hibitors described below, affinity labels do not require activation by catalysis at the enzyme's active site. Most often, the covalent bond formation occurs by an S_N2 alkylation-type mechanism, Schiff base formation, or acylation (156, 159).

Affinity labels, some of which have become successful therapeutic agents, are often used to identify catalytically important residues. In some cases, by examining the pH dependency of the rate of inactivation, it is possible to determine the pK_a of the labeled residue. Again, there are a number of excellent reviews on this topic (160–163), including a complete volume in the Methods in Enzymology series (159).

Recently, Pratt (164) and Krantz (165) have suggested that any inactivator that utilizes an enzyme's mechanism, in the broadest sense, should be described as a mechanism-based inhibitor. Although this is not unreasonable, we have, for the purposes of this chapter, adopted the more narrow view of Silverman (166). In this view, mechanism-based inhibitors (also called suicide substrates, Trojan horse inactivators, enzyme-induced inactivators, k_{cat} inhibitors, and latent inactivators) are described as unreactive compounds, the structure of which usually resembles that of a substrate or product of the target enzyme, and that undergo a catalytic transformation by the enzyme to species that, before release from the active site, inactivate the enzyme. Thus, these compounds usually contain a latent, reactive functional group that gets activated during the normal catalysis of the enzyme. Upon formation of the initial reversible enzyme-inhibitor complex E . I, the enzyme starts its normal catalytic cycle, leading in a usually rate-determining step to the formation of a highly reactive species, E . I' (Equation 17.46).

$$\mathrm{E} + \mathrm{I} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{E}\cdot\mathrm{I} \xrightarrow{k_2} \mathrm{E}\cdot\mathrm{I}' \begin{cases} \xrightarrow{k_3} \mathrm{E}\text{–}\mathrm{I}'' \\ \xrightarrow{k_4} \mathrm{E} + \mathrm{P} \end{cases} \qquad (17.46)$$

The reactive species can either react with one of the enzyme active-site amino acid residues, to form a covalent bond between the enzyme and the inhibitor (E-I"), or be released into the medium to form product (P) and free active enzyme (E). In some instances the reaction may occur between the reactive species and the enzyme's cofactor, again resulting in inactivation of the enzyme.

It should also be noted that the activation of a mechanism-based inhibitor by its target enzyme is, formally, an example of metabolic activation. However, there is a clear distinction between the activation of a mechanism-based inhibitor described above and the metabolic activation of a prodrug. In the latter case, an inactive precursor is metabolized in the body (either chemically or enzymatically) to metabolites that possess the desired activity. For example, Acyclovir (3a) must be metabolically converted to the triphosphate (3b) and released into the medium before it will inhibit viral DNA polymerase. Further discussion on prodrugs may be found in volume 2, chapter 14.

3.1 Evaluation of the Mechanism of Inactivation of Covalently Binding Enzyme Inhibitors

The inherent complexity of the inactivation mechanisms of covalently binding enzyme inhibitors makes it necessary to evaluate their proposed modes of action carefully. An overview of the criteria for the study of irreversible inhibitors is provided below.

3.1.1 Criteria for the Study of Affinity Labels. The evaluation of affinity labels is based on the fulfillment of the following criteria:

1. ***Irreversible inactivation.*** Inactivation by affinity labels leads to irreversible covalent bond formation between the enzyme and the inhibitor. Unlike the complex between and enzyme and a rapid, reversible inhibitor, the covalent enzyme-inhibitor complex is no longer in equilibrium with free enzyme and inhibitor. Therefore, exhaustive dialysis or gel filtration of the covalent enzyme-inhibitor complex cannot lead to the recovery of free, active enzyme. However, such experiments do not allow distinction among tight-binding, noncovalent inhibitors, affinity labels, and mechanism-based inactivators.
2. ***Time- and concentration-dependent inactivation showing saturation kinetics.*** The