Structural and Functional Aspects of Alcohol Dehydrogenase- A minireview

Phuntso Gombo[1], Pramod K Yadav[2] and Meera Yadav[1]*
[1]Department of Chemistry, NERIST, Nirjuli, Itanagar-791109(AP) India.
[2]Department of Life Sciences & Biotechnology, Chhatrapati Shahu Ji Maharaj University, Kanpur-208024
*Email: drmeerayadav@rediffmail.com

Abstract

Alcohol dehydrogenase, ADH (EC.1.1.1.1) is a zinc based metalloenzyme which catalyses the reduction of aldehydes and ketones to primary and secondary alcohols. These enzymes utilizes β-1,4-nicotinamide adeninedinucleotide (NAD$^+$) or β-1,4-nicotinamide adeninedinucleotide phosphate (NADP$^+$) as an electron acceptors. Alcohol dehydrogenases are exquisitely stereospecific; and by binding their substrate via a three-point attachment site. Details of structural and functional features of ADH have been discussed with respect to its catalytic active site, mechanism of action of ADH and functional ability in selective reduction of α,β-unsaturated carbonyl compounds to their corresponding unsaturated alcohols is very important chemical conversion for pharmaceuticals, flavor and fragrance industries.
Keywords: Metalloenzyme; Alcohol Dehydrogenase; Stereospecific; Co-factor; Biocatalyst

Introduction

Practice of light alcoholic beverage consumption for living up a social atmosphere is accepted in many cultures all over the globe and is perhaps the most common form of drug abuse in the world. Pleasant taste, relaxing nature and social beverage category makes it popular while its ill effects such as addictive nature, it dulls brain, confuses physical reactions are merely considered leading to numerous accidents, injury and even death. Alcohol affects almost every part of the human body, but it particularly affects the brain, heart, liver, pancreas and immune system [1].
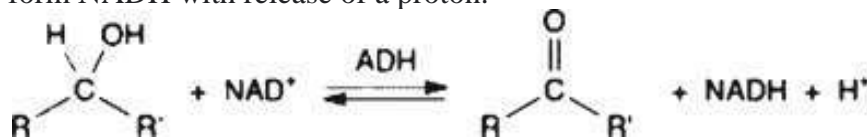
Apart from ingesting ethanol directly, human digestive system also produces approximately 3 grams of ethanol per day from digestion and fermentation of different food items that we eat [2]. So, our body must have a mechanism to eliminate these endogenous as well as exogenous alcohols. Thus, catabolic degradation of ethanol becomes important. Fortunately, a group of dehydrogenase enzymes collectively called Alcohol Dehydrogenases (ADHs) plays a vital role in catabolism of ethanol [3]. They are abundant in liver but are also present in different extents in other tissues [4].

In human and other mammals, ethanol is first converted to acetaldehyde using NAD$^+$ in presence of alcohol dehydrogenase enzymes, which is even more toxic than ethanol itself. In the second step acetaldehyde is further converted to acetic acid in presence of Aldehyde Dehydrogenase enzymes [5]. Finally with the help of two enzymes, viz., acyl-CoA synthetase and acetyl-CoA synthase 2, the acetic acid is converted to acetyl-CoA which then enters the normal citric acid cycle involving a series of reactions and releases energy [6].

Enzymes are biological catalyst that catalyzes specific biochemical reaction in plants and animals. Alcohol dehydrogenases are a group of dehydrogenase enzymes that occur in many plants, animals and in micro-organisms and facilitate the inter-conversion between alcohol (primary and secondary) and corresponding carbonyl compounds using oxidized and reduced form of nicotinamide adenine dinucleotide i.e., NAD$^+$ and NADH as co-factors for electron acceptance and donation.

Mechanism of action:

The alcohol dehydrogenase enzyme is a zinc based enzyme, and the reaction has two steps: Hydrogen is removed from the oxygen on the alcohol. A carbon-oxygen double bond is formed, making the aldehyde or ketone, and hydrogen from the carbon is added to NAD to form NADH with release of a proton.

