

Figure 15.1. Thermodynamic box relating equilibria for nonenzymatic (black) and enzymatic (red) transition-state formation (K_{non}^\ddagger and K_{enz}^\ddagger , respectively), dissociation of substrate from the Michaelis complex (K_d), and the hypothetical dissociation of the transition state from the enzyme (K_d^\ddagger). Assuming equal transmission coefficients, K_d^\ddagger is equal to K_d multiplied by the ratio of the reaction rate constants for nonenzymatic (k_{non}) and enzymatic (k_{enz}) reactions. E = enzyme; S = substrate.

A third explanation for enzymatic transition-state formation involves the substrate's adoption of a reactive conformation. According to Bruice and coworkers, a chemical reaction will occur only with a limited range of substrate conformers, termed *near-attack conformers* (NACs), which are characterized by having a geometric arrangement of reactive functional groups sufficient for formation of a transition state.⁹ An example of a NAC is given by the intramolecular cyclization of dicarboxylic acid monoesters to generate five- and six-membered rings [Figure 15.3(a)]. Computational modeling revealed that a NAC existed when the distance between the nucleophilic oxygen and carbonyl carbon was 2.8–3.2 Å and the angle of attack was within a 30° cone of the optimal angle of 15° [Figure 15.3(b)].¹⁰ The

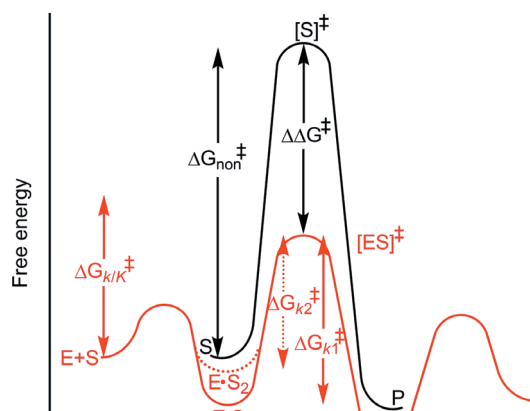


Figure 15.2. Enzyme catalysis by transition-state stabilization and ground-state destabilization. The free-energy profile for the nonenzymatic reaction (black) proceeds from substrate (S) to product (P) via the transition state $[S]^\ddagger$ with energetic barrier ΔG_{non}^\ddagger . In the enzymatic reaction (red), the transition state $[E\cdot S]^\ddagger$ is stabilized by $\Delta\Delta G^\ddagger$, resulting in a barrier $\Delta G_{k_1/K}^\ddagger$ on k_{cat}/K_m (that is, from $E+S$ to $[E\cdot S]^\ddagger$) and a barrier $\Delta G_{k_2}^\ddagger$ on k_{cat} (that is, from $E\cdot S_1$ to $[E\cdot S]^\ddagger$). With ground-state destabilization on the Michaelis complex ($E\cdot S_2$, dotted line), the barrier on k_{cat}/K_m remains unchanged, but the barrier on k_{cat} is reduced to $\Delta G_{k_2}^\ddagger$.

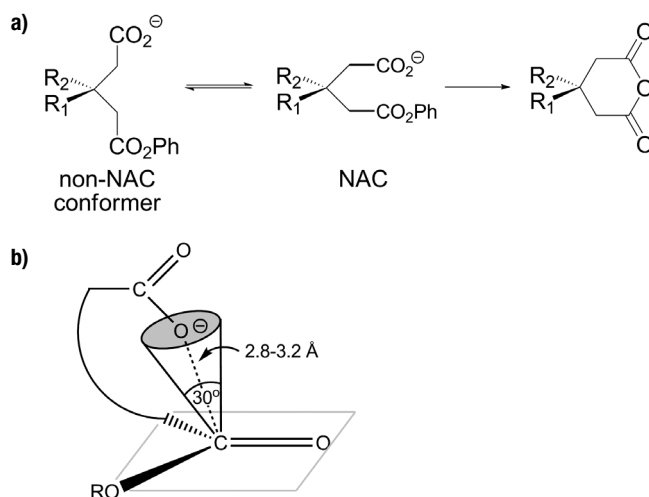


Figure 15.3. Proximity effect demonstrated by near-attack conformers (NACs). (a) Conformers of dicarboxylic acid monoesters with inappropriate geometry between the reactive groups must rotate into NACs to cyclize. (b) Geometric features of NACs for the cyclization reaction in (a) include an interatomic distance of 2.8–3.2 Å and an angle of approach within 30° from the optimal angle of 15° to the perpendicular to the carbonyl plane. Modified from Lightstone and Bruice.¹⁰

proximity effect demonstrated by the neighboring-group participation of these compounds, it is argued, is also created in the active sites of enzymes. The greater the population of substrate conformers existing as NACs, the greater the rate of reaction; hence, enzymes accelerate reaction rates, at least in part, by increasing the likelihood that an E·S complex is a NAC.

A recent theory of transition-state formation that has gained support is the coupling of dynamic motions to the reaction coordinate. It has been reasoned that certain discrete atomic vibrations, often called protein-promoting vibrations (PPVs), within the protein work in concert to cause bond cleavage and/or bond formation along the reaction coordinate.¹¹ These promoting motions are the result of the enzyme's dynamic excursions along the allowed vibrational modes. When PPVs function together, the substrate and enzymatic groups promoting catalysis are pushed toward the transition state as the chemical reaction proceeds. Examples for which there is evidence supporting PPVs include hydrogen-transfer reactions catalyzed by alcohol dehydrogenase,¹² dihydrofolate reductase,^{13,14} and lactate dehydrogenase,¹¹ as well as the phosphate-ribosyl transfer reaction catalyzed by purine nucleoside phosphorylase (PNP),¹⁵ which is the subject of discussion in the remaining sections of this chapter. Figure 15.4 illustrates reaction-coupled dynamic motions located in the His104Arg mutant of PNP, whereby the movement of Arg104 (magenta), a distant residue from catalytic site ligands (green), is coupled to Phe159 (red), which in turn affects the dynamics of the active site residues (orange).¹⁶ It is important to keep in mind that the above theories of transition-state formation are mutually compatible and any or all may be involved to varying degrees.