IIT Guwahati

Lecture 40

Course BT 631

Protein Structure, Function and Crystallography

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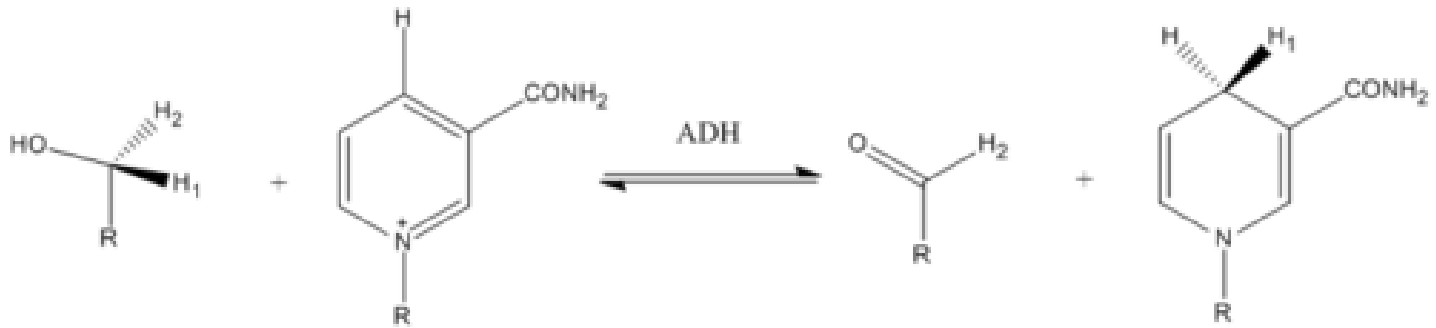
Dehydrogenases

Dehydrogenases are a class of oxido-reductase that mediate the removal of hydrogen atoms [H] by reduction of an electron acceptor which is usually usually $NAD^+/NADP^+$ or a flavin coenzyme such as FAD or FMN.

e.g. Alcohol dehydrogenase, Malate dehydrogenase, Glutamate dehydrogenase.



Alcohol **Electron Acceptor (NAD)** **Ketone** **NADH**

***Fig*. Alcohol Dehydrogenase (ADH).**

Classification of Dehydrogenases

Dehydrogenases constitute Class EC 1 of the IUBMB classification of enzyme-catalysed reactions.

- **EC: 1.1.1.X (With NAD^+ or $NADP^+$ as acceptor): 234 types of dehydrogenase.**
- **EC: 1.1.2.X (With a cytochrome as acceptor): 7 types of dehydrogenase.**
- **EC: 1.2.1.X (With NAD^+ or $NADP^+$ as acceptor): 69 types of dehydrogenase.**
- **EC: 1.3.1.X (With NAD^+ or $NADP^+$ as acceptor): 32 types of dehydrogenase.**
- **EC: 1.4.1.X (With NAD^+ or $NADP^+$ as acceptor): 19 types of dehydrogenase.**
- **EC: 1.5.1.X (With NAD^+ or $NADP^+$ as acceptor): 19 types of dehydrogenase.**
- **EC: 1.6.1.X (With NAD^+ or $NADP^+$ as acceptor): 3 types of dehydrogenase.**

Few Dehydrogenases

Alcohol Dehydrogenases: The alcohol dehydrogenase catalyses the reaction of alcohols to aldehydes and ketones by using NAD^+ as a coenzyme. The enzyme has Zinc ion sites in it. The zinc ion binds with NAD^+ during the catalysis.

L-Lactate Dehydrogenases: L-lactate dehydrogenase oxidizes the reversible reaction of L-lactate to pyruvate by using NAD^+ as a coenzyme. Lactate dehydrogenase helps in maintaining constant flow of glycolysis through conversion of NADH to NAD^+.

Malate Dehydrogenases: It catalyses, reversible dehydrogenation of malate to oxaloacetate or the decarboxylation of malate to pyruvate by using NAD^+ or $NADP^+$.

Glutamate Dehydrogenase: Glutamate dehydrogenase catalyses the conversion of glutamate to α-ketoglutarate and ammonia while reducing $NAD(P)^+$ to NAD(P)H. Glutamate dehydrogenase is capable of using either NAD^+ or $NADP^+$ as cofactor, in its catalytic reactions.

Functions of Dehydrogenases

Numerous Functions of Dehydrogenases:

- In human and other animals, dehydrogenases in respiratory electron chain transfer two hydrogen atoms from organic compounds to electron acceptors, thereby oxidize the organic compounds and generate energy.
- They participate in the generation of useful aldehyde, ketone or alcohol group during the biosynthesis of various metabolites. They also make important modifications to retinol, steroids, and fatty acids.
- Dehydrogenase can play a role in the diagnosis of diseases. Such as elevated blood serum Glutame dehydrogenase (GDH) levels indicate liver damage.
- In yeast, plant and many bacteria, some dehydrogenases catalyse the reverse reaction (NADH → NAD^+) as a part of fermentation to ensure a constant supply of NAD^+.