In other words, ethanol is oxidized into acetaldehyde and the hydrogen atoms are added to NAD. Recall that an alcohol is any carbon chain with a carbon attached to an OH group while
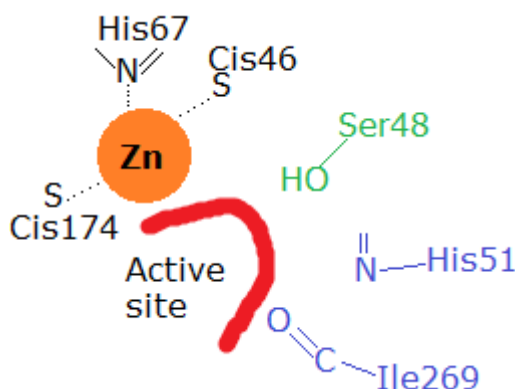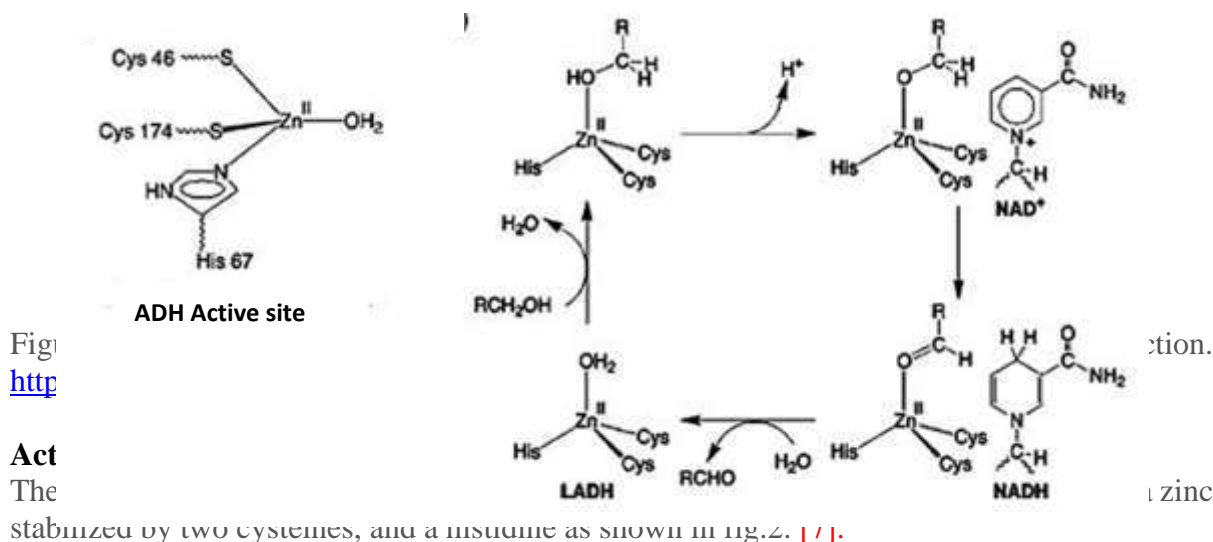
an aldehyde is any carbon chain with the terminal carbon forming a double bond with oxygen. The mechanism proposed for the catalytic cycle of ADH is shown in fig.1

ADH Active site

Fig... ...ction.
http...

Act...
The... ...zinc
stabilized by two cysteines, and a histidine as shown in fig.2. [7].

Figure. 2 : Active site of ADH. The oxygen in the alcohol binds to the zinc. The NAD is bound to the isoleucine. https://study.com/academy/lesson/alcohol-dehydrogenase-mechanism-pathway.html

The active site of human ADH1 (PDB:1HSO) consists of a zinc atom, His-67, Cys-174, Cys-46, Thr-48, His-51, Ile-269, Val-292, Ala-317, and Phe-319. In the commonly studied horse liver isoform, Thr-48 is a Ser, and Leu-319 is a Phe. The zinc coordinates the substrate (alcohol). The zinc is coordinated by Cys-46, Cys-174, and His-67. Leu-319, Ala-317, His-51, Ile-269 and Val-292 stabilize NAD^+ by forming hydrogen bonds. His-51 and Ile-269 form hydrogen bonds with the alcohols on nicotinamide ribose. Phe-319, Ala-317 and Val-292 form hydrogen bonds with the amide on NAD^+ [8].

