# Purine nucleoside phosphorylases as targets for transition-state analog design

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## INTRODUCTION

Among the most powerful enzyme-targeted drugs are those that bear a strong resemblance to the transition state of the chemical reaction undergoing catalysis. This chapter illustrates that experimental determination of enzymatic transition-state structure permits chemically stable analogs to be designed. Mimics of these transition states exhibit binding affinities exceeding those of the substrates by factors of greater than $10^6$. To appreciate this approach to drug design, it is necessary to understand the nature of transition-state formation and how it relates to the strong binding interactions between enzymes and transition-state analogs.

### Enzymatic transition-state formation

All chemical reactions proceed through at least one transition state, an unstable structure of maximal energy along the reaction coordinate. Having a lifetime of under 100 fs ($10^{-13}$ s), the time required for a single bond vibration, the transition state is the most unstable species along a chemical reaction coordinate. In the absence of a catalyst, the probability of transition-state formation is extremely low. Enzymes achieve great catalytic reaction rates by providing appropriately positioned functional groups within the active site, which interact with and distort the substrate toward the transition state by dynamic motions of the complex.

Although the physical means of enzymatic transition-state formation remain the subject of scientific debate, several theories have been proffered. In the early 1940s, Linus Pauling postulated that enzymes bind most optimally not to the normal substrate molecule but rather to the substrate molecule in a strained configuration corresponding to the "activated complex."[1] He suggested that various attractive forces with the enzyme cause the substrate to adopt the strained configuration, thereby favoring the chemical reaction and accounting for the lowered activation energy of the catalyzed reaction. Wolfenden later expanded this theory by considering a thermodynamic equilibrium between the nonenzymatic transition state and the enzyme-bound transition state (Figure 15.1).[2,3] A nonenzymatic reaction proceeds from substrate (S) to products with a rate constant $k_{non}$ via a transition state ($S^{\ddagger}$), governed by the equilibrium constant $K_{non}{}^{\ddagger}$. The corresponding enzymatic process proceeds first through a Michaelis complex (E·S), given by the dissociation constant $K_d$, followed by the enzymatic transition state ($E{\cdot}S^{\ddagger}$), which is given by rate constant $k_{enz}$ and equilibrium constant $K_{enz}{}^{\ddagger}$. A hypothetical binding equilibrium ($K_d{}^{\ddagger}$) between the enzyme and transition state completes the thermodynamic box. The rate acceleration for the enzyme-catalyzed reaction over the nonenzymatic reaction, $k_{enz}/k_{non}$, therefore indicates the degree of tightness to which the transition state is bound relative to the substrate, typically between $10^{10}$ and $10^{15}$.

A related factor that may play a role in transition-state formation is ground-state destabilization. In much the same way that the transition-state stabilization model of Wolfenden explains lowering of the activation-energy barrier through stabilizing interactions with the enzyme, Jencks and others have suggested that destabilizing interactions with the bound substrate could promote distortions toward the transition state (Figure 15.2).[4,5] Strategies by which these binding interactions can assist the chemical reaction include desolvation of substrate functional groups, positioning substrates in the active site, and geometrically or electrostatically destabilizing substrates.[6] This third strategy has been proposed for orotidine-5′-phosphate (OMP) decarboxylase,[7] in what Jencks has termed the "Circe effect," whereby the enzyme attracts the substrate by forming energetically favorable binding interactions at one region of the substrate but simultaneously destabilizes the reactive group that undergoes chemical transformation. Richard and coworkers have since observed formation of a carbanion intermediate that OMP decarboxylase stabilizes by at least 14 kcal/mol, suggesting transition-state stabilization is the dominant factor in the $10^{17}$-fold rate acceleration by this enzyme.[8] Thus, it is possible that transition-state stabilization and ground-state destabilization function in complementarity to achieve rate enhancements, as is depicted in Figure 15.2.