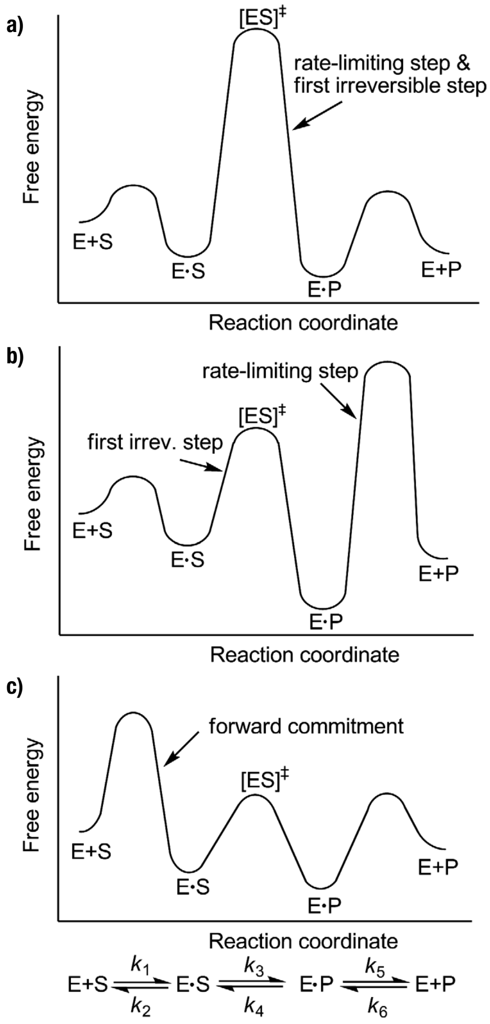

Figure 15.8. Free-energy diagrams for hypothetical enzymatic reactions, illustrating the effect of kinetic complexity on experimental kinetic isotope effects. **(a)** In the simplest scenario, the isotope-sensitive step is both the rate-limiting step and the first irreversible step. The intrinsic KIE will be fully expressed in the *V*/*K* KIE. **(b)** If the rate-limiting step occurs after the first irreversible step (which is the same as or follows the isotope-sensitive step), the intrinsic KIE will still be fully expressed in the *V*/*K* KIE. **(c)** If the substrate is sticky ($k_3 > k_2$), forward commitment will cause masking of the intrinsic KIE. Modified from Berti and Tanaka.[30]

Intrinsic isotope effects

To establish the transition state for a reaction, it is imperative that the KIEs be *intrinsic*; that is, they must reflect only the chemical step. The simplest case is where the chemical step is both the rate-limiting step and the first irreversible step [Figure 15.8(a)]. However, it is common for enzymatic reactions to involve different rate-limiting and first irreversible steps [Figure 15.8(b)] and to involve additional kinetically significant steps [Figure 15.8(c)]. A feature of the competitive method is that it reflects isotope effects on the enzyme's specificity constant, k_{cat}/K_m (or V_{max}/K_m; these isotope effects are commonly referred to as *V*/*K* KIEs) and therefore reports on atomic vibrational frequency changes between the unbound substrate and the first irreversible step. Following the first irreversible step, the bound species is committed to proceeding to completion and no further isotopic discrimination can occur; thus, situations such as that depicted in Figure 15.8(b) would not interfere with the measurement of *V*/*K* KIEs. A possibility that may prevent *V*/*K* KIEs from being intrinsic, however, is with a "sticky" substrate, which, once bound to the enzyme, has a greater tendency to partition toward products than to dissociate back into solution [i.e., $k_3 > k_2$ in Figure 15.8(c)]. This occurrence, called "commitment to catalysis" or simply "commitment," causes the magnitude of the observed *V*/*K* KIE to always be lower than the intrinsic KIE; as a result, one must choose alternative reaction conditions to reduce or eliminate commitments or conduct additional experiments (e.g., isotope trapping[31]) to quantitate and correct for this effect. It is of interest to note that more catalytically efficient enzymes have increasing commitments to catalysis, with "perfect enzymes" (i.e., $k_{cat}/K_m \sim 10^9\ M^{-1}\ s^{-1}$) being fully committed and therefore exhibiting no KIE.

Computational modeling of transition states

At present, there are no algorithms for generating transition-state structure directly from experimentally determined KIEs. Instead, one relies on iterative computational modeling of predicted structures to calculate KIEs that, when the structure is optimized, match the experimental values. This method requires modeling of both the substrate, whose structure can often be obtained from crystallographic, spectroscopic, or other means, and the transition state. The latter structure can be obtained by iterative model manipulation, such as fixing bond lengths and angles, and frequency calculation of all vibrational modes, with the assistance of structure software applications, such as Gaussian,[32] which can be run on common desktop computers but may require advanced computational capabilities for more complex systems. The vibrational frequencies can be then be analyzed by programs such as QUIVER[33] or ISOEFF98,[34] which are based on equations of the form in Equation (15.1),[35] originally derived in the midtwentieth century by Bigeleisen:[36–38]

$$\frac{k_L}{k_H} = \frac{v_L^{\ddagger}}{v_H^{\ddagger}} \times \prod_{i}^{3n-6} \frac{u_{iL}^{R} \sinh\left(u_{iH}^{R}/2\right)}{u_{iH}^{R} \sinh\left(u_{iL}^{R}/2\right)} \times \prod_{i}^{3n-7} \frac{u_{iH}^{\ddagger} \sinh\left(u_{iL}^{\ddagger}/2\right)}{u_{iL}^{\ddagger} \sinh\left(u_{iH}^{\ddagger}/2\right)}, \tag{15.1}$$

where the subscripts L and H indicate light and heavy isotope, respectively; the superscripts R and ‡ indicate reactant and transition state, respectively; n is the number of atoms in the system; $v^{\ddagger}$ is the imaginary frequency of bond lysis at the transition state; and $u_i = hv_i/k_B T$, where h is Planck's constant, v_i is the frequency of the ith vibrational mode in wave numbers, k_B is Boltzmann's constant, and T is the absolute temperature.[35] Systematic variation of model geometry is necessary to identify the ranges of bond lengths, bond angles, and dihedral angles that would give rise to the observed KIEs, within experimental error. The extent of computational space may be