Mammalian alcohol dehydrogenases also have a structural zinc site. This Zn ion plays a structural role and is crucial for protein stability. The structures of the catalytic and structural zinc sites in horse liver alcohol dehydrogenase (HLADH) as revealed in crystallographic structures, which has been studied computationally with quantum chemistry as well as with classical molecular dynamics methods. The structural zinc site is composed of four closely spaced cysteine ligands (Cys97, Cys100, Cys103, and Cys111 in the amino acid sequence) positioned in an almost symmetric tetrahedron around the Zn ion. A recent study showed that the interaction between zinc and cysteine is governed by primarily an electrostatic contribution with an additional covalent contribution to the binding [9].

These enzymes are usually zinc-containing enzymes present in mammals, high plants, fungi, yeast, and bacteria and are produced as homodimers or homotetramers with a monomer size of around 40 kDa. During catalysis, the zinc atom stabilizes the oxygen atom in the alcohol, making the hydroxy proton more acidic and favoring the hydride transfer.

Alcohol dehydrogenases are exquisitely stereo specific; and by binding their substrate via a three-point attachment site. They can distinguish between the two-methylene protons of the prochiral ethanol molecule.

Distribution of Alcohol dehydrogenases in prokaryotes and eukaryotes:

Generally speaking, alcohol dehydrogenases may be broadly classified into three major families, medium chain dehydrogenases/reductases (MDR family, ~370 residues, zinc containing),short chain dehydrogenases/reductases (SDR family, ~250 residues, lacks metal ions) and iron containing/activated alcohol dehydrogenases. The activity of first category of the enzymesis predominantly observe in higher animals, plants and in yeastwhereas the second category mainly observed in insects and the third category of dehydrogenases were isolated from thermophiles and hyperthermophiles [10-12].

MDR alcohol dehydrogenase family includes zinc-dependent dehydrogenases and are widely distributed in nature. Depending upon phylogenetic relationship, sequence alignment, gene expression pattern and catalytic properties, seven different classes of alcohol dehydrogenases (Class I-VII) have been described for animals by G. Duester et al in 1999. Among mammals, alcohol dehydrogenases associated with human body has been studied to the best extent. Mammalian alcohol dehydrogenases are group of enzymes in which Zn atom is firmly bounded with various residues (often two Cys and one His) and exists in dimeric form with subunits of approximately 40 kDa [13,14]. Out of seven classes, six different classes of alcohol dehydrogenases have been described for mammals, Class-I to Class-VI [15]. Class-I alcohol dehydrogenases is present in great amount in liver of almost all mammals and play a vital role in ethanol metabolism. However, only five classes of ADH enzymes have been found in human tissues. Out of five classes only class IV ADH is not expressed in liver and are fairly expressed in stomach and esophagus and contribute to ethanol metabolism [16]. Class-I alcohol dehydrogenase enzymes in human (ADH1) are coded by three genes, i.e., exists as three isozymes, ADH1A, ADH1B and ADH1C (Nomenclature recommended by Human Genome Organization Nomenclature Committee) and are more or less 90 % identical in amino acid sequences [17] Human ADH3 (class III ADH) is universally distributed in various tissues and because of its close resemblance with glutathione-dependent formaldehyde dehydrogenase found in plants and yeast, it is believed that all other ADH of human form were formed by genetic mutation of ADH3 [18,19]. Other classes, i.e., ADH2 and ADH5 are present in different concentrations in various tissues of human body and perform similar functions. ADH6 (a class VI ADH) activity have been observed in rat and deer mouse in addition to ADH1, ADH2, ADH3 and ADH4 in case of rat and ADH1 in case of deer mouse [20] ADH7 (a class-VII alcohol dehydrogenase) activity were found in chicken [21,22]. Other animal where ADH activity have been studied extensively includes horse liver ADH (ADH1E, ADH1S and ADH3), rabbit ADH (ADH1, ADH2A, ADH2B and ADH3), Ostrich ADH (ADH1 and ADH2) and many more.

