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Review

Oxidative aliphatic carbon–carbon bond cleavage reactions

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ABSTRACT

Over the past decade, several metalloenzymes have been characterized which catalyze dioxygenase-type aliphatic carbon–carbon bond cleavage reactions. The substrates for these enzymes vary from species that are stable with respect to O_2 under ambient conditions, to examples that in anionic form exhibit O_2 reactivity in the absence of enzyme. Described herein are advances from studies of the enzymes themselves and model systems. These combined investigations provide insight into novel mechanistic pathways leading to aliphatic carbon–carbon bond cleavage and/or the factors that influence regioselectivity in the oxidative carbon–carbon bond cleavage reactions.

Abbreviations: acac, acetylacetonate; Ala, alanine; ARD, acireductone dioxygenase; Asp, aspartic acid; bipy, bipyridine; Bn-BQA, benzyl bis(2-quinolinylmethyl)amine; B. subtilis, *Bacillus subtilis*; DAD, 2,4′-Dihydroxyacetophenone dioxygenase; DFT, density functional theory; DHAP, 2,4′-dihydroxyacetophenone; Dke1, diketone dioxygenase; DTBP, 2,4-di-tert-butylphenol; E. coli, *Escherichia coli*; en, ethylenediamine; EPR, electron paramagnetic resonance; ES, enzyme-substrate; Glu, glutamic acid; HEP, 2-hydroxyethylphosphonate; HEPD, hydroxyethylphosphonate dioxygenase; 3-Hfl, 3-hydroxyflavone; His, histidine; HMP, hydroxymethylphosphonate; HOMO, highest occupied molecular orbital; HPPD, 4-hydroxyphenylpyruvate dioxygenase; α-KG, α-ketoglutarate; MCD, magnetic circular dichroism; MLCT, metal-to-ligand charge-transfer; MTOB, 4-methylthio-2-oxobutanoate; NMR, nuclear magnetic resonance; OAc, acetate; pp, phenylpyruvate; QDO, quercetin dioxygenase; TOF, turnover frequency; TTBP, 2,4,6-tri-*tert*-butylphenol; XAS, X-ray absorption spectroscopy.

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1. Introduction

One of the most challenging reactions in chemistry and biology is the selective oxidative cleavage of aliphatic carbon–carbon bonds. Despite the prevalence of such reactions in biological systems, there is currently little known regarding the mechanistic details of such processes. These types of reactions are of considerable current interest due to their potential for a broad array of applications, including the utilization of biomass in fuel production, the removal of otherwise intractable organic contaminants during wastewater treatment and other bioremediation, and for improving understanding of the treatment of human disease [1,2].

In nature several types of oxidative carbon–carbon cleavage reactions are catalyzed by metalloenzymes and involve the incorporation of both atoms of dioxygen into their products. These reactions may be divided into two sets, those that result in the cleavage of carbon–carbon bonds within an aromatic π-system (aromatic carbon–carbon bonds), and those that result in the cleavage of other carbon–carbon bonds (aliphatic carbon–carbon bonds). The former, which includes reactions catalyzed by intradiol and extradiol catechol dioxygenases, as well as other ring-cleaving dioxygenases, have been extensively investigated in studies of both enzymes and synthetic model systems [3–5]. Important themes in these investigations included the elucidation of novel mechanistic pathways and the discovery of how the metal oxidation state and ligand environment influence the regioselectivity of the oxidative reaction.

In contrast to systems that catalyze oxidative aromatic carbon–carbon bond cleavage, the metalloenzymes that cleave aliphatic carbon–carbon bonds have received much less attention until recently (with the exception of the quercetin dioxygenases), and are notable for their diversity in structure and substrate (Scheme 1). Most of the enzymes that catalyze these reactions are cupin-type proteins. The metal cofactor is most commonly iron, but other first-row transition metals, such as nickel and copper, are also found (Table 1). Metal binding residues at the active sites of these enzymes vary from the common 2-His, 1-carboxylate facial triad (hydroxyethylphosphonate dioxygenase (HEPD)), to the 3-His (acetylacetone 2,3-dioxygenase (Dke1)) and 3-His, 1-carboxylate (quercetin dioxygenase (QDO), acireductone dioxygenases (ARD and ARD′)) binding motifs. The carbon–carbon bonds cleaved in these systems all have an accessible enol form, with the notable exception of hydroxyethylphosphonate. The relatively easily oxidizable quercetin and acireductone substrates are both oxygen-rich and are reminiscient of the common biological reductant ascorbic acid.

