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Review

Functionally diverse biotin-dependent enzymes with oxaloacetate decarboxylase activity

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ABSTRACT

Biotin-dependent enzymes catalyze carboxylation, decarboxylation and transcarboxylation reactions that participate in the primary metabolism of a wide range of organisms. In all cases, the overall reaction proceeds via two half reactions that take place in physically distinct active sites. In the first half-reaction, a carboxyl group is transferred to the 1-N′ of a covalently tethered biotin cofactor. The tethered carboxybiotin intermediate subsequently translocates to a second active site where the carboxyl group is either transferred to an acceptor substrate or, in some bacteria and archaea, is decarboxylated to biotin and CO_2 in order to power the export of sodium ions from the cytoplasm. A homologous carboxyltransferase domain is found in three enzymes that catalyze diverse overall reactions: carbon fixation by pyruvate carboxylase, decarboxylation and sodium transport by the biotin-dependent oxaloacetate decarboxylase complex, and transcarboxylation by transcarboxylase from *Propionibacterium shermanii*. Over the past several years, structural data have emerged which have greatly advanced the mechanistic description of these enzymes. This review assembles a uniform description of the carboxyltransferase domain structure and catalytic mechanism from recent studies of pyruvate carboxylase, oxaloacetate decarboxylase and transcarboxylase, three enzymes that utilize an analogous carboxyltransferase domain to catalyze the biotin-dependent decarboxylation of oxaloacetate.

Introduction

In 1933, Burk and colleagues described an accessory factor isolated from egg yolk that was essential for the growth of the nitrogen-fixing bacterium, *Rhizobium* [1]. Three years later, Kögl and Tonnis isolated a crystalline 'Bios' factor from duck egg yolks that they called biotin [2]. Shortly thereafter, biotin was recognized as an essential growth factor common to bacteria, yeast and mammals (reviewed in [3]). The structure of biotin was soon chemically deduced (Fig. 1; [4]) and it was shown to be involved in carboxylation reactions, providing a clue into its role in cellular physiology [5]. Eventually, biotin was found to be covalently tethered to and necessary for catalysis in acetyl coenzyme A (CoA)[1] carboxylase [6]. This critical observation established a biological role for biotin in CO_2 fixation and resulted in the discovery of a family of enzymes known today as the biotin-dependent enzymes.

In recent years, the description of structure and function in the biotin-dependent enzymes has advanced considerably, primarily as a result of an influx of new X-ray crystal structures. Two recent reviews [7,8] have summarized these structural advances, particularly in the Class I biotin-dependent enzymes, and the reader is referred to these sources for a complete and recent description of the carboxylase class of biotin-dependent enzymes. This review focuses, instead, on the structure and function of the carboxyltransferase domain from three enzymes that encompass the three classes of the biotin-dependent family: pyruvate carboxylase, oxaloacetate decarboxylase and transcarboxylase. These three enzymes catalyze very different overall reactions, but structural and kinetic data compiled over the past 10 years have provided significant insights into common active site features, shared aspects of chemical mechanism and similarities in the interactions between the carboxyltransferase domain, the biotin cofactor and associating functional domains.

Biotin-dependent enzymes

The biotin-dependent enzymes are present across all three domains of life, and phylogenetic analyses of the individual domains suggest an ancient evolutionary origin [9]. These enzymes are composed of two distinct active sites, with a covalently tethered

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[1] *Abbrevation used:* CoA, acetyl coenzyme A; BCCP, biotin carboxyl carrier protein; BC, biotin carboxylase; ODC, oxaloacetate decarboxylase; ACP, acyl carrier protein; PC, pyruvate carboxylase; OADC, oxaloacetate decarboxylase; TC, transcarboxylase; TCA, tricarboxylic acid; α-OADC, α-subunit of OADC.

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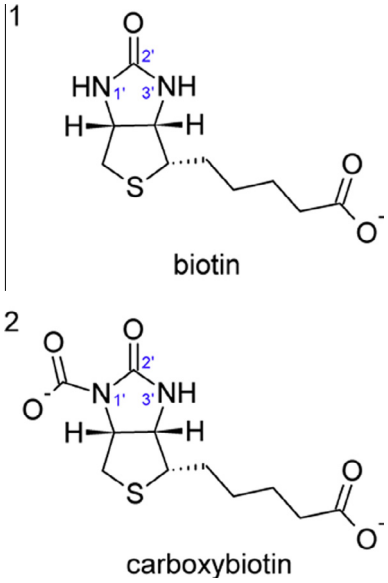

Fig. 1. Chemical structure of biotin and *N*-carboxybiotin. (1) Biotin, also known as vitamin H, vitamin B_7 or coenzyme R, is composed of a valerate side chain tethered to a bicyclic ring. The bicyclic ring consists of a ureido ring fused to a tetrahydrothiophene ring. (2) *N*-carboxybiotin is the reaction intermediate for all biotin-dependent enzymes. Biotin is carboxylated at the N-1 position.

