Enzyme Mechanisms

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Radical and Electron Recycling in Catalysis

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coenzymes · enzyme catalysis · radical enzymes · radicals · synthetic methods

An increasing number of enzymes are being discovered that contain radicals or catalyze reactions via radical intermediates. These radical enzymes are able to open reaction pathways that two-electron steps cannot achieve. Recently, organic chemists started to apply related radical chemistry for synthetic purposes, whereby an electron energized by light is recycled in every turnover. This Minireview compares this new type of reaction with enzymes that use recycling radicals and single electrons as cofactors.

1. Introduction

Leonor Michaelis, who together with Maud Menten introduced the famous Michaelis–Menten Equation to enzymology, proposed in 1939 that many enzymatic reactions involve one-electron steps.[1] The statement, which included mainly redox reactions, was challenged in the second half of the 20th century by the upcoming area of enzymology. The general opinion was that no reaction that could be formulated by two-electron steps proceeded via radicals. Today, the paradigm has changed again. We have experienced the discovery of an increasing number of enzymes that either contain radicals or are able to stabilize radical intermediates. These "radical enzymes" use the barrierless reactivity of radicals and tame them by specific interactions so as to avoid "free radicals", which could damage the protein or even the whole cell[2,3] (see Ref. [4]). Although the formation of radicals necessitates high energy and mechanistic expenditures, they open up new reaction pathways that two-electron steps cannot achieve. An example is the reversible hydration of crotonyl-CoA [**1**, coenzyme A thioester of (*E*)-2-butenoic acid, Scheme 1]. The "normal" two-electron product is (*S*)-3-hydroxybutyryl-CoA (**3**), whereas reactions via ketyl radicals lead to (*R*)-2-hydroxybutyryl-CoA (**2**)[5] or 4-hydroxybutyryl-CoA (**4**).[6]

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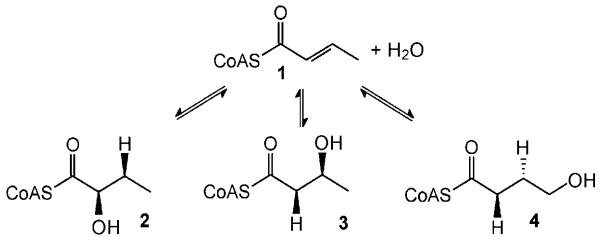
The question arises as to why organic chemistry does not apply such radical reactions to enlarge its synthetic potential. Recently, however, two publications appeared which describe the successful application of reductively formed ketyl radicals in organic synthesis.[7,8] This Minireview will demonstrate that some reductive biological radical reactions obey the same principle as these new synthetic approaches: it is the recycling of the electron that enables catalysis in one- and two-electron steps. Section 2 will cover those biological radical reactions in which the radical itself rather than the electron is recycled.

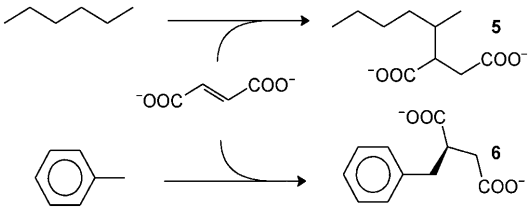


Scheme 1. Radicals open new reaction pathways.

2. Recycling Radicals with Enzymes

The majority of radical enzymes catalyze the homolytic cleavage of unactivated C–H bonds, which requires less energy than abstraction of a proton or hydride. Under aerobic conditions, the reactive species, which are able to functionalize hydrocarbons, are mainly derived in an irreversible manner from oxygen. The participation of radicals in these processes, however, is a matter of debate.[9,10] In contrast, there are several alternative pathways for the degradation of hydrocarbons under anaerobic conditions. A recently isolated bacterial consortium probably uses N_2O derived from the reduction of nitrite rather than oxygen for the oxidation of methane;[11] this process can also be found in industry, where CH_4 is applied to remove N_2O from industrial gases.[12] The oxidation of methane by sulfate to give CO_2 and H_2S in deep-sea sediments and in the anaerobic zone of the Black Sea

appears to require two organisms operating in a syntrophic (eating together) way. An archaeon catalyzes the oxidation of CH_4 to CO_2 and $4\,H_2$ by reverse methanogenesis (see Section 3) and the hydrogen is consumed by an H_2S-producing sulfate-reducing bacterium.[13] Hydrocarbons with more than one C atom are succinylated by anaerobic bacteria to provide a handle for further β-oxidation to acetyl-CoA and propionyl-CoA.[14] This leads to very characteristic intermediates, such as 2-(1-methylpentyl)succinate (**5**)[15] or (*R*)-2-benzylsuccinate (**6**, Scheme 2).[14,16–18] An exception is ethylbenzene, which—depending on the organism—is either succinylated to 2-methyl-2-benzylsuccinate[19] or hydroxylated to (*S*)-1-phenylethanol. In this case, the oxygen comes from water, mediated by a molybdopterin-containing dehydrogenase.[20]



Scheme 2. First step in a common pathway of anaerobic hydrocarbon degradation catalyzed by glycyl radical enzymes (GREs).

The alkyl and benzylsuccinate synthases (Scheme 2) belong to an enzyme family that in the resting state contains a stable glycyl radical (GRE, glycyl radical enzyme). During catalysis, the radical transfers to a specific cysteine residue to yield a thiyl radical (GRE-S•; see also Scheme 3), which removes a hydrogen atom from the substrate. The addition of fumarate to the substrate-derived radical affords the product-related radical (**5**• or **6**•) and regenerates the thiyl radical. The most prominent members of this extremely oxygen-sensitive enzyme family are pyruvate formate lyase[21] and ribonucleotide reductase,[22] which are only produced in *Escherichia coli* and some other bacteria under strictly anaerobic conditions. Other members are coenzyme B_{12} independent glycerol dehydratase[23] (see Scheme 7) and *p*-hydroxyphenylacetate decarboxylase from *Clostridium difficile*[24] which catalyze the formation of 3-hydroxypropanal and *p*-cresol, respectively.[25] In the case of *p*-hydroxyphenylacetate (**7**, Scheme 3), the thiyl radical most likely abstracts the phenolic

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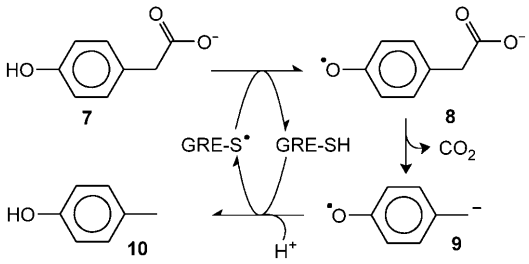
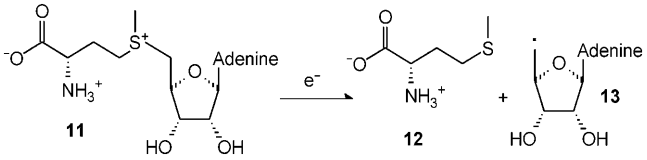


Scheme 3. Proposed mechanism of the decarboxylation of *p*-hydroxyphenylacetate (**7**) to *p*-cresol (**10**) catalyzed by a glycyl radical enzyme (GRE-S•).

hydrogen atom to yield the phenoxyradical **8**, which undergoes decarboxylation to give the resonance-stabilized *p*-cresolyl radical anion **9**. Protonation of **9** and regeneration of the thiyl radical affords *p*-cresol (**10**).[26]

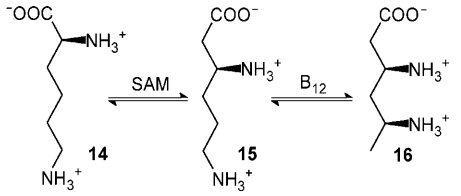
The bacterial cell synthesizes the glycyl radical enzymes as inactive proenzymes that are activated by so-called radical SAM enzymes. These activases require a strong one-electron reducing agent such as ferredoxin or flavodoxin to convert *S*-adenosylmethionine (**11**, SAM; Scheme 4), bound to an [4Fe-



Scheme 4. Reductive cleavage of *S*-adenosylmethionine (SAM, **11**) to form methionine (**12**) and 5′-deoxyadenosylradical (**13**).

