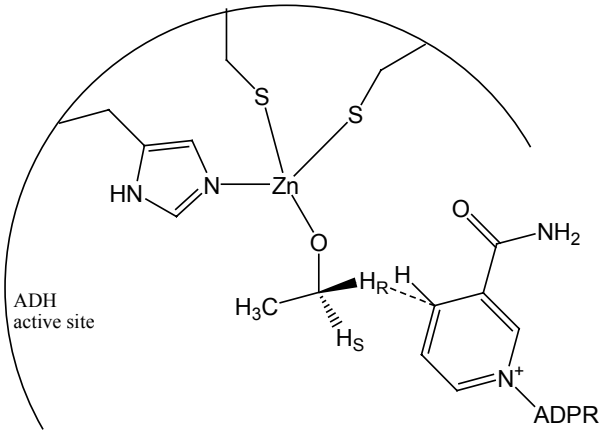
$$E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E.NAD^+$$

II. Solvent Exclusion

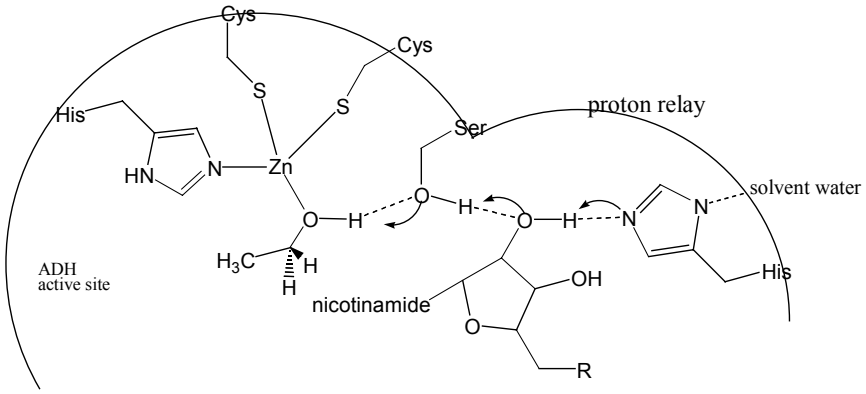
In general enzyme active sites reduce solvent accessibility and thereby lower the dielectric constant.

This results in more significant ionic interactions, remember $\mathbf{F = e_1e_2/Dr^2}$

III. Metal Ion in Active Site

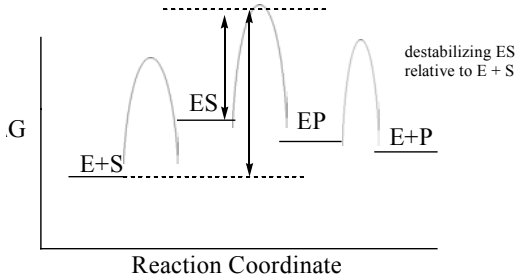


IV. General Acid/Base Catalysis



V. Entropy Effect (increase G and increased order)

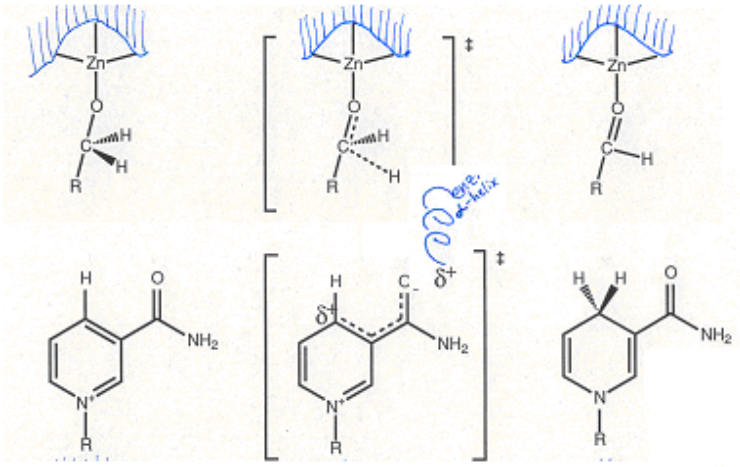
A. Loss of rotational and translational entropy upon E binding S



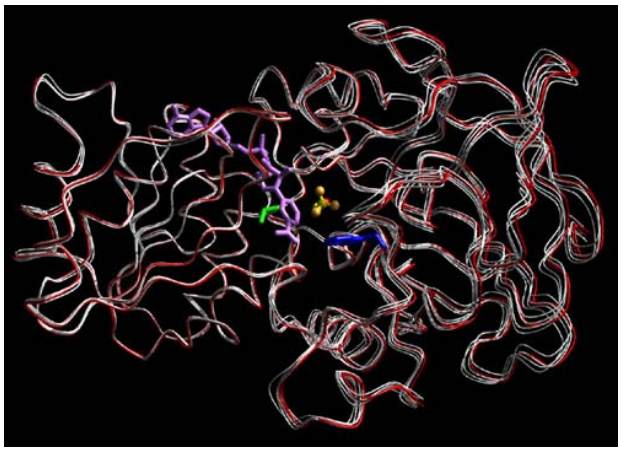
a related idea:

B. Proximity of donor and acceptor atom for hydride transfer in enzyme active site compared to what happens free in solution, more than offsets the destabilization due to increased order.

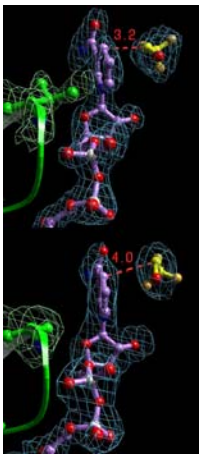
VI. Enzymes Bind Their Transition States



VII. Thermally Driven Protein Dynamics



WT



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