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Review

The shikimate dehydrogenase family: Functional diversity within a conserved structural and mechanistic framework

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ABSTRACT

Shikimate dehydrogenase (SDH) catalyzes the NADPH-dependent reduction of 3-deydroshikimate to shikimate, an essential reaction in the biosynthesis of the aromatic amino acids and a large number of other secondary metabolites in plants and microbes. The indispensible nature of this enzyme makes it a potential target for herbicides and antimicrobials. SDH is the archetypal member of a large protein family, which contains at least four additional functional classes with diverse metabolic roles. The different members of the SDH family share a highly similar three-dimensional structure and utilize a conserved catalytic mechanism, but exhibit distinct substrate preferences, making the family a particularly interesting system for studying modes of substrate recognition used by enzymes. Here, we review our current understanding of the biochemical and structural properties of each of the five previously identified SDH family functional classes.

Introduction

Early reports of an $NADP^+$-dependent enzyme from *Escherichia coli* that catalyzes the reversible oxidation of shikimate, and of a discrete, NAD^+-utilizing enzyme from *Aerobacter aerogenes* that catalyzes the oxidation of a similar molecule, quinate, appeared nearly simultaneously [1,2]. A half-century later, X-ray crystallographic studies would demonstrate that these enzymes possess remarkably similar three-dimensional structures, unambiguously illustrating their close evolutionary relatedness [3]. The two enzymes, shikimate dehydrogenase (SDH[1] or AroE) and quinate dehydrogenase (QDH or YdiB) were the first identified members of the SDH family. More recently, three additional SDH family proteins have been discovered. These enzymes are known as aminoshikimate dehydrogenase (RifI), SDH-like (SdhL), and AroE-like1 (Ael1). Different members of the SDH family exhibit ~20% to ~50% sequence identity at the amino acid level (Fig. 1). Although these proteins all share a highly conserved α/ß fold, they possess subtle variations in active site residue composition and geometry [3–6]. These variations result in significant changes in the biochemical properties of the enzymes, particularly in terms of their substrate preferences (Fig. 2). As such, the enzymes are an interesting system for studying mechanisms of substrate selectivity.

While SDH is strictly conserved among plants and microbes, other members of the SDH family exhibit more variable distributions [7]. In many cases, individual organisms possess multiple SDH family proteins. For example, *E. coli* [3] and the fungus, *Aspergillus niger* [8], both contain an SDH and a QDH enzyme, while *Populus trichocarpa* (poplar) encodes two SDH and three QDH isozymes [9]. The soil bacterium, *Pseudomonas putida*, possesses a representative of each of the five previously identified SDH family classes, making the organism a useful model for exploring SDH functional diversification [7]. In this review, we will discuss the biochemical and structural characteristics of the different members of the SDH family, with particular emphasis on their physiological roles, catalytic properties, and mechanisms of substrate recognition. SDH family proteins will be classified here based on their functional properties, although in some cases, functionally equivalent proteins from different kingdoms may exhibit relatively low overall sequence identity.

Shikimate dehydrogenase (SDH)

Physiological relevance of SDH

SDH (EC 1.1.1.25) catalyzes the reversible, NADPH-dependent reduction of 3-dehydroshikimate to shikimate (Fig. 3A). This

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[1] *Abbreviations used:* SDH/AroE, shikimate dehydrogenase; QDH/YdiB, quinate dehydrogenase; SdhL, shikimate dehydrogenase-like; RifI, aminoshikimate dehydrogenase; Ael1, AroE-like1; DHQ, dehydroquinate dehydratase; EPSP, 5-enolpyruvylshikimate-3-phosphate; IC_{50}, 50% inhibitory concentration; EGCG, epigallocatechin gallate; ECG, epicatechin gallate; PQQ, pyrroloquinoline-quinol; AHBA, 3-amino-5-hydroxybenzoic acid; DAHP, 3-deoxy-D-arabinoheptulosonate-7-phosphate.

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reaction represents the fourth step of the shikimate pathway, a conserved biosynthetic route in plants, fungi, bacteria, and apicomplexan parasites [10–13]. The shikimate pathway funnels erythrose 4-phosphate and phosphoenolpyruvate toward the production of chorismate, a precursor of the aromatic amino acids, vitamins B_9 and K_1, ubiquinone, and salicylate (Fig. 3A). The enzymes of the pathway are not found in metazoans, making them promising targets for non-toxic herbicides and antimicrobials. In addition, shikimate, the product of the SDH-catalyzed reaction, is a valuable chiral molecule used in the synthesis of the antiviral drug, oseltamivir (Tamiflu®) [14,15]. While shikimate has traditionally been isolated from plant sources, recent studies have exploited the activity of SDH as part of engineered biosynthetic schemes for microbe-based production of the compound [16–18].

The structural organization of SDH differs significantly across kingdoms. While the enzyme is monofunctional in bacteria, in plants such as *Arabidopsis thaliana* and *P. trichocarpa*, it is fused to an anabolic ('type I') dehydroquinate dehydratase (DHQ), forming a bifunctional protein known as the DHQ–SDH complex [19–21]. This complex catalyzes both the third and fourth reactions in the plant shikimate pathway. In fungi, such as *Aspergillus nidulans* [22], *Neurospora crassa* [23], and *Saccharomyces cerevisiae* [24,25], and in the apicomplexan parasite, *Toxoplasma gondii* [26], SDH activity is associated with the C-terminal domain of the AROM polypeptide. This large enzyme complex contains five functional domains that are equivalent to the monofunctional bacterial enzymes catalyzing reactions two through six of the shikimate pathway.

