Aldehyde Dehydrogenase Deficiency

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The lack of the normal mitochondrial enzyme aldehyde dehydrogenase (ALDH2) is prevalent in many people of Asian descent. Individuals without the proper form of aldehyde dehydrogenase suffer alcohol-flush syndrome due to their inability to properly catabolize alcoholic drinks. The skin of the individual becomes bright red and the respiratory tract is restricted. Alcohol-flush syndrome is the result of excessive acetaldehyde accumulation. Aldehyde dehydrogenase is produced in the liver and is metabolized by muscle cells throughout the body (Michinaga, 2012). Aldehyde dehydrogenase has the biochemical responsibility of oxidizing aldehydes to carboxylic acids in the human body (Crabb, 1989). This paper focuses on the biological importance of aldehyde dehydrogenase at the structural and biochemical levels and explains how the drug Alda-1 is able to reestablish normal aldehyde dehydrogenase function in deficient individuals.

![Figure 1: The oxidation of an aldehyde to carboxylic acid via aldehyde dehydrogenase.](image)
The mechanism of aldehyde dehydrogenase is shown in Figure 1. The entire reaction is dependent on the coenzyme NAD+. The active site of aldehyde dehydrogenase is bound to an aldehyde along with the cofactor nicotinamide adenine dinucleotide (NAD+). A Rossman fold in the active site keeps the active site functional while permitting the isomerization of the enzyme (Michinaga, 2012). A Rossman fold is just a modification in the protein structure that is specific to the binding of nucleotides. The coenzyme NAD+ is used in electron transfer. In alcohol metabolism, NAD+ acts as an oxidizing agent to accept electrons, producing NADH (Beretta, 2010). A cysteine residue uses the electrons from sulfur to interact with the aldehyde substrate. A hydride is released and bonds with NAD+ making NADH via the electron transfer reaction. Glutamate reacts with H₂O. The isomorphic change in the active site created by the Rossman fold allows the H₂O molecule to attack the carbonyl. The sulfur is released as the leaving group and a carboxylic acid is produced.

The structure of ALDH2 is considered to be a dimer of dimers. The tetrameric enzyme consists of four identical 54kDA subunits, each 500 amino acids in length (Larson, 2005). Each subunit consists of three bioactive domains: the coenzyme-binding domain, the catalytic domain, and the oligomerization domain (Wenzl, 2009). Two pleated sheet structures are formed when the oligomerization domains integrate with the catalytic domains. The structure of ALDH2 was determined using x-ray crystallography (Crabb, 1989). X-ray crystallography uses the diffraction of x-rays that bounce off crystalline atoms to produce a three-dimensional structure.
Aldehyde dehydrogenase activity was measured by the conversion of the coenzyme NAD$^+$ to NADH in the presence of acetaldehyde (Beretta, 2010). A spectrophotometer measured the UV light absorbance at 340 nm, which would signify the presence of NADH, and represent the normal functioning ALDH2 isoenzyme (Larson, 2005). The lack of absorbance at 340 nm characterizes the variant ALDH2*2 isoenzyme.

A faulty aldehyde dehydrogenase enzyme (ALDH2*2) is associated with individuals who have aldehyde dehydrogenase deficiency. This alternate form of aldehyde dehydrogenase is caused by a glutamate to lysine substitution at amino acid residue 487, located within the oligomerization domain of the tetrameric protein (Moreb, 2012). The crystal structure for ALDH2*2 shows a disoriented region located in the coenzyme binding cleft. There is a noticeable change in the amino acid sequence that forms the bottom of the active site (Larson, 2005). The alterations in the structure of the variant enzyme create a rigid body that hinders rotation of its catalytic and coenzyme-binding domains. This results in Lysine 487 not being able to hydrogen bond with arginines 264 and 475.

