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Reviews

Decarboxylation mechanisms in biological system

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ABSTRACT

This review examines the mechanisms propelling cofactor-independent, organic cofactor-dependent and metal-dependent decarboxylase chemistry. Decarboxylation, the removal of carbon dioxide from organic acids, is a fundamentally important reaction in biology. Numerous decarboxylase enzymes serve as key components of aerobic and anaerobic carbohydrate metabolism and amino acid conversion. In the past decade, our knowledge of the mechanisms enabling these crucial decarboxylase reactions has continued to expand and inspire. This review focuses on the organic cofactors biotin, flavin, NAD, pyridoxal 5′-phosphate, pyruvoyl, and thiamin pyrophosphate as catalytic centers. Significant attention is also placed on the metal-dependent decarboxylase mechanisms.

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1. Introduction

Decarboxylation is one of the most common processes in nature and one of the most fundamentally important reactions in biological systems. Essentially all of the carbon dioxide evolved in fermentation and respiration is generated by the decarboxylation of organic acids [1]. Decarboxylases are known for their roles in a wide variety of catabolic and anabolic pathways including decarboxylation of α- and β-keto acids, amino acid conversions, and carbohydrate synthesis [2]. Within the IUPAC classification scheme, decarboxylases are currently divided into at least 90 different classes [3]. Substantial efforts have been applied to the study of the origin and the mechanisms of production of metabolic carbon dioxide, and considerable knowledge has been accumulated regarding the decarboxylation mechanisms in biological systems. Enzymatic decarboxylation usually utilizes either an organic cofactor such as pyridoxal 5′-phosphate and biotin, or an inorganic cofactor, such as an iron or zinc complex, in the catalytic reaction (Table 1). Only

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Table 1
Catalytic strategy for biological decarboxylation.

Catalytic cofactor/coenzyme			Representative enzyme
None			Orotidine monophosphate decarboxylase
Organic		Biotin	Oxaloacetate decarboxylase, glutaconyl-CoA decarboxylase
		Flavin	Lantibiotic-biosynthesizing enzyme EpiD, 4-phosphopantethenoyl cysteine decarboxylase
		Glycyl radical	4-Hydroxyphenylacetate decarboxylase
		$NAD^+/NADP^+$	Methylmalonyl CoA decarboxylase
		Pyridoxal 5′-phosphate	Glycine decarboxylase; ornithine decarboxylase; DOPA decarboxylase
		Pyruvoyl	Arginine/aspartate/histidine decarboxylase; *S*-adenosylmethionine decarboxylase
		Thiamin diphosphate	Pyruvate dehydrogenase multienzyme complex; phosphonopyruvate decarboxylase
Inorganic	Alkaline earth metal	Mg^{2+}	3-Keto-L-gulonate 6-phosphate decarboxylase
	d-Block metal	Fe^{2+}/O_2 (oxidative)	Gallic acid decarboxylase; CloR decarboxylase; α-ketoglutarate-dependent dioxygenases
		Mn^{2+}/O_2	Oxalate decarboxylase
		M^{2+} (oxidant-independent) (M=Zn, Fe, Co, Cd, or Mn)	α-Amino-β-carboxymuconate-ε-semialdehyde decarboxylase

a handful of decarboxylases, e.g. orotidine monophosphate decarboxylase (OMPDC) [4,5] and methylmalonyl CoA decarboxylase from *Escherichia coli* [6], do not utilize any cofactors. In this review, representative decarboxylation mechanisms are outlined.

2. Enzymatic decarboxylation without an exogenous cofactor

The active sites of most decarboxylases bind organic or metal cofactors, which activate decarboxylation and/or stabilize the carbanion upon the elimination of carbon dioxide from the substrate [7]. Orotidine 5′-monophosphate decarboxylase (OMPDC) [5,8–10], 2-oxo-4-hydroxy-4-carboxy-5-ureidoimidazoline decarboxylase (OHCUD) [11,12], methylmalonyl CoA decarboxylase (MMCD) from *E. coli* [6], and malonate semialdehyde decarboxylase (MSAD) from *Pseudomonas pavonaceae* [13,14] are among a few decarboxlylases that do not contain any cofactors. These enzymes catalyze non-oxidative decarboxylation reactions.