The close proximity of domains in the DHQ–SDH and AROM complexes may facilitate substrate channeling between enzyme active sites, minimizing the loss of shikimate pathway intermediates to competing processes [27,28]. In plants, 3-dehydroshikimate from the shikimate pathway is thought to be the immediate precursor of gallate, a component of hydrolysable tannins [29–31]. Plants may also divert 3-dehydroquinate, 3-dehydroshikimate, and shikimate for use in the biosynthesis of the cell wall component, lignin, and the anti-herbivory compound, chlorogenate [32–35]. Enzymatic turnover by the SDH domain of some DHQ–SDH proteins has been observed to exceed that of the DHQ

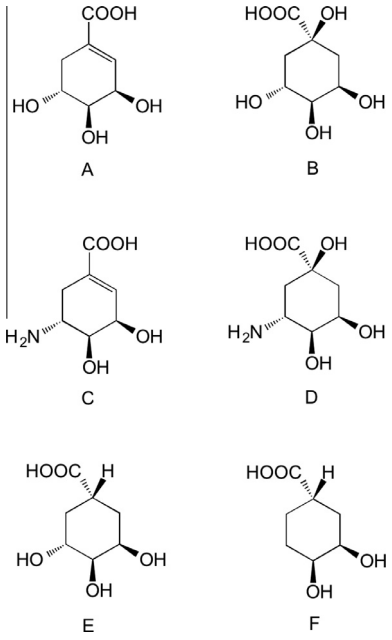

Fig. 2. Diversity of compounds utilized by SDH family proteins. (A) Shikimate, the product of the *in vivo* reaction catalyzed by SDH, and a substrate of some QDH enzymes; (B) quinate, a substrate for QDH; (C) aminoshikimate, a likely substrate of *Amycolatopsis mediterranei* RifI [136]; (D) aminoquinate, a potential alternative substrate of *A. mediterranei* RifI; (E) dihydroshikimate and (F) 3,4-dihydroxycyclohexanecarboxylate, possible substrates for a putative QDH-like protein, produced during the metabolism of quinate and shikimate by some species of *Lactobacillus* [115].

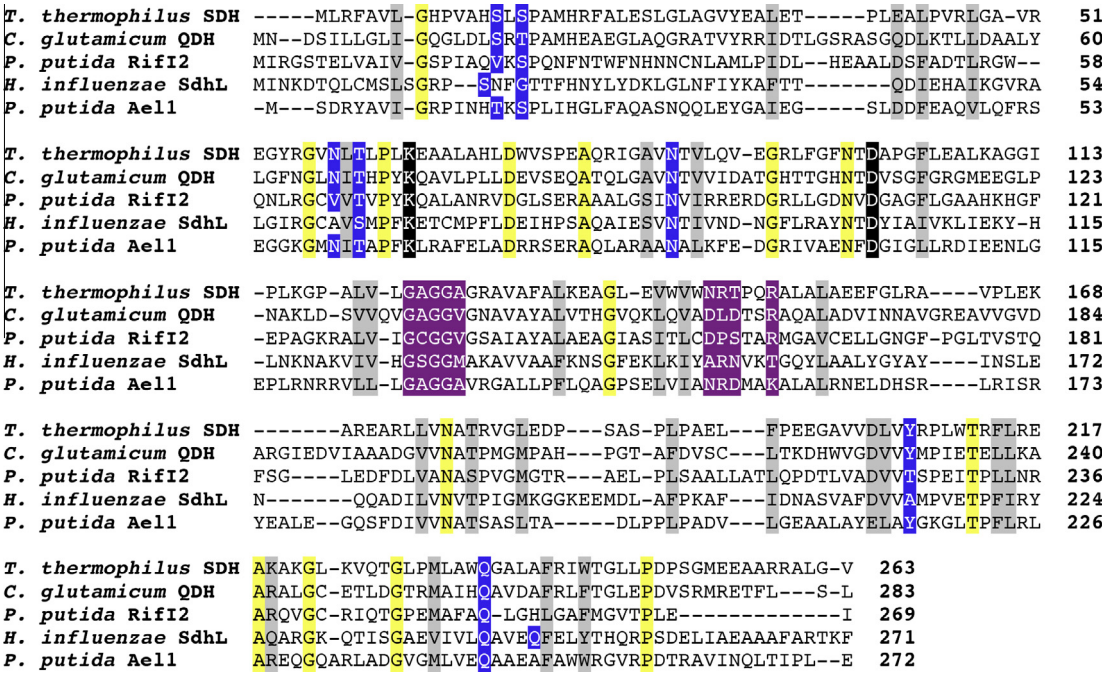

Fig. 1. Multiple sequence alignment of representative members of the SDH family. Key residues in the substrate and cofactor binding sites (discussed in text) are outlined in blue and purple, respectively. Catalytic groups are outlined in black. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)