Alcohol Dehydrogenase

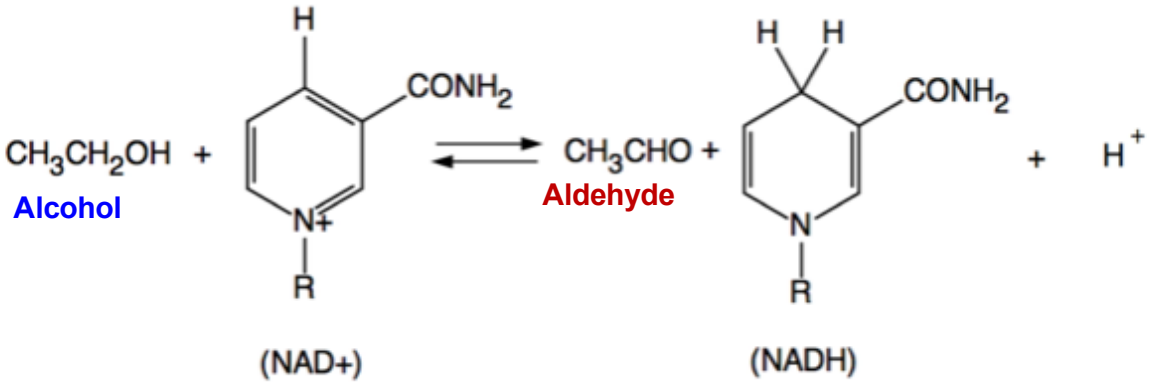
Alcohol Dehydrogenase (ADH, EC: 1.1.1.1)

- Alcohol dehydrogenases (ADH) facilitates the interconversion between alcohols and aldehydes or ketones with the reduction of nicotinamide adenine dinucleotide (NAD^+) to NADH.
- In human, ADHs are present in liver and lining of stomach.

Reaction Types:

(1) An Alcohol + NAD(+) → Aldehyde or Ketone + NADH.

(2) A Secondary Alcohol + NAD (+) → Ketone + NADH.



Why ADHs evolved in us?

- **Ripe fruits constitute more than 4% ethanol. Animals eating the fruit needed a system to metabolize exogenous ethanol that is present. This explains the need of Alcohol Dehydrogenases in the organism other than fermenting yeast.**

- **In animals, the metabolism of endogenous alcohol like Vitamin A, generates the hormone retinoic acid and retinol. The primary function of the enzyme is to eliminate the toxic level of retinol.**

- **ADHs oxidizes methanol to produce formaldehyde and ethylene glycol. That ultimately yield glycolic and oxalic acids. Glycolic acid is needed in tissue repair and oxalic acid in formation of uracil and orotic acid.**

If alcohols are toxic, why do yeasts produce so much ethanol??

Structure of Alcohol Dehydrogenase

- ADHs in higher eukaryotes (plants and animals) are usually dimeric, whereas those in prokaryotes and lower eukaryotes (yeast) are tetrameric.
- Medium-chain alcohol dehydrogenases contain 327–376 amino acid residues per chain and are usually zinc-dependent.
- In dimeric proteins, each subunit binds two Zn^{2+} ions, only one of which is catalytically active.
- The catalytic Zn^{2+} ion has distorted tetrahedral geometry, coordinated to 1 histidine (His-67) and 2 cysteine residues (Cys-174, Cys-46).
- The non-catalytic zinc plays a structural role and is coordinated tetrahedrally to 4 cysteine residues. This is the essential feature of the catalytic core that plays an important role in catalysis.

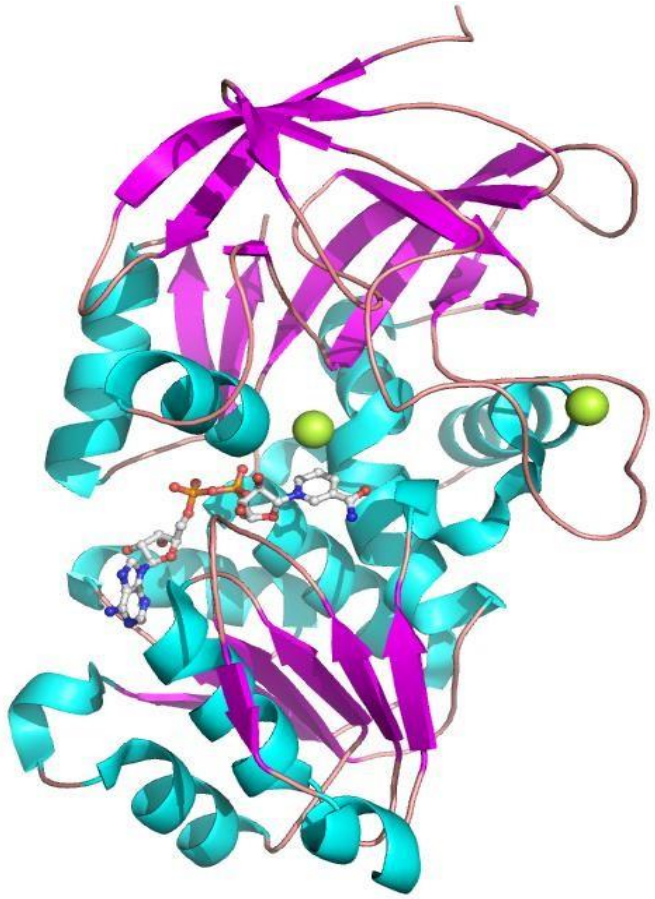


Fig. Horse Liver Alcohol Dehydrogenase.