4S] cluster, into methionine (**12**) and the first reactive species, the 5′-deoxyadenosyl radical (**13**). This radical abstracts a hydrogen atom from the conserved glycine residue of the proenzyme to irreversibly form the resting state of the active enzyme.[27,28] Upon addition of the specific substrate, for example, of pyruvate to afford pyruvate-formate lyase, the glycyl radical generates the thiyl radical from the conserved cysteine residue.[29]

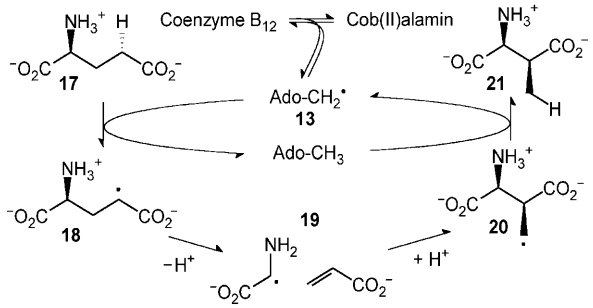
Bioinformatic analyses of genomes detected almost 3000 members of the SAM radical enzyme family.[30] Besides activation of glycyl radical enzymes, the 5′-deoxyadenosyl radical derived from SAM is involved in a large variety of enzyme activations, t-RNA maturations, and biosyntheses of cofactors.[30] Examples are the formation of the active sites of the enzymes [FeFe]hydrogenase, nitrogenase, and sulfatase as well as biosynthesis of the cofactors biotin, thiamine, protoporphyrin, and molybdopterin. All these reactions are irreversible and yield 5′-deoxyadenosine, which has to be degraded. There are only two exceptions, the spore photoproduct lyase—a DNA repair enzyme (see Section 3.2)—and α-lysine-2,3-aminomutase—the first step in the fermentation of (*S*)-α-lysine to ammonia, acetate, and butyrate by the anaerobic bacterium *Clostridium subterminale* (Scheme 5).[31] In both enzymes the 5′-deoxyadenosyl radical is recycled after each turnover. This radical is able to abstract either the unactivated H atom from the β carbon atom of α-lysine (**14**)



Scheme 5. The first two steps of anaerobic lysine fermentation.

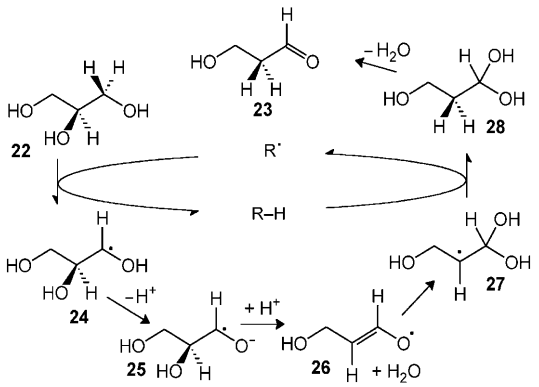
or the activated H atom from the α carbon atom of the product (*S*)-β-lysine (**15**). The migrating amino group is bound to pyridoxal-5′-phosphate.[4] The radical at the α carbon atom of β-lysine that is stabilized by the carboxyl group can be readily detected by EPR spectroscopy.[32]

Interestingly, **15** (Scheme 5) is converted further into (3*S*,5*S*)-3,5-diaminohexanoic acid (**16**), whereby the ε-amino group, also bound to pyridoxal-5′-phosphate, is shifted from the ε to the δ position. Although the reaction catalyzed by β-lysine-5,6-aminomutase appears to be identical to that catalyzed by lysine-2,3-aminomutase, the generator of the 5′-deoxyadenosyl radical (**13**) is coenzyme B_{12} rather than SAM.[33] Coenzyme B_{12}, also called adenosylcobalamin, contains a cobalt–carbon bond, which remains stable upon binding to the enzyme. Only after addition of the substrate to the enzyme does the substrate homolyze to radical **13** and cob(II)alamin. Each turnover regenerates the Co–C bond, as shown for the reversible rearrangement of the carbon skeleton of (*S*)-glutamate (**17**, Scheme 6) to (2*S*,3*S*)-3-methylaspartate (**21**). In the first step of the reaction catalyzed by glutamate mutase, radical **13** abstracts the 4-H_{Si} atom stereospecifically from glutamate. The resulting substrate-derived radical **18** undergoes exchange coupling with the unpaired electron of cob(II)alamin, as revealed by EPR spectroscopy.[34] Radical **18** fragments to a gylcyl radical and acrylate (**19**), which recombine to give the methyleneaspartate radical (**20**).[35,36] Finally, the initially abstracted hydrogen atom returns to **20** to regenerate **13** and afford the product **21**. A more common coenzyme B_{12} dependent reaction is the well-known rearrangement of the carbon skeleton of methylmalonyl-CoA to succinyl-CoA in bacteria and human mitochondria.[37]



Scheme 6. Proposed fragmentation mechanism of glutamate mutase in the anaerobic bacterium *Clostridium cochlearium*.

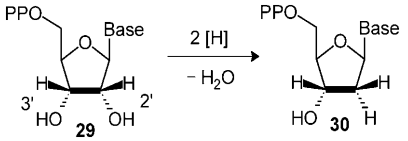
Coenzyme B_{12} has the advantage of much lower sensitivity than the glycyl radical and the [4Fe-4S] cluster/SAM radical enzymes towards oxygen. In addition, there is no known radical-generating alterative to coenzyme B_{12} for the "difficult" reversible rearrangements of the carbon skeleton and amino group migrations. Therefore, it has been postulated that in these reactions involving methylene radicals, cob(II)-alamin does not act as a spectator but as a conductor by actively stabilizing radicals, although in an as-yet unknown manner.[38,39] In contrast, the "easier" irreversible dehydration of glycerol (**22**) to 3-hydroxypropanal (**23**) is catalyzed by a coenzyme B_{12} dependent enzyme[40] as well as by a glycyl radical enzyme (Scheme 7).[41] The radical—either **13** or



Scheme 7. Proposed mechanism of glycerol dehydratase. R• is either the thiyl radical of a GRE-S• or radical **13** derived from coenzyme B_{12}. Alternatively, **24** may rearrange directly to **27**.[42]

GRE-S•—abstracts the hydrogen atom at the carbon atom neighboring the migrating hydroxy group. Deprotonation of radical **24** yields the ketyl radical **25**, which eliminates the hydroxy group at the adjacent carbon atom to afford the enoxy radical **26**. Re-addition of water leads to a more reactive radical **27** that is able to reabstract the hydrogen atom from 5′-deoxyadenosine or GRE-SH (R-H in Scheme 7) to recycle radical **13** or GRE-S•, respectively. Finally, the *gem*-diol **28** dehydrates to 3-hydroxypropanal (**23**).