Two of the systems that have received the most attention recently, acetylacetone 2,3-dioxygenase and the acireductone dioxygenases, highlight the diversity of substrate oxidation in these enzymes and attendant mechanistic questions. In the former system the oxidation of the substrate is a difficult reaction with no synthetic model systems yet devised that can oxidatively cleave the native acetylacetone substrate in a biomimetic reaction. Thus, investigations have focused on the unique coordination motif and the role of charge at the enzyme active site. By contrast, the substrate for acireductone dioxygenases is readily oxidatively cleaved by O_2 in the absence of catalyst by simply raising the pH. In studies of relevance to this system, efforts have focused on understanding the role that metal center may play in directing the regiospecificity of carbon–carbon bond cleavage.

Table 1
Aliphatic carbon–carbon bond cleaving dioxygenase enzymes, active site metal ions, and metal coordination environment.

Enzyme	Metal ion	Protein-derived ligands
Dke1	Fe(II)	3-His
HEPD	Fe(II)	2-His-1-carboxylate
DAD	Fe(II)	[a]
QDO	Cu(II)[b]	3-His/3-His-1-carboxylate
ARD/ARD′	Ni(II), Fe(II)	3-His-1-carboxylate

[a] Active site ligand environment not yet determined.
[b] Fungal quercetinases contain Cu(II). Bacterial quercetinases exhibit activity with a variety of metal ions including Mn(II), Fe(II), Ni(II) and Co(II).

Herein we summarize recent developments in understanding of metalloenzymes that catalyze oxidative aliphatic carbon–carbon bond cleavage via a dioxygenase-type reaction. Additionally, we examine model systems of relevance to these enzymes. Emphasis is placed on studies that have revealed mechanistic insight into overcoming the barrier to the spin-forbidden reaction between the organic substrate and O_2, and factors that influence regioselectivity in aliphatic carbon–carbon bond cleavage. We have limited the scope of this review to aliphatic carbon–carbon bond cleaving dioxygenases for which either mechanistic studies have been reported for the enzyme, or model systems have been reported. With this limitation, we have excluded systems such as carotenoid oxygenases, for which biochemical and computational studies have been summarized recently elsewhere [6]. Additionally, although the decarboxylation of 2-oxo acids such as α-ketoglutarate is a type of aliphatic carbon–carbon bond cleavage reaction that occurs in metalloenzymes, it is a chemically distinct process that is not included in this review. This is because it does not represent aliphatic carbon–carbon bond cleavage resulting from incorporation of both atoms of dioxygen into a single substrate [7].

2. Metalloenzymes that oxidatively cleave aliphatic C-C bonds and their model systems

2.1. Acetylacetone 2,3-dioxygenase (Dke1)

Acetylacetone is used industrially in the production of anti-corrosion agents, pharmaceutical compounds, and pesticides. It is also a precursor to several heterocyclic compounds (pyrazoles, diketamines) via condensation reactions. Simple metal acetylacetonate complexes are useful as catalysts. With its wide array of uses, it is important to note that acetylacetone is toxic to mammals [8], as well as to aquatic species [9] and microorganisms [10]. Therefore, bioremediation approaches toward the degradation of acetylacetone to less toxic byproducts are of current interest. In 2002, Straganz et al. reported the identification of a bacterium (*A. johnsonii*) that can grow with acetylacetone as its only carbon source [11,12]. This bacterium degrades acetylacetone to produce acetate and methyl glyoxal (Scheme 1(a)), the latter of which is converted to pyruvate. The enzyme that catalyzes this oxidative cleavage reaction is termed acetylacetone 2,3-dioxygenase, or diketone-cleaving enzyme (Dke1) [11,12]. It is a tetrameric cytosolic protein with each 16.6 kDa subunit containing a single non-heme Fe(II) center. Structural studies of the Zn(II)-containing form of the enzyme (PDB: 3bal) revealed Dke1 to be a member of the cupin superfamily [13] of proteins. The Fe(II) center is ligated facially by three histidine residues (His62, His64, His104). The coordination of these residues makes the active site of Dke1 distinct from the more typical two histidine, one carboxylate facial triad found in an array of mononuclear non-heme iron enzymes that utilize O_2 to oxidize organic substrates (e.g. alpha-ketoglutarate (α-KG) dependent dioxygenases) [3,14]. X-ray absorption spectroscopic studies of an Fe(II)-dependent cupin oxygenase protein (*Bxe*_A2876) from *Burkholderia xenovorans*, which similarly to Dke1 oxidatively cleaves β-diketones, revealed a five or six coordinate metal center in the resting state. Specifically, the Fe(II) center has three coordinated histidine residues at 1.98 Å, a carboxylate ligand (perhaps Glu98) at ~2.08 Å, and one or two coordinated water molecules at 2.04 Å [15]. CD, MCD and VTVH MCD spectroscopic studies of Dke1 are consistent with a six-coordinate metal center in