OMPDC catalyzes the critical final step in the pyrimidine biosynthetic pathway [5,8]. This enzyme has drawn much attention because its rate enhancement is one of the highest of any known enzymes [15]. The crystal structures of OMPDC from *Pyrococcus furiosus* with and without substrate have recently been determined at high resolution. These structures have helped to establish a well-defined substrate binding pocket [16]. An extremely hydrophobic pocket is believed to be a strong factor in the rate enhancement compared to rates in the absence of a catalyst [17]. The presence of a carbanionic intermediate is supported through mutagenic [18] and kinetic experiments [18,19], and a carbanion-like intermediate has been trapped in crystals using substrate analogs [20]. However, whether the protonated site occurs at C5 or C6 remains to be determined [9,21–23].

A stepwise mechanism has been proposed for the OMPDC catalysis mechanism: a conserved lysine protonates either the C5 or C6 carbon bound to the leaving carboxylate of the pyrimidine, then an aspartate destabilizes the substrate to prepare it for decarboxylation [10,18]. A concerted and more widely accepted mechanism has also been proposed for OMPDC based on high resolution crystal structures (Fig. 1A) [5,7,11,24]. In this mechanism, the negative charge region of the strictly conserved Asp positions the anionic carboxylate of OMP, which helps to destabilize the ground state of the substrate; meanwhile, the positively charged ammonium group of a strictly conserved lysine is positioned close to the carbon, connected to the leaving carboxylate of the pyrimidine, stabilizing the developing negative charge in the transition state. Thus the OMPDC catalyzed reaction proceeds by a bimolecular electrophilic substitution mechanism (*i.e.*, decarboxylation and protonation are concerted), which will avoid the development of a high energy carbanion intermediate (Fig. 1A). However, recent product deuterium isotope effect studies [25], and quantum mechanical and molecular mechanical simulations [26] bring this mechanism into question. Efforts have also been made to target this active site lysine residue with covalently binding inhibitors [27].

Unlike the mechanism proposed for OMPDC, in which decarboxylation and protonation occur simultaneously at the C5 or C6 carbon of orotidine, decarboxylation and protonation take place stepwise in the proposed mechanism for three other enzymes [11,12]. In the mechanism proposed for OHCUD, the decarboxylation reaction was postulated to occur directly by using the double bond between C5 and N1 as an electron sink to stabilize the negative charge carbanion. A Glu, analogous to the Asp in OMPDC, in the active site may function to destabilize the ground state of the substrate by electrostatic repulsion to facilitate the exit of the carboxylate group. A conserved His in the active site is believed to be involved in the subsequent deprotonation of the hydroxyl group at C4 and the protonation of C5 of the substrate, generating a stereospecific product (Fig. 1B). A similar mechanism in which the protonated imine is believed to be the electron acceptor during decarboxylation has also been proposed [28]. Structures of both the enzyme, and enzyme bound with allopurinol, a recently discovered inhibitor, were published in 2010 [28]. Notably, these structures show a reorganization of the active site upon substrate binding [28].

In the proposed mechanism of MMCD [6], a conserved Tyr forms a hydrogen bond to the leaving carboxyl group of the substrate and orients the substrate in a plane with the thioester carbonyl group. Besides this conserved Tyr, only hydrophobic residues reside close to the negative charge of the leaving carboxyl group. This configuration destabilizes the ground state of the substrate and thus facilitates decarboxylation to leave a neutral carbon dioxide molecule which is more favorable in this hydrophobic environment. Two backbone amide groups from two strictly conserved residues, a His and a Gly, form hydrogen bonds with the thioester carbonyl group of the substrate to produce the required polarization of this bond and to stabilize the proposedanionic intermediate and transition state (Fig. 1C).

A similar mechanism has been proposed for malonate semialdehyde decarboxylase (MSAD) from *P. pavonaceae*, which also catalyzes the decarboxylation of a β-keto acid. In the proposed mechanism based on crystal structure, mutagenesis and inactivation studies [13,14], conserved Pro and Asp residues create a hydrogen bonding network to polarize the β-keto group of the substrate and stabilize the enolate anionic intermediate by donating a proton to the enolate anion. Two Arg residues position the leaving carboxyl group of the substrate such that the scissile bond is in the plane