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Minireview

Reaction specificity in pyridoxal phosphate enzymes☆

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Abstract

Pyridoxal phosphate enzymes catalyze a wide variety of reaction types on amines and amino acids, generally by stabilizing carbanionic intermediates. This makes them very useful in cellular metabolism, but it also creates problems in controlling the reaction pathway that a given enzyme follows, i.e., in controlling reaction specificity. Stereoelectronic effects have been proposed to play a major role in determining the bond to Cα that gets broken in the external aldimine intermediate that is common to all PLP enzymes. Here, we discuss our work on dialkylglycine decarboxylase aimed at providing direct evidence for stereoelectronic control of external aldimine reactivity. Once a bond to Cα has been broken to form the carbanionic intermediate, enzymes must also carefully control the fate of this reactive species. Our studies with alanine racemase suggest that the enzyme selectively destabilizes the carbanionic quinonoid intermediate to promote higher racemization specificity by avoiding transamination side reactions.

Keywords: Reaction specificity; Pyridoxal phosphate; Dialkylglycine decarboxylase; Alanine racemase

Vitamin B_6 was first identified as pyridoxine, a catalytically inactive form, in 1938 while the catalytically active aldehyde (pyridoxal) and amine (pyridoxamine) forms and their phosphorylated derivatives (pyridoxal 5′-phosphate and pyridoxamine 5′-phosphate) were discovered in the early 1940s [1]. Snell discovered that heating pyridoxal and amino acids yielded the amino form of the coenzyme and proposed based on these observations that pyridoxal, or a derivative thereof, was the mediator of transamination reactions [1]. The following three decades saw the demonstration of the involvement of pyridoxal 5′-phosphate (PLP)[1] and pyridoxamine 5′-phosphate (PMP) in a wide variety of both nonenzymatic and enzymatic reactions. The enzyme-catalyzed reactions corresponding to the nonenzymatic ones were shown to follow the same basic chemical mechanisms, with the enzymes enforcing substrate and reaction specificity as well as enhancing catalytic power.

Fig. 1 illustrates the breadth of reaction specificity enabled by PLP, using serine as an example substrate. The first and common step for all PLP-dependent enzyme-catalyzed reactions is a Schiff base exchange reaction (transimination). All known PLP enzymes exist in their resting state as a Schiff base (internal aldimine) with an active site lysine residue. The incoming, amine-containing substrate displaces the lysine ε-amino group from the internal aldimine, in the process forming a new aldimine with the substrate (external aldimine). This is a multistep process that includes several facile steps and is frequently very rapid compared to the central steps in the reaction mechanism (e.g., deprotonation of Cα). The external aldimine is the common central intermediate for all PLP-catalyzed reactions, enzymatic and nonenzymatic. Divergence in reaction specificity occurs from this point. The great majority of pyridoxal phosphate-catalyzed reactions depend on the formation of

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[1] *Abbreviations used:* PLP, pyridoxal 5′-phosphate; PMP, pyridoxamine 5′-phosphate; DGD, dialkylglycine decarboxylase.

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a carbanionic intermediate, while the 1,2-aminomutases largely explored by Frey's group employ PLP in radical-based reactions [2,3].

From the external aldimine intermediate, carbanions formed by heterolytic cleavage of any one of the bonds to Cα (except for the C–N bond) can be stabilized. Loss of CO_2 gives a carbanion that is commonly reprotonated on Cα to give the corresponding amine as the product. Less commonly, for example with dialkylglycine decarboxylase (discussed below), the resulting carbanion is reprotonated on C4′ of the coenzyme to give oxidized substrate and the reduced, amino form (PMP) of the coenzyme. Proton abstraction is the most common forward step that external aldimines undergo since racemization, transamination, and β-elimination, three common reaction types, all require it. Retro-aldol cleavage of serine, central to one-carbon metabolism, is initiated by abstraction of a proton from the β-hydroxyl group followed by Cα–Cβ cleavage. Other known reaction types include β-decarboxylation of aspartate, β-elimination and replacement, γ-elimination and replacement, α/γ-elimination, cyclopropyl ring opening, radical-based 1,2-amino migrations, and others. This extraordinarily wide range of reaction types makes PLP enzymes extraordinarily useful to cells. The enzyme commission has more than 140 EC numbers assigned to PLP enzymes, and free living prokaryotes devote ~1.5% of their open reading frames to them [4].