A fascinating radical reaction is the reduction of ribonucleotides **29** to 2′-deoxyribonucleotides **30**, the building blocks of DNA (Scheme 8). There are four classes of enzymes that all catalyze this reaction. They exhibit related crystal structures, but differ in the generation of the thiyl radical in the active site.[43] Class I, which is present in humans as well as in *E. coli* under aerobic conditions, comprises a binuclear iron center that activates oxygen and forms a tyrosyl radical, the first stable radical detected in biological systems.[44] Upon addition of the substrate, the radical induces the generation of



Scheme 8. Reduction of ribonucleotides. PP = diphosphate.

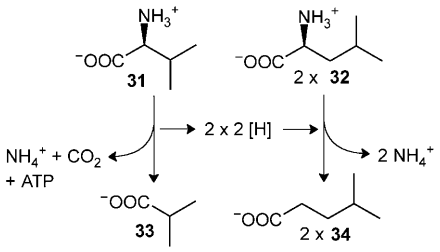
the thiyl radical over a distance of 40 Å, mediated by aligning tyrosine residues.[45] The distance is probably necessary to avoid interference of oxygen with the thiyl radical in the active site. In the enzyme from the bacterium *Chlamydia trachomatis* (class Ib), one iron atom is replaced by manganese, and the tyrosyl radical is missing. Apparently, manganese takes over the function of the conserved tyrosine residue.[46] Class II uses coenzyme B_{12} as the radical generator,[47] whereas class III is a gylcyl radical enzyme.[48] Similar to glycerol dehydratase (Scheme 7, **22**→**26**), the initial steps in the reduction of ribonucleotes are the abstraction of the 3′-hydrogen atom by the thiyl radical[49] and deprotonation of the 3′-OH group. The resulting ketyl radical expels the 2′-OH group and then undergoes two consecutive one-electron reductions via a disulfide radical anion[50] to form the product radical. The final return of the 3′-hydrogen atom recycles the thiyl radical.

3. Recycling of Electrons by Enzymes

Section 2 showed that the formation of radicals requires either oxygen or complex coenzymes such as *S*-adenosylmethionine or coenzyme B_{12}. The glycyl radical enzymes, the reversible SAM radical enzymes, and the coenzyme B_{12} dependent enzymes recycle the radical after each turnover (Schemes 3, 6, 7, and 16). On the other hand, the addition of just one high-energy electron to a carbonyl group generates a ketyl radical that enables catalysis in two-electron steps and recycles the electron for the next turnover. The 2-hydroxyacyl-CoA dehydratases[51] and DNA photolyases[52–54] use such a mechanism.

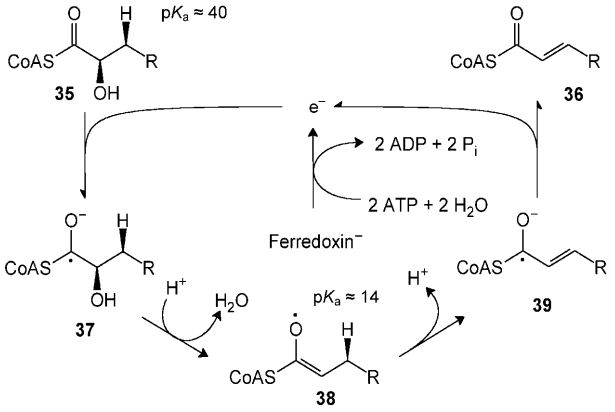
3.1. 2-Hydroxyacyl-CoA Dehydratases

Several anaerobic bacteria from the order *Clostridiales* and the genus *Fusobacterium* are able to ferment α-amino acids rather than sugars in a process known as the Stickland reaction.[55] They oxidize and decarboxylate one amino acid via the corresponding 2-oxoacid to a so-called "energy-rich" thioester, which leads to the formation of ATP through phosphorylation of the substrate. Another or the same amino acid regenerates the electron-accepting cofactors by reduction to a short-chain fatty acid (Scheme 9).



Scheme 9. Fermentation of the amino acids valine (**31**) as the donor and leucine (**32**) as the acceptor through the Stickland reaction to give isobutyrate (**33**) and isocaproate (**34**).

For the reduction of the amino acids, for example, L-leucin (**32**) to isocaproate (**34**), the amino group in the α position has to be eliminated together with the β hydrogen atom, which is not acidic except in the case of aspartate ($pK_a \approx 40$). For this difficult reaction, the amino acid in the hydroxyacid pathway, which occurs in the presence of strictly anaerobic bacteria in the human gut, is oxidized to an ammonium ion and to a 2-oxoacid, before being reduced to a (*R*)-2-hydroxyacid. The transfer of the $CoAS^-$ from an acyl-CoA molecule to the (*R*)-2-hydroxyacid delivers (*R*)-2-hydroxyacyl-CoA (**35**; Scheme 10), which is dehydrated in a *syn* conformation to



Scheme 10. Proposed mechanism for the *syn* dehydration of (*R*)-2-hydroxyacyl-CoA (**35**) to (*E*)-2-enoyl-CoA (**36**); R = H, CH_3, CH_2—COO^-, phenyl, or isopropyl; ADP = adenosine phosphate, P_i = inorganic phosphate.

(*E*)-2-enoyl-CoA (**36**). Finally, **36** is reduced to saturated acyl-CoA, and a second CoA transfer releases the fatty acid. Up to 12 of the 20 amino acids occuring in proteins can be reduced in a number of different organisms by this pathway to give the corresponding fatty acids with the same carbon skeleton as the original amino acid.[56]

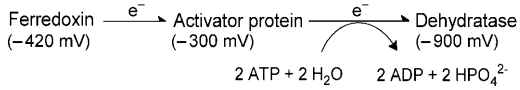
The most exciting enzyme in this pathway is 2-hydroxyacyl-CoA dehydratase, whose substrate encounters the same problem as the parent amino acid—the activation of the β hydrogen atom. To date, four such enzymes have been characterized which catalyze the following dehydrations:

1) (*R*)-lactyl-CoA derived from alanine, serine, and cysteine to acryloyl-CoA or (*R*)-2-hydroxybutyryl-CoA (**2**; Scheme 1) from methionine and threonine to crotonyl-CoA (**1**);
2) (*R*)-2-hydroxyglutaryl-CoA from glutamate, glutamine, and histidine to (*E*)-glutaconyl-CoA;
3) (*R*)-2-hydroxyisocaproyl-CoA from leucine to 2-isocaprenoyl-CoA (probably the *E* isomer);
4) (*R*)-3-phenyllactyl-CoA from phenylalanine to (*E*)-cinnamoyl-CoA.

