How Kinetic Isotope Effects Can Be Used to Understand Enzymatic Transition States

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Understanding enzymatic transition states is a powerful tool in biochemistry, especially in the context of designing transition-state analogues to act as inhibitors of undesirable or pathological biochemical reactions. Despite the common misrepresentation of a protein crystal structure as a static molecule, proteins are highly dynamic, as are enzymatic transition states. Even mutations that occur far away from enzymatic catalytic sites have the power to change the overall vibrational structure of a protein and, by extension, change the nature of its transition state. The rationale for wanting to understand enzymatic transition states lies in the possibility of designing extremely effective and specific inhibitors.

The "thermodynamic box" (conceptualized by Wolfenden) provides a way for thinking about the energies of transition states. We know from chemical studies that the rates of reactions without catalysts are very slow. Additionally, scientists can measure the rates of enzyme catalysis (turnover) as well as Michaelis constants/dissociation constants, which give us information about how tightly substrate molecules bind to enzymes. If, in theory, all four sides of the "box" are connected in thermodynamic equilibrium, the product of any two sides of the square is equal to the product of the other two sides. Therefore, one can solve for the dissociation constant (K_d) of the transition state (TS). This tells us how much energy it takes at the instant of the TS to pull out the transition-state species and put it back in. Energetic calculations show that TS analogues can bind 10^{10} to 10^{15} times tighter than the substrate. These are huge energies, especially because substrates already bind with micromolar dissociation constants. These energies essentially represent the irreversibility of binding. Therefore, scientists have set out to make TS analogues that should capture part of the binding energy. In theory, when a perfect TS analogue inhibitor binds an enzyme, the same enzymatic conformational changes occur as when the substrate binds, but all the energy of activation is converted into energy of binding, represented graphically in figure 1 by falling into a stable TS energy well (bolded dot). Since these TS analogues can bind 10^{10} to 10^{15} times tighter than the substrate, they would make exceptionally potent inhibitors.

Understanding enzymatic transitions states is no easy task. Kinetic isotope effects (KIE) have been an extraordinary resource in helping us comprehend the nature of transition states. Generally, KIE report on bond vibrations at the instant of catalysis, or the instant when a bond is being broken. (Binding isotope effects are different from KIE in that they report on the difference in bond vibration between two stable states.) When an enzyme catalyzes a reaction, there are various steps involved: binding of the substrate, distortion of the substrate, motions, TS barrier, and release of products. KIE help elucidate what happens at the instant of the TS by reporting if bond-breaking is a rate-limiting step of the process.

First, a target enzyme is selected and its substrate is synthesized with isotopically labeled atoms. By measuring the isotope effect, a pattern is obtained that reports on the bond vibrational environment of every atom at the instant of the TS. For example, if it is determined that the carbon-14 at a certain position reports a number of 1.026 in measuring the KIE, this means that the isotopic substitution slows the reaction rate by 2.6 percent. The reported number of 1.026 is the ratio of the reaction rate of a normal substrate divided by the reaction rate of the labeled substrate. The ratios reported for each atom tell us about what bond vibrational environment each atom has at the instant of the TS. This is the significance of the KIE.

Now that the various ratios that provide information about how every atom is vibrating at the instant of the TS have been obtained, we need to back up and figure out what kind of TS gives rise to those isotope effects. When the molecular structures are entered into a computer, software programs can predict where the TS is by calculating the probability of every electron at every possible point in space. The resulting structure is indicative of the *most probable* transition state a reaction will have. What kind of isotope effect would that TS give us? One can calculate backward and get a family of isotope effects. Next, one would compare those calculated isotope effects (IE) to the experimental IE from the actual TS of the enzyme. By comparing experimental and electronic results, scientists can make changes until they find a match between the computer-driven TS and the actual TS measured in the lab. From there, a geometric model can be built around two parameters: geometry and electrostatics of the TS. The model will tell us what we need to know about the instant of the TS: the geometry, electron distribution, Van der Waals surface, charge interaction, hydrophobic forces, hydrogen bonding, and so on. By using this model as a blueprint, scientists can design a chemically

stable molecule that looks like the TS, which can act as a potent inhibitor. Therefore, by measuring the KIE, and hence the vibrational environment, of each atom at the instant of the TS, investigators are able to build models of highly-probable transition states that can be used for subsequent testing for TS analogue inhibitor designs.

REFERENCES

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