Unlike in mammals, ADH in plants, yeast and bacteria do not convert alcohols to carbonyl compounds, rather they play an important role in fermentation and initiate the reverse reaction i.e., carbonyl compounds to alcohols to ensure constant supply of NAD^+ (vital coenzyme for glycolysis), thereby maintaining balanced redox reaction [23]. Among eukaryotes, yeast ADH have been studied extensively and yeast ADH consists of three isoenzymes: YADH-1, YADH-2 and YADH-3 [24]. YADH-1 exists in tetrameric form having four identical subunits, 347 amino acid residues are present in each subunit, its molecular mass is of 36 kDa [25]. YADH-1 is the first ever isolated ADH which was purified and crystalized in 1937 [26].

In contrast to detail studies on animal and yeast ADHs, plant ADHs have been studied to much less extent even though they play important roles in plants during various environmental stresses such as drought and water logging [27]. These enzymes also play role in seed germination, pollen development and fruit ripening. Abdel et al. in 2014 studied the activity of ADH during water stress on three legume plants (*Phaseolus vulgaris L, Pisum Sativum L and Vicia faba L)* and found that during water logging the activity of ADH enzymes are greatly enhanced as in such cases of O_2 deficiency, plants shifted to anaerobic respiration via fermentation [28]. Also, during excessive drought enhancement in ADH activity in *Arabidopsis* plants has been reported [29]. ADHs plays very important role during seed germination when the energy stored in seeds as starch, proteins, fats etc. were anaerobically

break down and provide nourishment to the growing embryo [30].This is the reason that most of plant ADHs were extracted and studied from germinating seeds. The molecular weight of pea and lentil ADH were reported as 60 kDa and 70 kDa. Numerous forms of alcohol dehydrogenase enzymes have been isolated from various tissues of plant and studied which include plants like pea, bean, lentil, peanut, tea, maize, rice, wheat, tomato, potato, *arabidopsis*, *eucalyptus, pyrus,* switchgrass and many more [31-33].

SDR alcohol dehydrogenases mainly exists as dimer having two subunits of molecular mass of approximately 27.5 kDa, however, its tetrameric forms are also known [34]. When we talk about alcohol dehydrogenases of SDR family, ADH activity found in *Drosophila*has been studied extensively. Drosophila *Melanogaster* ADH are homodimer with two identical subunits, each with a molecular mass of 27400 Da and perform detoxification as well as nutritional functions during ethanol metabolism [35].
Two isozymes (ADH 1 and ADH 2) have been isolated from Mediterranean fruit fly (*Ceratitis capitata*)**,** the activity of ADH 2 was found greater than activity of ADH 1 during the entire life cycle. ADH of SDR category from Flesh fly, *Sarcophaga peregrina* [36] Olive fly [37] and many more insects are available in literature.

The last and also the least studied category of alcohol dehydrogenases, i.e., iron containing/activated ADHs have been mainly isolated and purified from thermophilic archaea that grow optimally at elevated temperature of more than 80^0C. Iron dependent ADHs are homodimer with two subunits, each with a molecular mass of 40000 ± 1000 Da [38]. Unlike the catalytic importance of Zn in MDR superfamilies have been well explained, the catalytic importance of Fe in such enzymes have not been fully resolved. However, their thermostability and broad range of substrate specificity properties attracts the present-day researchers a lot.

Applications

Reduction reactions are very important and powerful tools in all areas of chemistry. Selective reduction of α,β-unsaturated carbonyl compounds to their corresponding unsaturated alcohols is very important chemical conversion for pharmaceuticals, flavor and fragrance industries [39-41]. However, selective hydrogenation of the C=C bond to obtain saturated carbonyl compounds is comparatively easy to achieve because it is thermodynamically favored but the selective hydrogenation of the C=O bond to obtain unsaturated alcohols is difficult to accomplish as a consequences side reactions leading to unwanted products are very common by conventional methods. Because of these reasons research effort were directed towards finding a route to improve selectivity to unsaturated alcohols.