Phenyllactyl-CoA dehydratase probably also catalyzes the dehydration of the two 3-aryllactyl-CoA derived from tyrosine and tryptophan to arylacryloyl-CoA, which are then reduced to 3-arylpropionates. All these dehydratases consist of two extremely oxygen-sensitive proteins: a homodimeric

activator with one [4Fe-4S] cluster between the two subunits, and the actual heterodimeric dehydratase with one [4Fe-4S] cluster in each subunit. The crystal structure of the activator protein of 2-hydroxyglutaryl-CoA dehydratase from *Acidaminococcus fermentans* (2×27 kDa) shows that four cysteine residues, two from each subunit, coordinate to the [4Fe-4S] cluster that is located between two helices, thereby forming a helix-cluster-helix angle of 105°.[57] Each subunit contains an ATP binding site related to that found in acetate and sugar kinases, heat shock protein Hsp70, and actin (ASKHA).[58] The two [4Fe-4S] clusters of the dehydratase are probably similar to that in the aconitase cluster of the Krebs cycle[59] or to that in SAM radical enzymes.[60] Mössbauer spectroscopy revealed two different iron species in a ratio of 3:1. Hence, three cysteine residues could coordinate to the cluster through three iron atoms, while the substrate binds at the fourth iron center.[89]

These data and chemical model reactions (see Section 4) lead to the following mechanism (Scheme 10): Ferredoxin, the most powerful reductant of the anaerobic cell, or the artificial reducing agents Ti^{III} citrate and dithionite spontaneously reduce the $[4Fe\text{-}4S]^{2+}$ cluster of the activator to $[4Fe\text{-}4S]^{+}$, which in the presence of 2 ATP and Mg^{2+} transfers the electron further to the dehydratase (Scheme 11). A direct oxidation of ferredoxin by the dehydratase is probably not



Scheme 11. Activation of the 2-hydroxyacyl-CoA dehydratases by electron transfer. The numbers in brackets are the approximate redox potentials ($E^{\circ\prime}$) versus the standard hydrogen electrode.

possible because of the large negative potential. Probably, this transfer occurs with a large conformational change, with the helix-cluster-helix angle of 105° opening up to 180° upon hydrolysis, thus allowing the activator cluster to approach one of the $[4Fe\text{-}4S]^{2+}$ clusters of the dehydratase and transfer the electron.[61] This hypothesis could be examined from the crystal structure of the complex formed between the activator protein and dehydratase and stabilized by ADP-AlF_4^-. Unfortunately, the crystals so far obtained have not been suitable for X-ray analysis.[62]

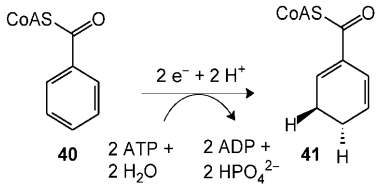
It has been proposed that the electron transferred to the dehydratase reduces the thioester carbonyl group of the substrate **35** to a ketyl radical **37** (Scheme 10). This radical acts as a nucleophile and expels the adjacent hydroxy group to yield the enoxy radical **38**.[63,64] This elimination increases the acidity of the β proton by 26 units from $pK_a \approx 40$ to $pK_a \approx 14$, as shown by theoretical calculations.[65] As with 3-hydroxybutyryl-CoA dehydratase (crotonase),[66] the observed *syn* geometry[67,68] suggests that the eliminated hydroxy group acts as the base that abstracts the β proton.[69] The thus formed allylic ketyl radical (**39**, R = isopropyl) could be detected by EPR spectroscopy, although only at a concentration of 1% compared to that of the enzyme. The EPR spectrum exhibits the expected coupling with the two hydrogen atoms at C-3 and C-4 identified by substitution with deuterium. Freeze-quench EPR studies revealed that the radical is catalytically competent; it is formed at the same rate [(140 ± 30) s^{-1}] as the overall turnover ($150\ s^{-1}$).[51] The final step recycles the electron back to the dehydratase and releases the unsaturated product, (*E*)-enoyl-CoA (**36**). The recycled electron in the dehydratase is then ready for the next turnover. After about 10^4 turnovers, the enzyme becomes inactive, probably by an accidental second electron transfer to the substrate. Another "shot" by the activator, however, reactivates the dehydratase immediately.[70]

This mechanism is reminiscent in part of that of nitrogenase, the enzyme system that catalyzes the reduction of dinitrogen to ammonia and hydrogen in many organisms [Eq. (1)]. Nitrogenase is a two-component enzyme system

$$N_2 + 8\,e^- + 10\,H^+ + 16\,ATP + 16\,H_2O \rightarrow 2\,NH_4^+ + H_2 + 16\,ADP + 16\,\text{phosphate} \quad (1)$$

composed of the iron protein, which resembles the activator of the 2-hydroxyacyl-CoA dehydratases, and the iron–molybdenum protein, where dinitrogen is reduced. Driven by the hydrolysis of two ATP molecules per electron, the iron protein transfers eight electrons, one by one, to the iron–molybdenum protein.[71] Although not phylogenetically related to the activator protein of 2-hydroxyacyl-CoA dehydratases, the nitrogenase iron protein contains a [4Fe-4S] cluster between the two subunits and contains a similar helix-cluster-helix architecture with an angle of 150°. The crystal structure of the complex formed between the two proteins stabilized by ADP-AlF_4^- showed that the angle opened to almost 180°, most likely during electron transfer.[72]

A system phylogenetically related to the 2-hydroxyacyl-CoA dehydratases is benzoyl-CoA reductase, which catalyzes the formation of cyclohexadienecarboxy-CoA (**41**, Scheme 12). The enzyme uses two electrons from ferredoxin

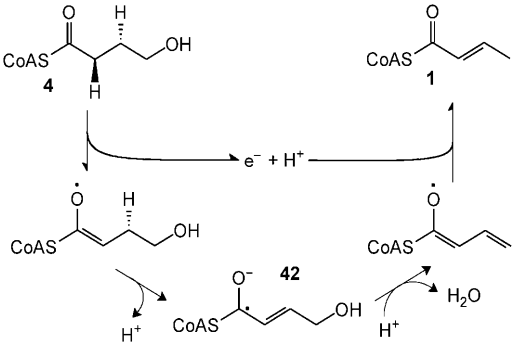


Scheme 12. Reduction of benzoyl-CoA (**40**) to cyclohexadienecarboxyl-CoA (**41**).

and two protons that are added in an *anti* configuration to the aromatic ring.[73,74] The first electron probably reduces the thioester of benzoyl-CoA (**40**) to a ketyl radical intermediate, driven by the hydrolysis of two ATP molecules. This radical anion is protonated at the *para*-position, and the second electron reduces the transient enoxy radical to an enolate, with final protonation at the *meta* position to yield **41**. The enzyme from the facultative anaerobic bacterium *Thauera aromatica* is a complex of four subunits, from which two resemble the activator protein with one [4Fe-4S] cluster and two the dehydratase with two [4Fe-4S] clusters. Despite these structural and functional similarities, nitrogenase and benzo-

yl-CoA reductase require stoichiometric amounts of electrons that cannot be recycled as in 2-hydroxyacyl-CoA dehydratase.