In an experiment by Larson in 2007, a glutamine substitution for Arginine at amino acid 475 produced another alternate form of aldehyde dehydrogenase (ALDH2*3). The binding of the coenzyme NAD$^+$ in ALDH2*3 resulted in the enzyme reestablishing its structure and enzymatic properties to wild-type levels (Larson, 2007). In the experiment ALDH2*2 was not so lucky; the active site was only partially restored. This lysine 487 mutant has an increased $K_m$ for its coenzyme NAD$^+$. $K_m$ is the substrate concentration that is required for the reaction to occur at 1/2 $V_{\text{max}}$. The variant enzyme also had a decreased $K_{\text{cat}}$, which is the turnover number and is calculated by dividing
$V_{\text{max}}$ by the enzyme concentration. An increased $K_m$ and decreased $K_{\text{cat}}$ results in the lysine 487 variant having reduced biochemical functionality (Larson, 2005). Having a glutamine at amino acid 487 to hydrogen bond with arginines 264 and 475 plays an important role in linking the structure of the NAD+ binding site to that of the active site. The hydrogen bonds created with arginines 264 and 475 help stabilize the alpha helical structure of the protein. Glutamate 487 places cysteine 302 in the correct position of the active site to help oxidize aldehydes (Wenzl, 2009). Aldehydes can be broken down much faster when cysteine 302 is in the active site due to the sulfur in cysteine.

Figure 2: Human aldehyde dehydrogenase complexed with Alda-1 (image credit to RCSB Protein Data Bank).
The drug Alda-1 has been reported to reestablish normal aldehyde dehydrogenase activity in individuals with aldehyde dehydrogenase deficiency (Beretta, 2010). A 2010 study by Beretta showed that Alda-1 increased acetaldehyde oxidation by 150% in the normal ALDH2 enzyme and 600% in the variant ALDH2*2 enzyme. Aldehyde dehydrogenase activity was measured by the conversion of the coenzyme NAD+ to NADH in the presence of acetaldehyde (Beretta, 2010). A spectrophotometer measured the UV light absorbance at 340 nm, which would signify the presence of NADH, and represent the normal functioning ALDH2 isoenzyme (Larson, 2007). The results indicate that Alda-1 stimulates aldehyde dehydrogenase activity by improving the binding of the coenzyme NAD+ in both isoenzymes. Hence, Alda-1 is basically a repair mechanism that fixes the defective enzyme by acting as a chemical chaperone for the ALDH2*2 variant. A tubular channel in the center of ALDH2 is used to oxidize aldehydes. In the variant ALDH2*2 enzyme the channel is misshaped, so the enzyme cannot oxidize the aldehydes (Moreb, 2012). Alda-1 attaches to the activator site in each of the four subunits of the enzyme, shown by the blue dots in figure 2. The binding of the drug induces an allosteric structural change in the enzyme, which activates the channel and reestablishes the enzyme’s ability to oxidize aldehydes to carboxylic acids (Moreb, 2012).

In conclusion, normal aldehyde dehydrogenase activity is vital for the regulation of acetaldehyde concentration. The aldehyde dehydrogenase mechanism is dependent on the coenzyme NAD+. Without the necessary cysteine residues in the active site, NAD+ is not able to bind. A single glutamate to lysine substitution at amino acid residue 487 results in a rigid body that hinders the rotation of its catalytic and NAD+ binding domains. The tetrameric structure of aldehyde dehydrogenase was determined using x-
ray crystallography. Aldehyde dehydrogenase activity was determined using a spectrophotometer that measured the conversion of the coenzyme NAD+ to NADH. The use of Alda-1 increased the oxidation of aldehydes by 600% in the variant ALDH2*2 enzyme by binding to the activator site and inducing an allosteric structural change, which activates the channel and reestablishes the enzyme’s ability to oxidize aldehydes to carboxylic acids. Now that the cause of aldehyde dehydrogenase deficiency is understood and drugs are proving to be effective, more research can be done to develop a treatment for alcohol-flush syndrome and a long-term solution to prevent aldehyde dehydrogenase deficiency.


RCSB Protein Data Bank.

<http://www.rcsb.org/pdb/explore/explore.do?structureId=3INJ>