The first model and historic example of selective hydrogenation of α,β-unsaturated carbonyl compounds was carried out by Tuley and Adams in 1925. They used unsupported Pt catalyst promoted by Zn and Fe chlorides for the liquid phase hydrogenation of Cinnamaldehyde to Cinnamyl alcohol. However the target product i.e., Cinnamyl alcohol could not formed in satisfactory amount, moreover, the process produces large number of by-products which need separation to recover the target molecule. In addition, a high temperature reaction condition and high hydrogen pressure requirement makes these procedures less suitable for such transformations. Thereafter, numerous numbers of such attempts were made by chemists all over the world to improve selectivity towards unsaturated alcohols.

No doubt, there exists a number of research papers and review papers available in the literature for selective hydrogenation as well as transfer hydrogenation of α,β-unsaturated carbonyl compounds using inorganic catalysts but selective enzymatic biotransformation of α,β-unsaturated carbonyl compounds to corresponding unsaturated alcohols has been reviewed hardly. P Gallezot and D Richard, 1998, reviewed the factors to improve the selectivity to unsaturated alcohols in hydrogenation of α, β-unsaturated carbonyl aldehydes, such as mechanistic pathways, metal catalysts specificities, stearic effects on metal surfaces, electronic effects of supports and ligands, influence of reaction conditions etc [42]**.** Ronald et al., 2018, reviewed the selective transfer hydrogenation of α,β-unsaturated carbonyl compounds to unsaturated alcohols using homogeneous as well as heterogeneous catalysis [43]**.** In this review we will provide the literature for high selective biotransformation of α,β-unsaturated carbonyl compounds to unsaturated alcohols using alcohol dehydrogenase enzymes.

α,β-unsaturated carbonyl compounds and other carbonyl compounds are reversibly reduced to corresponding alcohols in presence of alcohol dehydrogenase enzymes (ADHs). In the reaction reduced form of nicotinamide adinine dinucleotide (NADH) or nicotinamide adinine dinucleotide phosphate (NADPH) acts as cofactor [44]. Most of the researches were carried out for the biotransformation of Cinnamaldehyde to Cinnamyl alcohol because of its vital role in perfume and flavor industries.When it comes to catalytic reduction of unsaturated carbonyl compounds, ADH enzymes are of best choice as the reaction is very much efficient with high yield and is free from any side products, reducing separation cost, also the products are free from unnecessary odors which usually results from side product. However, there exist a major problem in the use of $NAD(P)^+$ - dependent hydrogenases as catalyst in organic synthesis, i.e., the high expensive cost of $NAD(P)^+$ coenzyme. Therefore, in this review we will discuss various approaches adopted in researcher papers on recycling of the expensive cofactor.

Paolo et al., described cofactor recycling for enzymatic biotransformation of cinnamaldehyde to cinnamyl alcohol using ADH from yeast *saccharomyces cerevisiae* in which the NAD^+ produced in the reaction is recycled back to NADH by the same enzyme at the expense of ethanol. In the reaction, ethanol acts as co-solvent for both CMA and CMO and is always present in large excess to force the whole process towards CMA reduction, moreover, elimination of acetaldehyde (low boiling point of 21^0C) drove CMA reduction towards completion. The result was appreciated as CMA was almost completely reduced only after 3 hours with 100% CMO selectivity.

Today, both the academic and the industrial community see biocatalyst as a highly promising area of research, especially for the development of sustainable technologies for the production of chemical and more selective and complex active ingredients in pharmaceuticals and agrochemicals.

Conclusion

ADH enzymes are biologically important catalyst that catalyzes the specific biochemical reaction in plants and animals. ADH are a group of dehydrogenase enzymes which facilitates the inter-conversion between alcohol (primary and secondary) and corresponding carbonyl compounds using oxidized and reduced form of nicotinamide adenine dinucleotide (NAD^+ and NADH) as co-factors. High selectivity and hence enantiomerically pure products are the particularly attractive features of bio-catalysis. This situation may be attributed to several perceived limitations of bio-catalysis, including the availability of biocatalyst, their substrate scope and the operational stability.

Acknowledgement

References

1. Deepanjali Mishra; Impact of Alcohol Consumption on Health; *Research Chronicler: International Multidisciplinary Refereed Peer Reviewed Indexed Research Journal*, 2018, Volume VIII.