Interestingly, the reversible hydration of crotonyl-CoA (**1**) to 4-hydroxybutyryl-CoA (**4**) proceeds by a mechanism opposite to that of the hydration to 2-hydroxybutyryl-CoA (**2**) (Scheme 1 and Scheme 13). Here the allylic ketyl radical **42** is

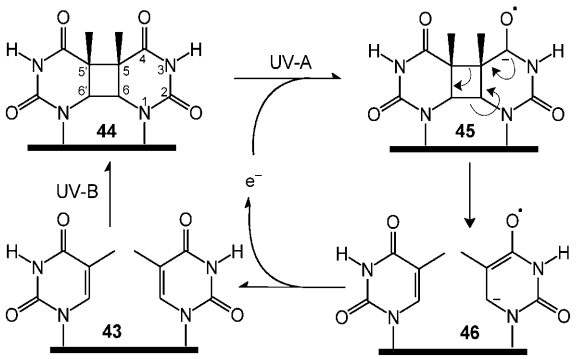


Scheme 13. Proposed mechanism for the dehydration of 4-hydroxybutyryl-CoA (**4**) to crotonyl-CoA (**1**).

generated by one-electron *oxidation* and two stereospecific deprotonations.[26,75] The homotetrameric enzyme from *Clostridium aminobutyricum* contains in each subunit one flavin-adenine dinucleotide (FAD) and a [4Fe-4S] cluster coordinated by three cysteine residues and one histidine residue. The hydroxy group of 4-hydroxybutyryl-CoA most likely binds to the [4Fe-4S] cluster, thereby replacing the histidine residue that abstracts the α proton.[76] FAD oxidizes and deprotonates the formed enolate to yield **26**, which expels the hydroxy group. EPR spectroscopy enabled the FADH· to be identified, but the signal of the substrate-derived radical can not yet be assigned.[77] The intermediate product **42** formed eliminates a hydroxy group to give a dienoxy radical, which is again reduced by FADH· and protonated at C-4 to afford product **1**. Dehydration of stereospecifically labeled **4** with deuterium and tritium at C-4 led to the stereogenic methyl group of **1**, whose stereochemical analysis showed there to be a retention of configuration in the substitution of the hydroxy group by a proton.[75]

3.2. DNA Photolyase

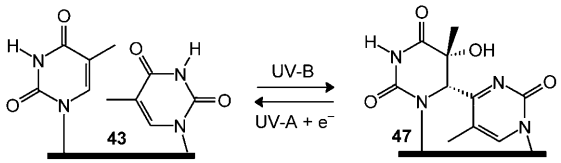
Upon irradiation of DNA with UV-B light (290–320 nm), two adjacent thymidine residues (**43**, Scheme 14) form a cyclobutane-pyrimidine dimer (CPD, **44**) by [2+2] cyclophotoaddition. DNA photolyase repairs this lesion in a radical reaction driven by blue light (350-450 nm).[52,53] The enzyme from *Escherichia coli* contains methylenetetrahydrofolate (CH_2=FH_4) and FAD as prosthetic groups. For catalysis, FAD needs to be in the reduced anionic state ($FADH^-$), which absorbs almost no blue light. The CH_2=FH_4 group ($\lambda_{max} = 380$ nm) acts as light antenna and transfers the energy to $FADH^-$ to yield the excited state $FADH^{-}$*. Binding of the lyase to the DNA lesion results in the CPD flipping into the active site of the enzyme.[78] Then, $FADH^{-}$* transfers a



Scheme 14. Formation and cleavage of the CPD lesion (**44**) in DNA.

high-energy electron to CPD to form a ketyl radical at C-4, adjacent to the cyclobutane ring (**45**). Similar to 2-hydroxyacyl-CoA dehydratases, the ketyl radical may act as a nucleophile that cleaves the 5–5′ and 6′–6 bonds of the cyclobutane ring to give a resonance-stabilized ketyl radical and one restored thymidine residue (**46**). Finally, the electron recycles back to the transiently formed FADH· semiquinone to yield the second restored thymidine and regenerated $FADH^-$. Thus, the next photon can drive another turnover.

A similar mechanism cleaves the (6–4) lesion (**47**, Scheme 15) in DNA.[54] Again, one high-energy electron from $FADH^{-}$* repairs the lesion in two-electron steps.



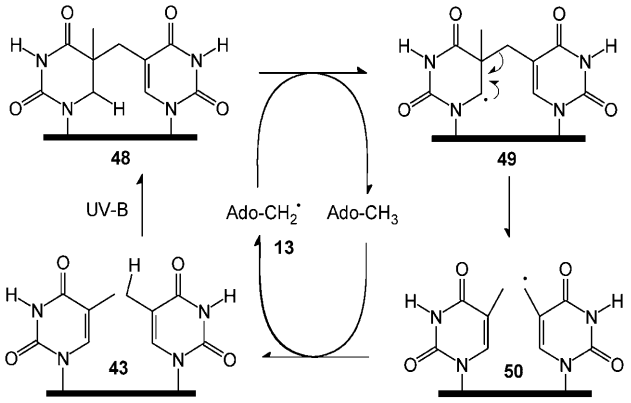
Scheme 15. Formation and cleavage of the (6–4) lesion (**47**) in DNA.

Thereby the oxygen is transferred without solvent exchange. The lesion derives from [2+2] cyclo-photoaddition of the 5′–6′ double bond of one thymidine residue at the C-4 carbonyl group of the other thymidine residue, probably with a four-membered oxetane ring as an intermediate.

Hence, the mechanisms of the photolyases and 2-hydroxyacyl-CoA dehydratases are very similar. A high-energy electron forms a substrate-derived ketyl radical, and the most likely concerted reaction proceeds to a product-related ketyl radical that recycles the electron back for the next turnover. Only in one aspect do the mechanisms differ: Whereas the photolyases require light energy for every turnover, the dehydratases are able to recycle the electron 10^4 times without external energy input. With the photolyase, the energy difference between the two radicals (**45** and **46**) is probably too high to allow recycling in the dark as in the dehydratase. On the other hand, the lifetime of $FADH^{-}$* might not be sufficient for the enzyme to find another lesion.

The irradiation with UV-B light of dry DNA present in bacterial spores leads to radical addition of the thymidine methyl group at the double bond of the neighboring thymidine residue rather than to CPD.[79] The corresponding

lyase requires the 5′-deoxyadenosine radical (**13**, Scheme 4) derived from SAM to repair this lesion, called a spore photoproduct (**48**, Scheme 16). In this reaction, **13** abstracts a hydrogen atom from **48** to yield radical **49**, which fragments



Scheme 16. Formation and cleavage of the spore photoproduct thymidine dimer (**48**) in DNA.

into thymidine and a thymidine radical (**50**; compare with the mechanism of glutamate mutase; Scheme 6). The latter radical re-abstracts a hydrogen atom from the methyl group of the transiently formed 5′-deoxyadenosine to yield the second thymidine residue and to regenerate **13**.[80] Hence, in this reaction, the radical rather than the electron is recycled. Furthermore, fragmentation of **49**→**50** is a radical reaction, whereas in photolyases the cleavage of **45**→**46** appears to involve two-electron steps.

