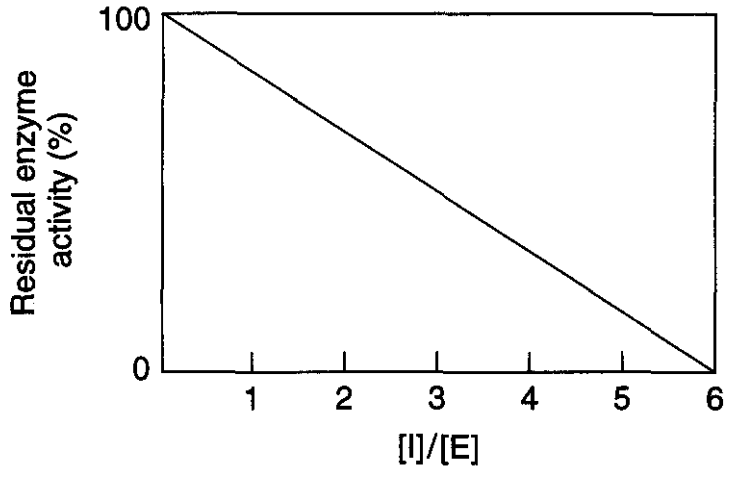

Figure 17.29. Determination of the partition ratio.

the initial concentration of inhibitor, it will depend on factors such as the rate of diffusion of the reactive species from the active site, its reactivity, and the proximity of the target for covalent bond formation. A number of different methods have been used to determine the partition ratio. For example, if, under the experimental conditions, the rate of inactivation is relatively fast compared to the chemical stability of the enzyme or the inhibitor, the partition ratio can be determined kinetically by titration of the enzyme activity. The titration measures the number of inhibitor molecules required to completely inactivate the enzyme. In an experiment of this type, increasing amounts of inhibitor are added to a known, fixed amount of enzyme, and the reaction is allowed to go to completion. After gel filtration or dialysis, a plot of the amount of inhibitor per enzyme active site and the remaining enzyme activity is drawn (Fig. 17.29). The intercept with the x-axis represents the minimum number of equivalents of inhibitor necessary to inactivate the enzyme completely (turnover number). A turnover number of 6, such as that shown in Fig. 17.29, indicates that on average 5 equivalents of inactivator are converted to product and only every sixth equivalent of inhibitor leads to irreversible covalent bond formation (i.e., the partition ratio equals the turnover ratio minus 1). Unfortunately, there are a number of factors associated with this method that may lead to misleading results (166). Another method for determining partition ratios is equilibrium dialysis of the enzyme with radiolabeled inactivator, followed by determination of the amount of radiolabeled metabolites produced per radiolabeled enzyme. Perhaps the simplest method is for cases where the rate of product formation (i.e., $k_{cat} = k_4$ in Equation 17.46) can easily be measured. In this instance, both k_{cat} and k_{inact} are measured directly, with k_{cat}/k_{inact} being the partition ratio (166, 167).

A more detailed discussion of the requirements for mechanism-based inhibition can be found in a recent review by Silverman (166).

3.2 Affinity Labels

Affinity labels are potentially good drugs, although the presence of a reactive functional group can make them somewhat nonselective and prone toward reaction with other proteins and metabolites. If the affinity label is highly selective toward its target enzyme and has a great affinity for the enzyme's active site, this drawback can be overcome kinetically. Once the inhibitor is bound, the unimolecular reaction between the inhibitor and an amino acid residue in close proximity is entropically quite favorable compared to a bimolecular reaction between two free molecules in solution. This proximity effect has resulted in rate enhancements as great as 10^8 (172) and means that a reagent that is, in itself, only weakly active, may be highly reactive when it is reformulated as an affinity label. More in-depth discussion on this topic can be found elsewhere (39, 173, 174).

The design of a potent affinity label requires the study of the initial requirements for the inhibitor to bind to the active site. Next, regions of bulk tolerance are determined that are useful for the introduction of a reactive functional group. In some cases, it might be advantageous to place the reactive group at the end of a spacer arm, particularly if no nucleophilic amino acid residue is in close proximity to the reactive group. However, not only the location and orientation, but also the size and inherent reactivity of the reactive functional group are critical for its potential as an affinity label.

Perhaps the archetypical example of an affinity label is TPCK (66) (Fig. 17.30). This