have led to the general conclusion that electron-hole pair excitations can be ignored or play at most a very minor role in low-energy adsorption. There are, however, important reasons to be cautious about this conclusion. There are small but systematic discrepancies between experiments and theory, and most simulations use semiempirical potentials with many adjustable parameters. Recent calculations show that electron-hole pair excitations can play a substantial role in some systems (*9*).

Direct measurements of electron-hole pair excitation can add much to our knowledge of the adsorption process. Gergen *et al.* (*1*) report such measurements for adsorption energies of 0.2 to 3.5 eV. The authors use a Schottky diode, which enables the detection of electrons from adsorption-induced electron-hole pair excitations, provided that they have energies greater than the Schottky barrier. The energy distribution of the electrons produced is not well known, and it is thus difficult to estimate the absolute detector sensitivity. Notwithstanding these difficulties, the probability of electron-hole pair excitations in these systems is estimated to range from 6 to 100%, implying that electron-hole pairs can indeed play an important role in energy dissipation for a wide variety of adsorption systems.

Gergen *et al.*'s report represents an important step in obtaining systematic information on electron-hole pair generation in atomic and molecular interactions with surfaces. With improved detector fabrication and the use of detectors with variable energy thresholds, it should be possible to map the energy distributions of electrons generated and to measure excitation probabilities more quantitatively. The use of molecular beam techniques to control the energy and angle of incident species can potentially provide great detail about the dynamics of electron-hole pair generation.

Electron-hole pair generation has recently been invoked as a mechanism for vibrational relaxation at surfaces (*10*). The use of Schottky diode detectors will enable a direct test of this hypothesis and provide much needed detail of the electron-hole pair generation. The prospects for future advances are bright.

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PERSPECTIVES: ENZYMOLOGY

Coenzymes and Radicals

Perry A. Frey

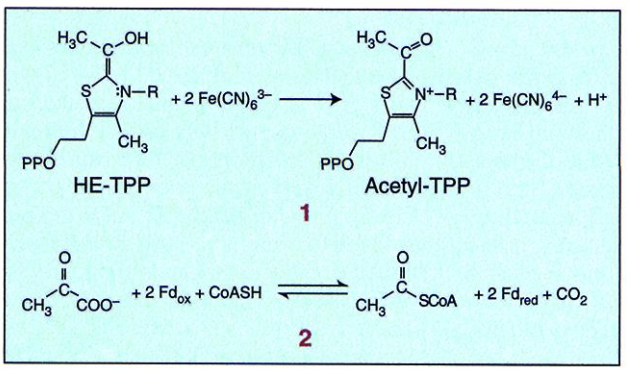
In recent years, organic radicals have come into their own as transient intermediates in enzymatic reactions. Spectroscopic data from electron paramagnetic resonance (EPR) implicate these highly reactive species in the actions of enzymes that catalyze a wide range of biological reactions, including electron transfer, isomerization, oxidation and oxygenation, reduction, DNA repair, and biosynthesis of antibiotics and DNA.

Because of their transient nature, little is known about the structure of the radical species and their mode of action. On page 2559 of this issue, Chabrière *et al.* (*1*) provide a rare view of the structure of a biological radical within its catalytic site. They present the crystal structure of the enzyme pyruvate:ferredoxin oxidoreductase (PFOR), crystallized with the intermediate radical form of hydroxyethylidene–thiamine pyrophosphate (HE-TPP). The structure of the radical shows that the thiazole ring is puckered, a feature that affects its electronic structure.

The participation of organic radicals in enzymatic reactions requires the action of coenzymes, which bind to the enzyme and are essential for its activity but are not permanently altered by the reaction. Many of these coenzymes traditionally facilitate polar (nonradical) reactions. Evidence that traditional coenzymes such as TPP do double duty as radical initiators or facilitors of radical reactions continues to accumulate (*2–10*). Many coenzymes induce substrate radical formation in enzymatic reactions and/or appear at least transiently in radical forms. Some participate in protein radical formation or participate in the biosynthesis of coenzymes such as biotin, heme, and thiamine, presumably by initiating radical formation in precursors (*11*).

TPP has long been known to react through radical intermediates. Most TPP-dependent enzymes can be assayed by observing the reduction of ferricyanide. The enzymatic intermediates are aldehyde derivatives of TPP such as HE-TPP. Two equivalents of ferricyanide oxidize HE-TPP to acetyl-TPP (see reaction 1 in the first figure), which then undergoes hydrolysis to acetate and TPP. As a compulsory one-electron acceptor, ferricyanide must first produce an oxidized, radical form of HE-TPP as an intermediate, which then undergoes a second round of one-electron oxidation to acetyl-TPP. The radical intermediate has not been observed spectroscopically but must have been present, presumably at a low concentration. It has been generated in electrolytic experiments (*10*).

The enzyme PFOR catalyzes the TPP-dependent reversible reaction of pyruvate with coenzyme A (CoA) and ferrredoxin to produce CO_2, acetyl CoA, and reduced ferredoxin (see reaction 2 in the first figure). During this reaction, TPP-dependent decarboxylation of pyruvate first produces HE-TPP, which then reacts with CoASH and two molecules of oxidized ferredoxin to produce acetyl CoA and two molecules of reduced ferredoxin. Just like ferricyanide, ferredoxin must accept electrons one at a time. The two-electron oxidation must therefore proceed in one-electron steps, and a radical form of HE-TPP must exist at least transiently. This radical has long been known from spectroscopic studies and has been repeatedly reported in the literature as a stable species (*4–6*).



Key reactions. Ferricyanide oxidizes HE-TPP to acetyl-TPP (reaction 1). PFOR catalyzes the TPP-dependent reversible reaction of pyruvate with coenzyme A (CoA) and ferredoxin (Fd) to produce CO_2, acetyl CoA, and reduced ferredoxin (reaction 2).