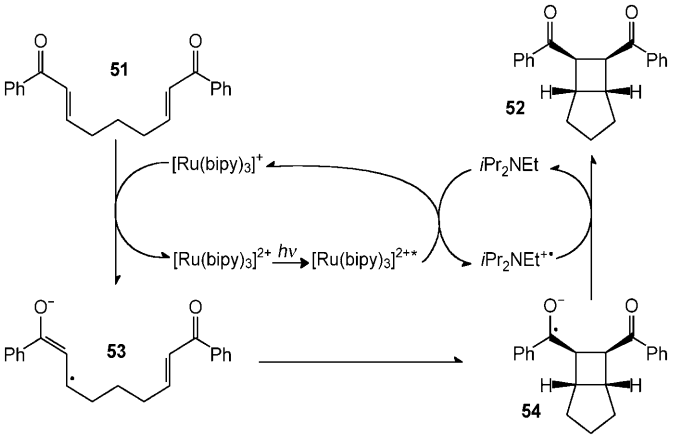
3.3. Other Electron-Recycling Enzymes

In methanogenesis and anaerobic methane oxidation, the reversible formation of CH_4 from methyl-S-CoM and CoB-SH (Co = coenzyme) catalyzed by methyl-CoM reductase most likely proceeds by a radical mechanism.[81] The recycling electron resides on the starting Ni^I center of the porphyrinoid cofactor F_{430}. Another example is the $FMNH_2$ (reduced riboflavin-5′-phosphate), which requires chorismate synthase to catalyze the *anti*-1,4-elimination of phosphate from 5-enolpyruvylshikimate 3-phosphate, whereby the unactivated 6*Re* proton has to be removed.[82,83] The most plausible mechanism of this key step in the biosynthesis of aromatic amino acids involves one-electron transfer from the cofactor $FMNH^-$ to the double bond to yield a radical anion that expels the phosphate. The resulting allyl radical acidifies the 6*Re* proton that is removed by a base of the enzyme to yield an allylic ketyl radical. Finally, the electron recycles back to the transiently formed $FMNH^•$ semiquinone.[84,85] Since both mechanisms are not firmly established, a more detailed description is not given here.

4. Recycling of Electrons in Organic Synthesis

A model reaction that helped to formulate the mechanism of 2-hydroxyacyl-CoA dehydratases[63] was the reduction of α-hydroyketones to ketones with one-electron donors such as metallic zinc in acetic acid, Cr^{II}, or dithionite.[86] The reduction probably proceeds via a ketyl radical that acts as a nucleophile and eliminates the adjacent hydroxy group. The transiently formed enoxy radical is further reduced to the unsubstituted ketone by a second electron. Recently two reports appeared that described the use of light to form ketyl radicals for application in organic synthesis.[7,8]

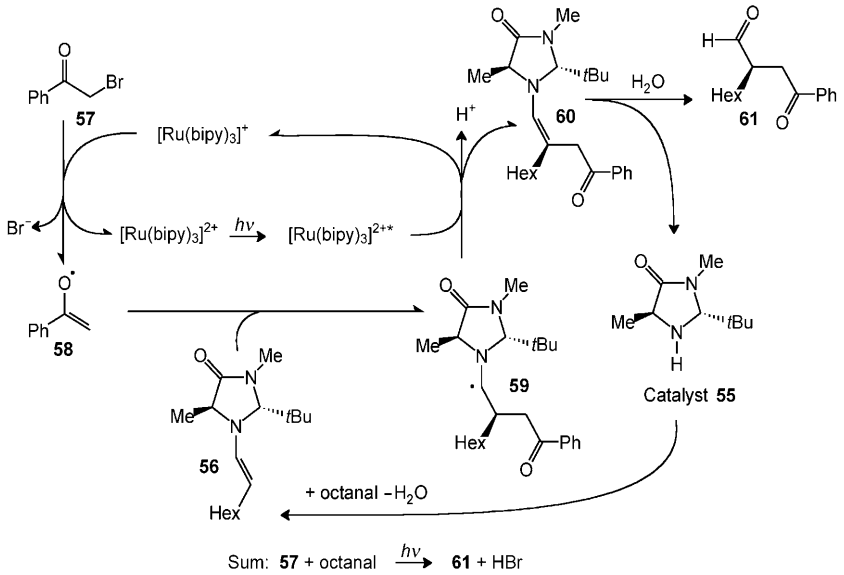
The intramolecular [2+2] cycloaddition of a bis(enone) **51** (Scheme 17) to a cyclobutane **52** is reminiscent of the



Scheme 17. Light-driven [2+2] enone cycloaddition.

formation and decomposition of thymidine dimers (**44**, CPD, Scheme 14). Organic radicals or cathodic electron transfer induce the cycloaddition that proceeds via resonance-stabilized allylic ketyl radicals.[87] Ischay et al. have used $[Ru(bpy)_3]Cl_2$ (bpy = 2,2′-bipyridine) as a photocatalyst in acetonitrile.[7] Irradiation of this chromophore with visible light ($\lambda_{max} = 452$ nm) yields the excited state $[Ru(bpy)_3]^{2+}*$, a strong oxidant that abstracts an electron from the tertiary amine iPr$_2$NEt. The resulting strong reductant $[Ru(bpy)_3]^+$ transfers one electron to the bis(enone), thereby generating an allylic ketyl radical that is additionally stabilized by the phenyl substituent and with Li^+ from the required $LiBF_4$ acting as a Lewis acid. After cycloaddition to **54**, which occurs in high yield and diastereoselectively, the electron recycles back to the transiently formed iPr$_2$NEt$^{+•}$ radical cation.

The second report, by Nicewicz and MacMillan, describes a more elaborate but widely applicable organic synthesis, in which one electron catalyzes the enantioselective α-alkylation of aliphatic aldehydes with α-bromocarbonyl compounds (Scheme 18).[8] An organocatalyst, a chiral imidazolidinone **55**, and again the photoredox catalyst $[Ru(bpy)_3]^+$ together mediate the reaction. The imidazolidinone condenses with the aldehyde to give an enamine **56**, which acts as a radical acceptor. $[Ru(bpy)_3]^+$ donates an electron to the bromocarbonyl **57** to give a ketyl radical that expels bromide, and the



Scheme 18. Light-driven α-alkylation of octanal by bromoacetophenone (**57**).

resulting enoxy radical **58** adds stereospecifically to the open *Si* side of the enamine double bond. The thereby formed $[Ru(bpy)_3]^{2+}$ oxidizes the new radical **59** to the product-related enamine **60** and regenerates $[Ru(bpy)_3]^{+}$ for the next turnover, whereby the electron is recycled. Final hydrolysis yields the enantioenriched *R* enantiomer of the α-alkylated aldehyde **61** and the regenerated organocatalyst **55**. As an example, octanal reacts with α-bromoacetophenone (**57**) in the presence of lutidine in DMF to give (*R*)-2-(2-oxo-2-phenylethyl)octanal (**61**) just by irradiation with a 125 W fluorescent light bulb at 25 °C for 6 h (84 % yield, 95 % *ee*). Thus, two single-electron transfers combine two cycles. The photoredox cycle acts by "lending an electron"[88] to the organocatalytic cycle that produces a radical and catalyzes the stereospecific addition of a radical at the enamine double bond.