The commonly accepted mechanism for stabilization of the resulting carbanion is resonance delocalization within the extended conjugated π system. This is illustrated in Fig. 2 where the three most significant resonance forms are shown. The rightmost resonance structure is referred to as the "quinonoid" since its structure resembles that of a quinone. It has strong absorption at ~500 nm (ε_{500}~40,000 $M^{-1}cm^{-1}$) and is sometimes but not always spectroscopically observable in enzyme-catalyzed reactions. This quinonoid resonance structure is commonly considered the major species responsible for the catalytic power of PLP since the electrons from Cα are neutralized by the protonated pyridine nitrogen. This simple view of PLP-catalyzed reactions may not be wholly accurate, as discussed below.

Model studies

The degree to which the Schiff base vs. the pyridine ring contributes to the stabilization of Cα carbanions has been debated recently. Computational studies performed by our group [5] and that of Bach [6] both predict that the pyridine ring does not play the largest role in carbanion stabilization as is commonly accepted in the literature [1,7,8]. Our studies used semi-empirical molecular orbital methods (PM3) to study both model aldimines and the intermediates formed in the active site of dialkylglycine decarboxylase (DGD). The four model aldimines studied are shown in Fig. 3. We demonstrated that the stereoelectronic (i.e., resonance) stabilization of the decarboxylation transition state could be separated energetically into the Schiff base and pyridine ring contributions. For example, the stereoelectronic contribution of the Schiff base in **B** is 11 kcal/mol while the pyridine ring contributes only ~2 kcal/mol. On the other hand, in **D** the Schiff base contributes 18 kcal/mol stereoelectronically while the pyridine ring contributes only 10 kcal/mol. The much larger total stereoelectronic effect for **D** vs. **B** is readily explained by the protonated Schiff base in **B**, which provides a large amount of ylide-type electrostatic stabilization of the developing negative charge on Cα, which is not dependent on p–p orbital interaction angles as are stereoelectronic effects.

These remarkable results, and those of Bach, challenge the prevailing view among biochemists that the delocalization of an electron pair onto the protonated pyridine nitrogen is the major source of the catalytic power of PLP. Rather, they suggest that the Schiff base makes the greatest contribution to transition state stabilization, even when the pyridine ring is protonated. The major role of the Schiff base receives circumstantial support from the very existence of a class of amino acid decarboxylases that employs a simple pyruvoyl group in the active site [9], which condenses with the substrate to form a Schiff base intermediate. Additionally, the lysine-dependent aldolases [10] provide further demonstration of the ability of enzyme-bound Schiff bases to stabilize carbanions readily.

Our semi-empirical calculations [5] suggest that protonation of the pyridine ring has a small effect (2–3 kcal/mol) on the activation energy either in the gas phase or the SM3 water model. This is remarkably consistent with experimental results from Bruice's [11] and our [12] laboratory, which show that protonation of the pyridine nitrogen increases reactivity by ~75-fold and quaternization of the pyridine nitrogen with a methyl group increases reactivity by ~20-fold. Dixon and Bruice [13] estimated the pK_a values of Cα for three protonation states of the 3-hydroxypyridine-4-aldehyde Schiff base with alanine. Their estimate is that pyridine nitrogen protonation decreases the pK_a of the Cα–H bond from 14 to 12, a similar factor of ~100-fold. Considering the pK_a value of ~29 estimated for glycine [14], pyridine ring protonation would appear to make a small contribution even to the thermodynamic stability of a carbanion formed on Cα.

This small rate enhancement is a far cry from the total effect of $\sim 10^{10}$-fold for PLP in amino acid decarboxylation [12]. We discuss below our idea that pyridine nitrogen protonation is a means of fine-tuning the reaction energetics, possibly for the purpose of selectively stabilizing the carbanionic intermediate in reaction