Did Enzymes Evolve to Capitalize on Quantum Tunneling?

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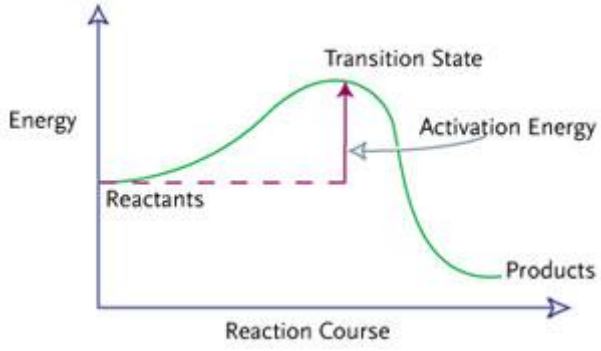
The activation energy barrier separates reactants from products. The top of the barrier is the transition state. Putting the right amount of energy into the reaction allows the reactants to surmount the barrier. Occasionally, the particle can appear in the probability area on the other side of the activation barrier. In effect, hydrogen "tunnels" through the activation barrier. Particles of different energies tunnel at various heights. The thinner barrier at the top promotes tunneling, but few particles have sufficient energy at these heights.



In the early years of the 20th century, a new theory, quantum mechanics, revolutionized physicists' understanding of nature. But delving into the subatomic realm meant rethinking some fundamental assumptions: Here, information passes instantly between particles; protons can be in two places at once; and hydrogen can defy classical conservation of energy.

Today, researchers use quantum mechanics to refine their understanding of the physical laws governing life. A phenomenon called quantum tunneling (QT), for example, lets hydrogen pass from reactant to product when there isn't enough energy in the mix to let the reaction occur by classical routes. QT is more than an esoteric biochemical byway; it allows some reactions to occur quickly enough to sustain life. But whether enzymes evolved to make the most of QT is hotly contested, with recent studies yielding contradictory results.

JUMPING THE BARRIER

According to the classical view of enzyme action, an activation barrier (see Fig. 1) separates reactants from products. Reactants require sufficient energy to surmount the barrier. The old view holds that enzymes function primarily by lowering the energy barrier. A more recent revision suggests that enzymes form distinct pathways involving intermediates that introduce multiple smaller barriers.

Several observations don't fit this model, however. For example, hydrogen (proton) transfer is important in many enzymatic reactions. In some cases, researchers found that hydrogen moved from the reactant to the product, despite the fact that the reaction did not have sufficient energy to get over the activation barrier. Moreover, replacing protons by deuterons, which have double the mass, reduces the rates of some reactions up to 100-fold, notes Willem Siebrand at the National Research Council of Canada. This effect is much more marked than one would expect from the classical model. "These large kinetic isotope effects [KIEs] are a typical quantum phenomenon for which there is no alternative classical explanation," says Siebrand. So enzymologists now often include QT when modeling enzyme actions.

In quantum mechanics, particles obey probability laws: One can find a quantum particle anywhere within a certain area, although the probability of detecting it varies across the space. For light atoms, such as hydrogen, this probability area can extend through activation barriers. So occasionally, hydrogen appears in the part of the probability area on the other side of the energy hurdle, in effect tunneling through the activation barrier. "In thermal processes, the system jumps over a barrier by borrowing thermal energy from its environment. In QT it goes straight through the barrier every now and then," says Gerard Milburn from the University of Queensland, Australia.

Although it may seem esoteric, QT is essential. In rhodopsin, Milburn notes, QT enables the molecule to change from a cis to a trans isomer. This switch triggers the cascade of electrical stimulation that ultimately ends in vision. "If all the mobile hydrogen atoms in our [bodies] were replaced by deuterium, we would undoubtedly die," Siebrand adds. "Some essential enzymatic processes would become too slow to sustain life."

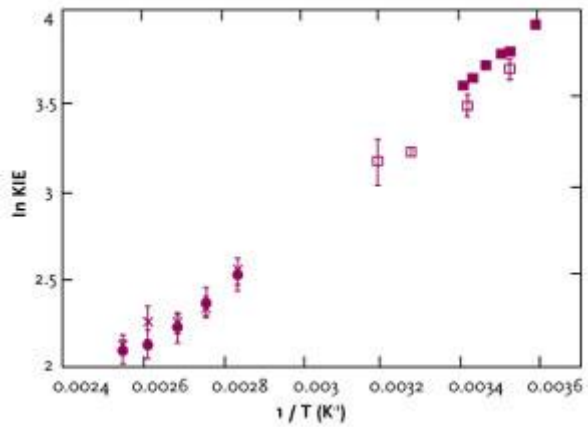
CONSENSUS AND CONTROVERSY

Judith Klinman of the University of California, Berkeley, pioneered studies into QT's role in enzyme action. She began her research in the mid-1980s by exploring anomalies in the hydride-transfer reaction catalyzed by alcohol dehydrogenase. Her work proved central to the current consensus that QT contributes to enzyme catalysis under physiological conditions.