This exciting synthesis parallels the enzymatic reactions catalyzed by 2-hydroxyacyl-CoA dehydratases and photolyases. Organic chemists can now apply the same principle that nature invented probably three billion years ago. Catalysis by one-electron recycling or by lending an electron is a hitherto barely recognised principle in enzymology and a previously elusive tool in "green" organic synthesis.

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[1] L. Michaelis, *Cold Spring Harbor Symp. Quant. Biol.* **1939**, *2*, 33 – 49.
[2] J. Stubbe, W. A. van der Donk, *Chem. Rev.* **1998**, *98*, 705 – 762.
[3] W. Buckel, B. T. Golding, *Annu. Rev. Microbiol.* **2006**, *60*, 27 – 49.
[4] P. A. Frey, A. D. Hegeman, G. H. Reed, *Chem. Rev.* **2006**, *106*, 3302 – 3316.
[5] A. E. Hofmeister, W. Buckel, *Eur. J. Biochem.* **1992**, *206*, 547 – 552.
[6] U. Scherf, W. Buckel, *Eur. J. Biochem.* **1993**, *215*, 421 – 429.
[7] M. A. Ischay, M. E. Anzovino, J. Du, T. P. Yoon, *J. Am. Chem. Soc.* **2008**, *130*, 12886 – 12887.
[8] D. A. Nicewicz, D. W. MacMillan, *Science* **2008**, *322*, 77 – 80.
[9] J. T. Groves, *J. Inorg. Biochem.* **2006**, *100*, 434 – 447.
[10] S. Shaik, S. P. de Visser, F. Ogliaro, H. Schwarz, D. Schröder, *Curr. Opin. Chem. Biol.* **2002**, *6*, 556 – 567.
[11] K. F. Ettwig, T. vanAlen, K. T. van de Pas-Schoonen, M. S. Jetten, M. Strous, *Appl. Environ. Microbiol.* **2009**, *75*, 3656 – 3662
[12] H. Jiang, H. Wang, F. Liang, S. Werth, T. Schiestel, J. Caro, *Angew. Chem.* **2009**, *121*, 3027 – 3030; *Angew. Chem. Int. Ed.* **2009**, *48*, 2983 – 2986.
[13] S. Shima, R. K. Thauer, *Curr. Opin. Microbiol.* **2005**, *8*, 643 – 648.
[14] J. Heider, *Curr. Opin. Chem. Biol.* **2007**, *11*, 188 – 194.
[15] R. Rabus, H. Wilkes, A. Behrends, A. Armstroff, T. Fischer, A. J. Pierik, F. Widdel, *J. Bacteriol.* **2001**, *183*, 1707 – 1715.
[16] T. Biegert, G. Fuchs, J. Heider, *Eur. J. Biochem.* **1996**, *238*, 661 – 668.
[17] L. Li, E. N. Marsh, *J. Am. Chem. Soc.* **2006**, *128*, 16056 – 16057.
[18] C. J. Krieger, W. Roseboom, S. P. Albracht, A. M. Spormann, *J. Biol. Chem.* **2001**, *276*, 12924 – 12927.
[19] O. Kniemeyer, T. Fischer, H. Wilkes, F. O. Glöckner, F. Widdel, *Appl. Environ. Microbiol.* **2003**, *69*, 760 – 768.
[20] D. P. Kloer, C. Hagel, J. Heider, G. E. Schulz, *Structure* **2006**, *14*, 1377 – 1388.
[21] J. Knappe, F. A. Neugebauer, H. P. Blaschkowski, M. Gänzler, *Proc. Natl. Acad. Sci. USA* **1984**, *81*, 1332 – 1335.
[22] X. Sun, S. Ollagnier, P. P. Schmidt, M. Atta, E. Mulliez, L. Lepape, R. Eliasson, A. Graslund, M. Fontecave, P. Reichard, B. M. Sjöberg, *J. Biol. Chem.* **1996**, *271*, 6827 – 6831.
[23] C. Raynaud, P. Sarcabal, I. Meynial-Salles, C. Croux, P. Soucaille, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 5010 – 5015.
[24] T. Selmer, P. I. Andrei, *Eur. J. Biochem.* **2001**, *268*, 1363 – 1372.
[25] T. Selmer, A. J. Pierik, J. Heider, *Biol. Chem.* **2005**, *386*, 981 – 988.
[26] W. Buckel, B. T. Golding, *FEMS Microbiol. Rev.* **1999**, *22*, 523 – 541.
[27] M. Frey, M. Rothe, A. F. Wagner, J. Knappe, *J. Biol. Chem.* **1994**, *269*, 12432 – 12437.
[28] J. L. Vey, J. Yang, M. Li, W. E. Broderick, J. B. Broderick, C. L. Drennan, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 16137 – 16141.
[29] W. Plaga, G. Vielhaber, J. Wallach, J. Knappe, *FEBS Lett.* **2000**, *466*, 45 – 48.
[30] P. A. Frey, A. D. Hegeman, F. J. Ruzicka, *Crit. Rev. Biochem. Mol. Biol.* **2008**, *43*, 63 – 88.
[31] B. W. Lepore, F. J. Ruzicka, P. A. Frey, D. Ringe, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 13819 – 13824.
[32] M. D. Ballinger, P. A. Frey, G. H. Reed, *Biochemistry* **1992**, *31*, 10782 – 10789.
[33] F. Berkovitch, E. Behshad, K. H. Tang, E. A. Enns, P. A. Frey, C. L. Drennan, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 15870 – 15875.
[34] H. Bothe, D. J. Darley, S. P. Albracht, G. J. Gerfen, B. T. Golding, W. Buckel, *Biochemistry* **1998**, *37*, 4105 – 4113.