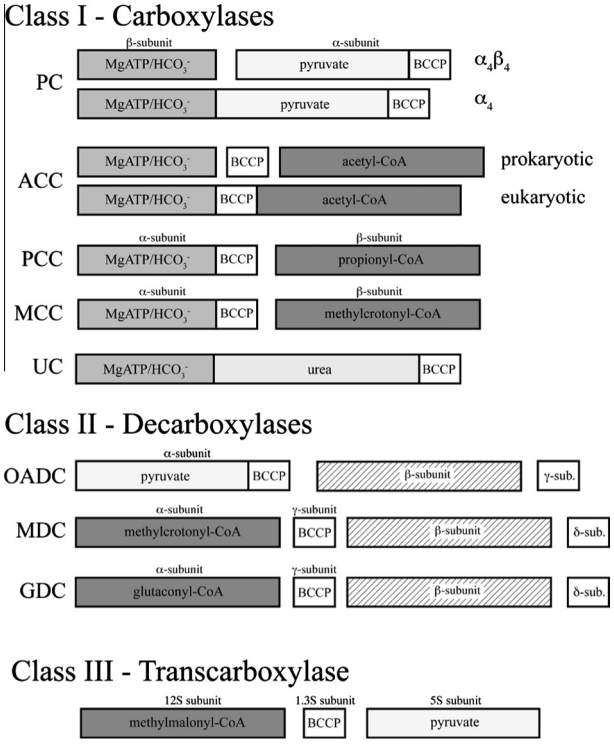

Fig. 2. Schematic diagram of the biotin-dependent enzyme family showing the arrangement of catalytic and biotin carrier domains. The biotin-dependent enzyme family is subdivided into three classes. The *Class I carboxylases* include pyruvate carboxylase (PC), acetyl-CoA carboxylase (ACC), propionyl-CoA carboxylase (PCC), methylcrotonyl-CoA carboxylase (MCC), urea carboxylase (UC) and geranyl-CoA carboxylase (not shown). Within this class, both PC and ACC are further subdivided into distinct structural arrangements. As described in Section 2.1, the majority of PC enzymes are of the α_4 form, with all domains arranged on a single polypeptide chain, while a few "subunit type" PC enzymes are composed of separate α- and β-subunits. The ACC enzymes of prokaryotic origin are composed of individual subunits while eukaryotic ACC is a multifunctional enzyme, with all domains originating from a single polypeptide chain. The *Class II decarboxylases* include oxaloacetate decarboxylase (OADC), methylcrotonyl-CoA decarboxylase (MDC), glutaconyl-CoA decarboxylase (GDC) and malonate decarboxylase (not shown). The *Class III transcarboxylase* consists of a single enzyme, transcarboxylase from *P. shermanii*, described in the section titled "Transcarboxylase". The biotin carboxyl carrier protein domain is abbreviated as BCCP. The domains are colored to highlight the homology among the individual domains.

biotin cofactor traveling between active sites via a so-called swinging-arm mechanism [10]. All enzymes in this family must coordinate the biotin-dependent transfer of a carboxyl moiety between remote active sites by way of two independent half-reactions. As such, they require multiple catalytic domains to accomplish catalysis.

Biotin serves as the conduit for carboxyl transfer and is carboxylated at the 1'-*N* position in the first active site (Fig. 1; [11–13]). The resulting carboxybiotin product is relatively stable, with a half-life exceeding 100 min at pH 8 [14]. The stability of 1'-*N*-carboxybiotin is critical, since energy is expended in carboxylating biotin and because carboxybiotin must physically translocate to a second active site prior to transferring the carboxyl moiety to an acceptor substrate.

The biotin cofactor is covalently attached, via an amide linkage, to the ε-amino group of a specific, conserved lysine in the biotin carboxyl carrier protein (BCCP) domain by the enzyme biotin protein ligase [15]. Several NMR and X-ray crystal structures of the BCCP domain reveal two sets of four antiparallel β-strands arranged around an approximate twofold axis [16–19]. The site of biotinylation is located on a hairpin turn that connects two β-strands near the C-terminal end of the domain. In most cases, the modified lysine residue is bracketed within a conserved sequence consisting of Ala–Met–Lys–Met. In pyruvate carboxylase and transcarboxylase, this identity extends to Ala–Met–Lys–Met–Glu–Thr. The BCCP domain physically translocates between catalytic domains and assists in inserting biotin into the individual active sites. The primary sequence and tertiary structure for the C-terminal ~80 amino acids of the BCCP domain is conserved for all enzymes within the family, suggesting that they are descended from a common ancestor [20]. In *Escherichia coli* acetyl-CoA carboxylase, however, the BCCP subunit includes an additional N-terminal domain of ~80 amino acids which may serve to impede the catalytic half-reactions prior to assembly of the complete enzyme complex [21,22].

The biotin-dependent enzymes are divided into three classes, depending on the overall reaction that they catalyze [23]. These are briefly described below.

Class I – carboxylases

Class I enzymes are unique within the biotin-dependent family in catalyzing carbon fixation. On the basis of chemical reactivity, enzymes involved in carbon fixation would be predicted to favor nucleophilic attack on the electrophilic carbon of carbon dioxide. However, at physiological pH and temperature, the concentration of dissolved carbon dioxide is twenty times less than bicarbonate ion (10 μM vs. 200 μM), making dissolved carbon dioxide availability low by comparison [24]. While there are enzymes that utilize dissolved carbon dioxide as a substrate (ribulose-1,5-bisphosphate carboxylase oxygenase, or RuBisCO, is a well-known example), these enzymes often suffer from low catalytic activity. Biotin-dependent carboxylases have evolved to effectively utilize the more abundant bicarbonate ion for carbon fixation [25,26].

The biotin-dependent carboxylases include pyruvate carboxylase (EC 6.4.1.1), acetyl CoA carboxylase (EC 6.4.1.2), propionyl CoA carboxylase (EC 6.4.1.3), 3-methylcrotonyl CoA carboxylase (EC 6.4.1.4), geranyl CoA carboxylase (EC 6.4.1.5), and urea carboxylase (EC 6.3.4.6) (Fig. 2). All enzymes within this class use a common mechanism to carboxylate biotin in the structurally conserved biotin carboxylase (BC) domain. A detailed description