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Carbonyl Reduction by Carboxylic Acid Reductases: An unsurprising Surprise?

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PURPOSE OF THE ABSTRACT

Carboxylic acid reductase enzymes (CARs) are well known for the reduction of a wide range of carboxylic acids to the respective aldehydes. The current understanding is that CARs release aldehydes, because they intrinsically circumvent the kinetic preference of aldehyde versus carboxylic acid reduction, owing to their reaction mechanism. CARs are comprised of three domains: an adenylation domain, a phosphopantetheinyl-binding domain and a reductase-domain and acid reduction is a multi-step cascade starting with carboxylate activation, which is followed by the formation of an enzyme-tethered thioester and finally, this thioester is reduced.

One of our long-term goals is to gain deep insight into the structure-function-relationship of CARs and we subjected various single-domains, di-domains and full-length CARs to crystallization trials. We solved the structure of the R-domain of a fungal CAR from Neurospora crassa (NcCAR, (PDB-code 8AEP). Its resemblance to short chain dehydrogenases (SDRs) triggered the question, why this R-domain releases a carbonyl compound while SDRs reduce carbonyl compounds to alcohols although both active sites were highly similar. Others postulated an on/off mechanism triggered by a particular amino acid that differed between SDRs and a crystallized R-domain of a bacterial CAR [1]. This particular amino acid, however, is not present in the NcCAR R-domain or any other R-domains of fungal CARs. We therefore sought to proof that the NcCAR R-domain was not acting as carbonyl reductase by incubation of a few carbonyl compounds in the presence of highly pure R-domain and NADPH. The unsurprising surprise was that the R-domain is in fact able to reduce carbonyls, including aldehydes, which are typically considered to be the final product of carboxylic acid reduction by CAR. We discovered that the respective full length NcCAR was able to reduce aldehydes [2]. Herein, we shed light on the structural elements, substrate requirements and an extended substrate scope of CARs (Figure 1).

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FIGURES

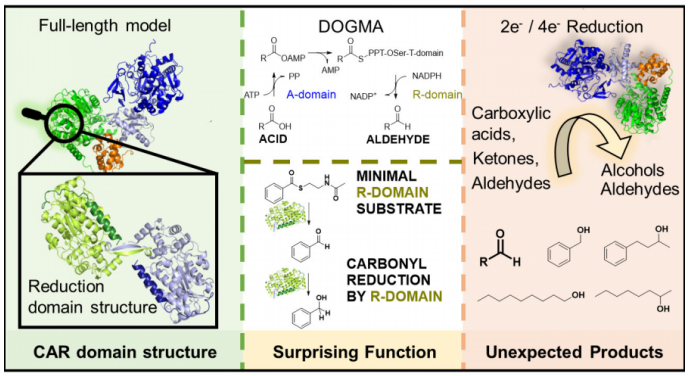

FIGURE 1

Figure 1

Structure and function of a CAR R-domain

FIGURE 2

KEYWORDS

Carboxylic Acid Reductase (CAR) | aldehyde | alcohol | enzymatic synthesis

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