Mechanism of Alcohol Dehydrogenase

1. A catalytic Zn^{2+} ion is coordinated in the active site by a Ser, Cys and His residue and functions to position the alcohol group of ethanol.
2. Before ethanol enters, a water molecule is initially positioned at the active site. After binding of NAD^+ the water molecule is displaced from the zinc atom by the incoming alcohol substrate.
3. Next deprotonation of alcohol happens, that gives the zinc bound aldehyde and NADH.
4. A water molecule then displaces the aldehyde to regenerate the original catalytic zinc centre, and finally NADH is released to complete the catalytic cycle.

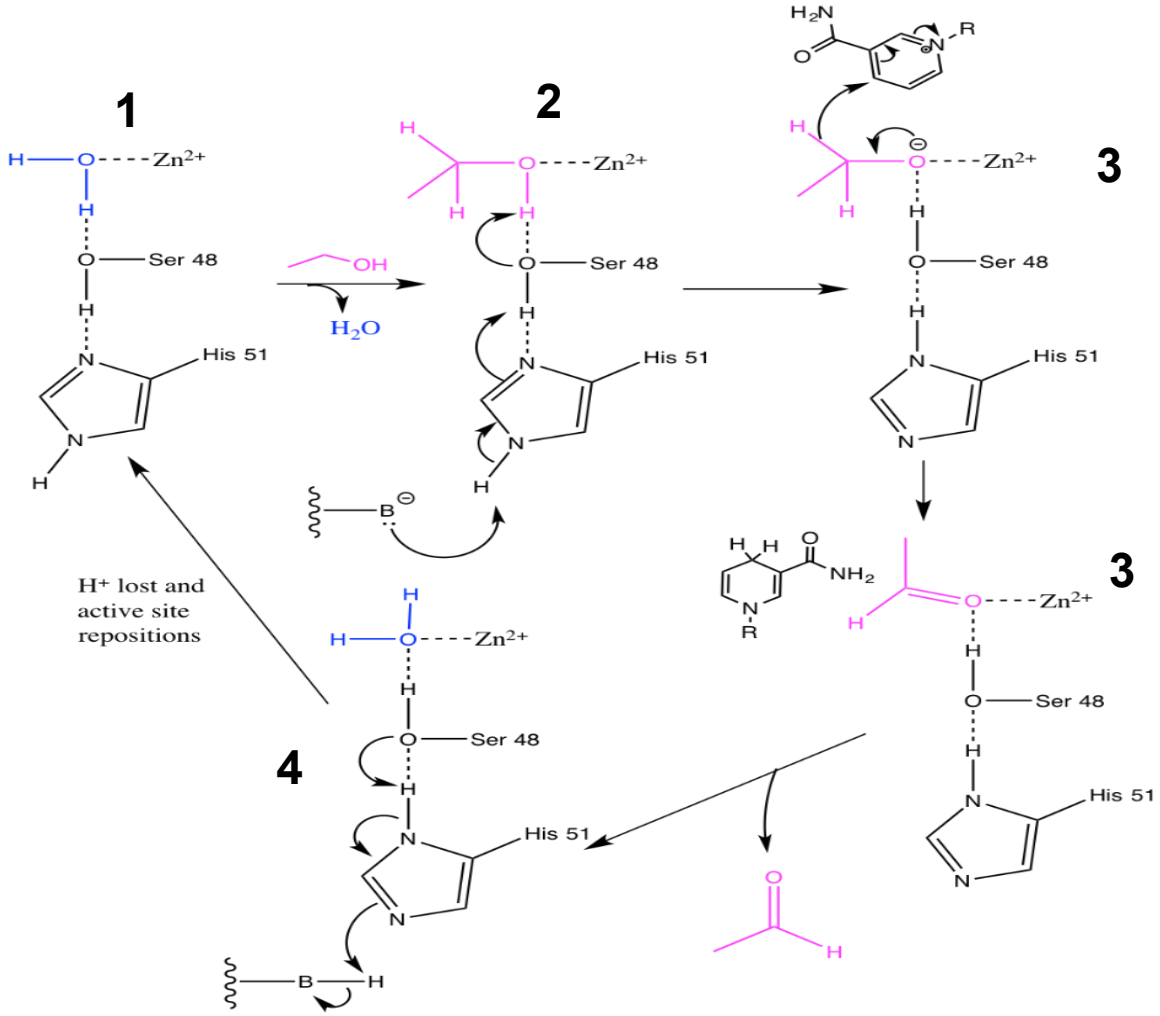


Fig. Mechanism of action of Alcohol Dehydrogenase.