The electronic structure of the HE-TPP radical can be formulated at two protonation levels. Removal of one electron from HE-TPP leads to a cation radical, in which

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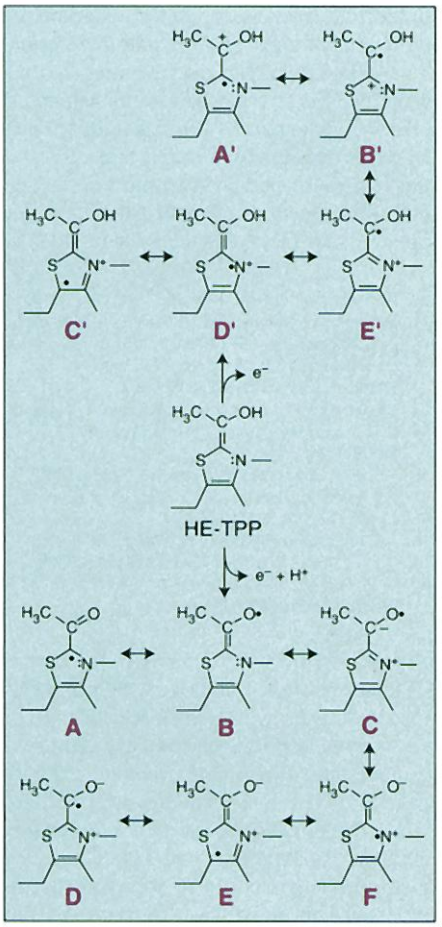

Principal resonance form for cation and neutral HE-TPP radicals. One-electron oxidation of HE-TPP gives a cation radical in which both the positive charge and the unpaired electron are highly delocalized. The delocalization is represented by the principal resonance forms **A′** to **E′ (top)**. One-electron oxidation coupled with loss of a proton gives a neutral radical, with the resonance forms **A** to **F (bottom)**. It is not known whether the radical in PFOR is the cation or neutral radical.

the cationic charge and the unpaired electron are delocalized (see structures **A′** to **E′** in the second figure). Removal of one electron and one proton from HE-TPP leads to a neutral radical, in which only the unpaired electron is delocalized (see structures **A** to **F** in the second figure).

We do not know which protonation level pertains to the radical in PFOR. In radicals in which the unpaired electron is delocalized, the resonance forms generally do not contribute equally to the structure, and one or a few are dominant. The puckered ring observed by Chabrière *et al.* rules out resonance forms with two double bonds in the ring (**E′**, **C**, and **D** in the second figure) as dominant forms and relegates them to minor status. Further clues come from the fact that incorporation of ^{13}C or deuterium into the exocyclic carbons of the radical leads to very modest perturbations of the EPR signal (*4*–*6*). If the unpaired electron were localized on the exocyclic carbons, dramatic changes in the signal would have been expected (*12*).

Therefore, the unpaired electron is likely to be largely confined to the ring. The resonance forms **A′**, **C′**, and **D′** for the radical cation or **A**, **E**, and **F** for the neutral radical may be the principal forms. These groups differ mainly by the presence of the proton in the cation radical. Detailed EPR analysis in H_2O and D_2O, with ^{13}C labeling at positions in the thiazole ring and with deuterium labeling in the methyl and methylene carbons, by available methods (*13*) will answer the remaining questions about the electronic structure.

Chabrière *et al.* suggest structures for the HE-TPP radical that include tautomeric forms resulting from labilization of protons from the methyl group of TPP. These unprecedented tautomeric isomerizations would lead to deuterium incorporation into the methyl group whenever the enzymatic reaction is conducted in D_2O. Analysis of TPP recovered from such experiments will provide an essential future test for their hypothesis.

Because CoA is required for the second electron transfer in PFOR, it is likely that CoA itself donates the second electron to the iron-sulfur centers in PFOR (*4*) and is transformed transiently into a thiyl radical, which undergoes radical coupling with the HE-TPP radical to form acetyl CoA and TPP. CoA may therefore be added to the list of coenzymes that participate as radicals in enzymatic reactions.

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PERSPECTIVES: GLOBAL CHANGE

Sharing the Garden

Christopher B. Field

Human activities have profound impacts on our planet, from the extinction of once-abundant species, to changes in the composition of the atmosphere, to the strong likelihood of effects on climate. There is no doubt that these human impacts are large, but it can be difficult to say how large in a way that is accurate, meaningful, and easy to explain.

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Among the most useful measures proposed to date is the fraction of Earth's total plant growth or net primary production (NPP) that is appropriated by humans. NPP is the energy transferred from plants to other levels in the food chain. It provides support for nearly all of Earth's heterotrophs (organisms that require preformed organic compounds for food), including humans. In an influential 1986 paper, Vitousek *et al.* (*1*) estimated that human appropriation of NPP was 32% of the land total with a conservative definition and 40% using the most reasonable definition of human appropriation. This is a huge fraction. If we already control two-fifths of the land's productive capacity, then the prospects for future increases are strongly constrained, especially if there is to be anything left for other species.

Since 1986, research in global ecology has increased dramatically, with improved data sets, simulation models, and analytical techniques. On page 2549 of this issue, Rojstaczer *et al.* (*2*) use some of the new products to revisit human appropriation of land NPP. Their mean value of 32%, using the conservative definition of (*1*), is the same as the comparable value from the 1986 study (*1*). Partly, this reflects the insight and judgment of the authors of that study. Partly, it is luck, as upward revisions of some appropriations nearly balance downward revisions in others.

The new numbers are based on a huge body of information, but the estimate for the core quantity—human appropriation of land NPP—is still uncertain. The magni-