Poster

Alcohol Dehydrogenases as model enzymes to investigate nucleotide cofactor specificity

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NAD(P)H is one of the most important molecules for cell physiology since it is used by a wide variety of proteins as cofactor. As such, the homeostasis of this cofactor must be tightly regulated by the cell, in particular the equilibrium between oxidized and reduced form [1]. Alcohol dehydrogenases (ADHs) are enzymes that can use either NAD^+ or $NADP^+$ as a coenzyme whereas others can use both. For this reason, they have been used as model tools for cofactor studies [2].

In this work, we focused on ADH1 from the model plant *Arabidopsis thaliana* to elucidate the structural requisites that determine cofactor preference of this enzyme. ADH1 catalyses both the reduction of toxic aldehydes and the reverse oxidation of alcohols. Despite its capability to perform both reactions, ADH1 is more efficient both *in vitro* and *in vivo* in reducing acetaldehyde using NADH [3-4].

Indeed, ADH1 shows a greater affinity for NADH over NAD^+ that differ only for the planarity of the nicotinamide ring. Moreover, ADH1 can use NADPH with a significant lower catalytic efficiency and affinity with respect to NADH.

We obtained the crystal structures of ADH1 by co-crystallizing the protein with various forms of the nucleotide cofactors at different starting concentration or by soaking preformed protein crystals at different concentration or incubation times. In addition, the crystal structure of ADH1 with NADHX, an inhibitor form of NADH formed *in vivo* during metabolic and heat stress, was also determined [5]. Our crystallographic studies shed new light on the elements that determine the cofactor specificity in a model enzyme such as ADH1.

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