[35] B. Beatrix, O. Zelder, F. Kroll, G. Örlygsson, B. T. Golding, W. Buckel, *Angew. Chem.* **1995**, *107*, 2573 – 2576; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 2398 – 2401.
[36] H.-W. Chih, E. N. G. Marsh, *J. Am. Chem. Soc.* **2000**, *122*, 10732 – 10733.
[37] R. Banerjee, *Chem. Rev.* **2003**, *103*, 2083 – 2094.
[38] W. Buckel, C. Kratky, B. T. Golding, *Chem. Eur. J.* **2006**, *12*, 352 – 362.
[39] P. M. Kozlowski, T. Kamachi, T. Toraya, K. Yoshizawa, *Angew. Chem.* **2007**, *119*, 998 – 1001; *Angew. Chem. Int. Ed.* **2007**, *46*, 980 – 983.
[40] M. Yamanishi, M. Yunoki, T. Tobimatsu, H. Sato, J. Matsui, A. Dokiya, Y. Iuchi, K. Oe, K. Suto, N. Shibata, Y. Morimoto, N. Yasuoka, T. Toraya, *Eur. J. Biochem.* **2002**, *269*, 4484 – 4494.
[41] J. R. O'Brien, C. Raynaud, C. Croux, L. Girbal, P. Soucaille, W. N. Lanzilotta, *Biochemistry* **2004**, *43*, 4635 – 4645.
[42] T. Toraya, *Chem. Rev.* **2003**, *103*, 2095 – 2127.
[43] S. Licht, G. J. Gerfen, J. Stubbe, *Science* **1996**, *271*, 477 – 481.
[44] C. L. Atkin, L. Thelander, P. Reichard, G. Lang, *J. Biol. Chem.* **1973**, *248*, 7464 – 7472.
[45] M. R. Seyedsayamdost, J. Xie, C. T. Chan, P. G. Schultz, J. Stubbe, *J. Am. Chem. Soc.* **2007**, *129*, 15060 – 15071.
[46] W. Jiang, D. Yun, L. Saleh, J. M. Bollinger, Jr., C. Krebs, *Biochemistry* **2008**, *47*, 13736 – 13744.
[47] H. P. Hogenkamp, *Pharmacol. Ther.* **1983**, *23*, 393 – 405.
[48] R. Eliasson, M. Fontecave, H. Jornvall, M. Krook, E. Pontis, P. Reichard, *Proc. Natl. Acad. Sci. USA* **1990**, *87*, 3314 – 3318.
[49] J. Stubbe, M. Ator, T. Krenitsky, *J. Biol. Chem.* **1983**, *258*, 1625 – 1631.
[50] C. C. Lawrence, M. Bennati, H. V. Obias, G. Bar, R. G. Griffin, J. Stubbe, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 8979 – 8984.
[51] J. Kim, D. J. Darley, W. Buckel, A. J. Pierik, *Nature* **2008**, *452*, 239 – 242.
[52] A. Sancar, *Chem. Rev.* **2003**, *103*, 2203 – 2237.
[53] L. O. Essen, T. Klar, *Cell. Mol. Life Sci.* **2006**, *63*, 1266 – 1277.
[54] M. J. Maul, T. R. Barends, A. F. Glas, M. J. Cryle, T. Domratcheva, S. Schneider, I. Schlichting, T. Carell, *Angew. Chem.* **2008**, *120*, 10230 – 10234; *Angew. Chem. Int. Ed.* **2008**, *47*, 10076 – 10080.
[55] L. H. Stickland, *Biochem. J.* **1934**, *28*, 1746 – 1759.
[56] J. Kim, M. Hetzel, C. D. Boiangiu, W. Buckel, *FEMS Microbiol. Rev.* **2004**, *28*, 455 – 468.
[57] K. P. Locher, M. Hans, A. P. Yeh, B. Schmid, W. Buckel, D. C. Rees, *J. Mol. Biol.* **2001**, *307*, 297 – 308.
[58] K. A. Buss, D. R. Cooper, C. Ingram-Smith, J. G. Ferry, D. A. Sanders, M. S. Hasson, *J. Bacteriol.* **2001**, *183*, 680 – 686.
[59] H. Beinert, M. C. Kennedy, *Eur. J. Biochem.* **1989**, *186*, 5 – 15.
[60] G. Layer, J. Moser, D. W. Heinz, D. Jahn, W. D. Schubert, *Embo J.* **2003**, *22*, 6214 – 6224.
[61] J. Kim, Y. Lu, W. Buckel, *C. R. Chim.* **2007**, *10*, 742 – 747.
[62] M. Hetzel, PhD thesis, Philipps-Universität (Marburg), **2005**.
[63] W. Buckel, R. Keese, *Angew. Chem.* **1995**, *107*, 1595 – 1598; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 1502 – 1506.
[64] U. Müller, W. Buckel, *Eur. J. Biochem.* **1995**, *230*, 698 – 704.
[65] D. M. Smith, W. Buckel, H. Zipse, *Angew. Chem.* **2003**, *115*, 1911 – 1915; *Angew. Chem. Int. Ed.* **2003**, *42*, 1867 – 1870.
[66] P. Willadsen, H. Eggerer, *Eur. J. Biochem.* **1975**, *54*, 247 – 252.
[67] W. Buckel, *Eur. J. Biochem.* **1980**, *106*, 439 – 447.
[68] C. Pitsch, H. Simon, *Hoppe Seyler's Z. Physiol. Chem.* **1982**, *363*, 1253 – 1257.
[69] B. J. Bahnson, V. E. Anderson, G. A. Petsko, *Biochemistry* **2002**, *41*, 2621 – 2629.
[70] J. Kim, D. Darley, W. Buckel, *Febs J.* **2005**, *272*, 550 – 561.
[71] D. C. Rees, J. B. Howard, *Curr. Opin. Chem. Biol.* **2000**, *4*, 559 – 566.
[72] H. Schindelin, C. Kisker, J. L. Schlessman, J. B. Howard, D. C. Rees, *Nature* **1997**, *387*, 370 – 376.
[73] H. Möbitz, M. Boll, *Biochemistry* **2002**, *41*, 1752 – 1758.
[74] B. Thiele, O. Rieder, B. T. Golding, M. Müller, M. Boll, *J. Am. Chem. Soc.* **2008**, *130*, 14050 – 14051.
[75] P. Friedrich, D. J. Darley, B. T. Golding, W. Buckel, *Angew. Chem.* **2008**, *120*, 3298 – 3301; *Angew. Chem. Int. Ed.* **2008**, *47*, 3254 – 3257.
[76] B. M. Martins, H. Dobbek, I. Çinkaya, W. Buckel, A. Messerschmidt, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 15645 – 15649.
[77] U. Näser, A. J. Pierik, R. Scott, I. Çinkaya, W. Buckel, B. T. Golding, *Bioorg. Chem.* **2005**, *33*, 53 – 66.
[78] A. Mees, T. Klar, P. Gnau, U. Hennecke, A. P. Eker, T. Carell, L. O. Essen, *Science* **2004**, *306*, 1789 – 1793.
[79] J. E. Donnellan, Jr., R. B. Setlow, *Science* **1965**, *149*, 308 – 310.
[80] A. Chandor-Proust, O. Berteau, T. Douki, D. Gasparutto, S. Ollagnier-de-Choudens, M. Fontecave, M. Atta, *J. Biol. Chem.* **2008**, *283*, 36361 – 36368.
[81] B. Jaun, R. K. Thauer, *Met. Ions Life Sci.* **2007**, *2*, 323 – 356.
[82] R. K. Hill, G. R. Newkome, *J. Am. Chem. Soc.* **1969**, *91*, 5893 – 5894.
[83] D. K. Onderka, H. G. Floss, *J. Am. Chem. Soc.* **1969**, *91*, 5894 – 5896.
[84] A. Osborne, R. N. Thorneley, C. Abell, S. Bornemann, *J. Biol. Chem.* **2000**, *275*, 35825 – 35830.
[85] O. Dmitrenko, H. B. Wood, Jr., R. D. Bach, B. Ganem, *Org. Lett.* **2001**, *3*, 4137 – 4140.
[86] G. A. Molander, G. Hahn, *J. org. Chem.* **1986**, *51*, 1135 – 1138.
[87] J. Yang, G. A. Felton, N. L. Bauld, M. J. Krische, *J. Am. Chem. Soc.* **2004**, *126*, 1634 – 1635.
[88] Editorial, *Science* **2008**, *322*, 12.
[89] J. Kim, W. Buckel, E. Bill, unpublished results.