

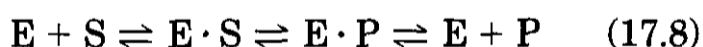
pected carboxylate anion, provided evidence that **cation- π** interactions may play an important role in ligand binding (65, 66). Model systems suggest that, energetically, the **cation- π** interaction can compete with full aqueous **solvation** in binding cations (61), and there is now significant effort being expended in studying the contribution of these interactions to molecular recognition (62, 66).

In summary, the K_i provides an indication of the relative stability of the **enzyme-inhibitor** complex compared to stability of the enzyme and inhibitor free in solution. Moreover, it is clear that entropy, enthalpy, and water will all have a major impact on the binding of an inhibitor to an enzyme.

2.2 Steady-State Enzyme Kinetics

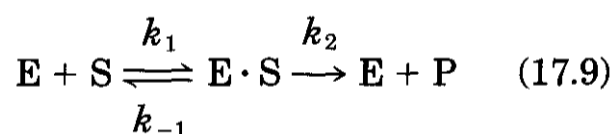
Just as an appreciation of the forces involved is essential to comprehending the binding of an inhibitor to an enzyme, so is an understanding of the **kinetic** analysis of an enzyme-catalyzed reaction essential to any **kinetic** evaluation of an inhibitor. In this section we provide a brief introduction to the study of enzyme **kinetics**, particularly steady-state **kinetics**. Regardless, the reader is advised to refer to other sources for more in-depth reviews of the **kinetic** equations and mathematical derivations involved (38, 60, 67–71).

2.2.1 The Michaelis-Menten Equation. In the simplest case, an enzyme-catalyzed reaction involves the conversion of a single substrate to a single product, as shown in Equation 17.8.



The free enzyme (**E**) binds the substrate (**S**) to form a noncovalent enzyme-inhibitor complex (**E · S**). This is assumed to be a rapid, reversible process, not involving any chemical changes, and with the affinity of the substrate for the enzyme's active site being determined by the binding forces discussed above. A chemical transformation of substrate to product (**P**), initially in complex with enzyme (**E · P**), then takes place. Finally, the product (**P**) is released into the medium with concomitant regeneration of free enzyme (**E**).

As can be seen from the following discussion, it is not difficult to carry out a kinetic analysis of a single-substrate reaction such as that described in Equation 17.8. However, as more substrates are added the task becomes more complex. Fortunately, **kinetic** analysis of enzymatic reactions involving two or more substrates can be made easier by varying the concentration of only one substrate at a time. By keeping all but one of the substrates at **fixed**, saturating concentrations, the reaction rate will depend only on the concentration of the varied substrate. This permits the use of the **kinetic** analysis employed for **enzyme-catalyzed**, single-substrate reactions even for complex multisubstrate reactions. In a further simplification, the dissociation of the **E · P** complex is assumed not to be rate limiting, and the reversion of product to substrate is assumed to be negligible. The latter assumption is valid under what are known as initial velocity conditions, that is, when less than about 5% of substrate has been consumed. Under these conditions, the concentration of **P** is low, and Equation 17.8 simplifies to Equation 17.9.



Generally, kinetic analyses are carried out by studying the reaction under **steady-state** conditions, that is, when the concentration of the enzyme is well below that of the substrate. Under those circumstances, following a brief preequilibrium period, the concentrations of the various enzyme-bound species, **E · S** and **E · P** in Equation 17.8, become effectively constant and the rate of conversion of substrate to product will greatly exceed the change in concentration of any enzyme species. This is an approximation but, provided the substrate concentration does not greatly change (e.g., under initial velocity conditions), it is a very useful approximation. Given steady-state conditions, the Michaelis-Menten equation (Equation 17.10) is a quantitative description of the reaction described by Equation 17.9.