While QT contributes to all hydrogen-transfer reactions, its importance depends on several factors, such as the particle's mass, the temperature, and the reaction. Siebrand explains that when the barrier is low, compared to the kinetic energy available at the ambient temperature, tunneling will be relatively insignificant. This is the case when protons transfer along a strong hydrogen bond. On the other hand, tunneling may be the dominant transfer mechanism when the reaction involves breaking a C-H bond. In this case, the energy barrier is high. Klinman adds that reaching this consensus was an uphill battle. "We have moved on to a new stage where we are investigating the degree to which the enzyme influences tunneling," she says.

Klinman's group found that altering the amino acid residues in the active sites of enzymes influences QT. Klinman interpreted the studies as suggesting that enzymes, by being flexible, could optimize the distance between the hydrogen donor and acceptor.[12] "Decades of studies show that enzymes generally use all available physical properties of nature to achieve their spectacular catalysis," she says.

A co-plot of KIE vs. 1/T for the reactions assessed by Finke is linear within experimental error over a 110°C temperature range. This co-plot includes published data ○, X, and □ from K.M. Doll, R.G. Finke, Inorg Chem, 42:4849-56, 2003) and unpublished data ■ (filled squares)provided by Finke. It reveals that there is no statistical difference within experimental error of the enzyme-free and enzymatic KIEs vs. temperature. The simplest "Ockham's Razor" interpretation is that evolution has not enhanced the degree of tunneling for this particular B_{12}-dependent enzyme.



Nevertheless, not all studies support suggestions that enzymes evolved to optimize QT. At Colorado State University, Richard Finke expected to confirm Klinman's hypothesis, so he says that the results came as a surprise. In vivo, methylmalonyl-CoA mutase converts methylmalonyl-CoA to succinyl-CoA using adenosylcobalamin (coenzyme B_{12}). Another B_{12}-dependent enzyme, diol dehydratase, uses ethylene glycol as a substrate. In vitro, adenosylcobalamin and a chemical relative, beta-neopentylcobalamin, catalyze the transfer of hydrogen or deuterium when the isotope is bonded to carbon in ethylene glycol.

Finke obtained KIEs over a 110°C range. He compared the in vitro data to KIEs measured when methylmalonyl-CoA extracts hydrogen or deuterium. He notes that hydrogen transfer rates often correlate "pretty well" with bond energies plus tunneling effects. This approach, Finke says, allowed his group to compare, for the first time, QT in enzymatic and nonenzymatic reactions at physiologically relevant temperatures.

Finke found that temperature-dependent KIEs all lie on the same line (see Fig. 2), irrespective of whether the reaction is enzyme-catalyzed. In other words, no statistically significant increase in QT occurred in the presence of the enzyme, and the tunneling parameters did not change (within experimental error) when the enzyme was removed. "The enzyme-enhanced tunneling hypothesis appears to be disproven in this particular case," he says.

Other researchers also question whether enzymes evolved to enhance QT. Klinman had suggested that some proteins might change shape to shorten the transfer distance. "This claim was made to account for the very short transfer distances she [Klinman] deduced from her experiments, distances much shorter than normally found for these atoms in crystals," Siebrand says. He adds that the distances in enzymes could not be measured directly and were calculated from "highly simplified" models. "We believe that there is no need to invoke such special protein dynamics of a type never experimentally observed or theoretically predicted," he says. Siebrand developed a model of QT, which he used to analyze Finke's data. His unpublished analysis found that the rates, isotope effects, and temperature dependence agree with the results calculated for transfer distances derived from known structural parameters.

Nevertheless, Finke's study is subject to several caveats. Finke admits, for example, that he cannot definitely rule out alternative hypotheses, such as that the data are coincidental for some unknown reason. Finke's papers also note that the model used ethylene glycol rather than the natural substrate. Furthermore, cleavage of the cobalt-carbon bond, rather than hydrogen transfer, is largely responsible for determining the rate, so this enzyme might not have been under much evolutionary pressure to improve QT. Finke says that it is still unclear if other enzymes enhance tunneling. "There is no experimentally documented case where enzyme-enhanced [quantum] tunneling has been unequivocally demonstrated, and there is now one case where the data show ... no enhancement of [quantum] tunneling within experimental error."[3]

NATURE DOING CHEMISTRY

To help settle the controversy, Klinman's group is studying several reaction types, using mutagenesis to vary the enzymes' active sites. The group is also comparing thermophiles to

mesophiles and psychrophiles to link enzyme dynamics with hydrogen transfer. Meanwhile, Finke suggests testing systems in which enhanced hydrogen removal should have been under evolutionary pressure. But he adds that neither he nor others have yet identified suitable enzymes. For instance, Steven Schwartz at Albert Einstein School of Medicine adds that Finke "for good experimental reasons" chose a model in which QT's importance is not as clear as some other systems. "Alcohol dehydrogenase would be a great candidate," Schwartz suggests, but "unfortunately, it would take centuries in solution."

Finke says that advocates of the evolution hypothesis need to reexamine their published data. "At least some of the key studies claiming evidence for the enzyme-enhanced tunneling hypothesis interpret their data only in terms of their original hypothesis. Science is about multiple alternative hypotheses and disproof," Finke emphasizes.

Until the controversy is settled, researchers will continue to debate whether enzymes evolved to enhance QT. The phenomenon undoubtedly contributes to enzymatic hydrogen transfer: "QT is just nature doing chemistry," Schwartz says. "The amazing thing is not that particles tunnel at physiologic temperatures, but that you have to work so hard to find experimental evidence for it."