MAGIC MIRROR IN MY HAND, WHICH IS THE FAIREST ALDEHYDE DEHYDROGENASE IN THE LAND?

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The first enzyme in the oxalocrotonate branch of the naphthalene-degradation lower pathway in Pseudomonas putida G7 is NahI, which belongs to the NAD(P)⁺-dependent aldehyde dehydrogenase superfamily. While NahI is thought to participate only in the conversion of 2-hydroxymuconate semialdehyde to 2-hydroxymuconate using NAD⁺ as a cofactor, XylG, another 2-HMSD involved in toluene and xylene degradation, oxidizes not only 2-hydroxymuconate semialdehyde, but also aromatic substrates such as benzaldehyde and its analogs. Our aim was to characterize kinetically and structurally the NahI enzyme. The nahI gene was subcloned into a T7 expression vector and the enzyme was overexpressed in Escherichia coli ArcticExpress as a hexa-histidine-tagged fusion protein. After purification by affinity and size-exclusion chromatography, small-angle X-ray scattering experiments were conducted to analyze the oligomeric state and the overall shape of the enzyme in solution. Kinetic assays with its biological substrate and salicylaldehyde, another intermediate in the pathway, were performed. NahI was also crystallized and X-ray diffraction data were collected. The protein is a tetramer in solution formed by two dimers of dimers that are oriented anti-parallel to each other to form a compact structure. The kinetic assays confirmed the preference of NahI for 2-hydroxymuconate semialdehyde, with a $k_{cat}/K_M$ value similar to that of another dehydrogenase (XylG) from the same subfamily. Surprisingly, NahI showed no activity with salicylaldehyde, in contrast to XylG, which has activity with different aromatic substrates. This difference might be associated with a specific 21-amino acid fragment, which is remarkably different for the two enzymes and located near the catalytic site. Comparison of nahI and xylG nucleotide sequences revealed that an insertion and a deletion mutation might have produced a reading frameshift, leading to a dramatic change in the catalytic properties of a 2-hydroxymuconate semialdehyde dehydrogenase. Therefore, this region located around the catalytic site suggests a possible role in substrate specificities.

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Key Words
3D structure, semialdehyde dehydrogenase, Pseudomonas putida