2. JyrkiTillonen; Ethanol, Acetaldehyde and Gastrointestinal Flora, Regulatory factors and pathophysiological consequences of microbial ethanol oxidation and acetaldehyde production in the digestive tract, ISBN 952-91-2603-4

3. Tanaka Furnika, Shiratori Yasushi, Yokosuka Osarnu, ImazekiFurnio, Tsukada Yoshio, Omata Masao;"Polymorphism of Alcohol-Metabolizing Genes Affects Drinking Behavior and Alcoholic Liver Disease in Japanese Men"; Alcoholism: *Clinical and Experimental Research, 1997, 21 (4): 596–601.*

4. Hans Jornvall and Jan-Olov Hoog; "Nomenclature of Alcohol dehydrogenases"; Alcohol and Alcoholism, 1995, Vol. 30. No. 2, pp. 153-161.

5. Hector Riveros-Rosas, Adriana Julian-Sanchez, Enrique Pina; Enzymology of Ethanol and Acetaldehyde metabolism in Mammals; *Archives of medical Research,* 1997, Volume 28, No. 4, pp.453-471.

6. BjoernSchwer, JakobBunkenborg, Regis O Verdin, Jens S Andersen, Eric Verdin; Reversible Lysine Acetylation Controls the activity of the Mitochondrial Enzyme Acetyl-CoA Synthetase; *ProcNatl Acad Sci USA, 2006, 103(27): 10224-1-229.*

7. E. Torres, M. Ayala,6.24 - Biocatalysis by Metalloenzymes, Editor(s): Jan Reedijk, Kenneth Poeppelmeier, Comprehensive Inorganic Chemistry II (Second Edition),Elsevier, 2013, Pages 685-735,ISBN 9780080965291, https://doi.org/10.1016/B978-0-08-097774-4.00625-2.

8. Hammes-Schiffer S, Benkovic SJ (2006). "Relating protein motion to catalysis". *Annual Review of Biochemistry.* 75: 519–41.

9. Brandt EG, Hellgren M, Brinck T, Bergman T, Edholm O (February 2009). "Molecular dynamics study of zinc binding to cysteines in a peptide mimic of the alcohol dehydrogenase structural zinc site". *Physical Chemistry Chemical Physics.* 11 (6): 975–83.

10. Hans Jornvall and Jan-Olav Hoog; Nomenclature of Alcohol dehydrogenases; *Alcohol & Alcoholism,* 1995, Volume 30, No. 2, pp 153-161.

11. Matthew F. Reid and Charles A. Fewson; Molecular Characterization of Microbial AlcoholDehydrogenases; *Critical Reviews inMicrobiology*, 20 (I): 13-56, 1994.

12. Aida Hernandez-Tobias, Adriana Julian-Sanchez, Enrique Pina, Hector Riveros-rosas; Natural alcohol exposure: Is ethanol the main substrate for alcohol dehydrogenases in animals?; *Elsevier;Chemico-Biological Interactions,*191, 14-25, 2011.

13. Vallee BL and Bazzone TJ; Isozymes of human liver alcohol dehydrogenases; *Current topics in biological and medical research 8,* 1983, pp. 219-244.

14. Jyrki Tillonen; Ethanol, Acetaldehyde and Gastrointestinal flora; Academic desertation, Research Unit of Alcohol Diseases, University of Helsinki,Finland, 2000.

15. Olle Danielsson et al.; Fundamental molecular difference between alcohol dehydrogenase classes; *Proceeding of the National Academy of Sciences of the United States of America,*1994, Volume 91, pp 4980-4984.

16. Howard J. Edenberg and Jeanette N. McClintick; Alcohol dehydrogenases, aldehyde dehydrogenases and alcohol use disorders: a critical review; *Wiley Online Library,* 2018, pp 2281-2297

17. Duester et al.; Recommended Nomenclature for vertebrate Alcohol Dehydrogese gene family; *Biochemical pharmacology,* 1999, Volume 58, pp 389-395.

18. William G. Gutheil, Barton Holmquist, and Bert L. Vallee; Purification, Characterization and Partial Sequence of the Glutathione-Dependent Formaldehyde Dehydrogenase from *Escherichia coli:* A Class III Alcohol Dehydrogenase; *Biochemistry* 1992, *31,* 475-481

19. Olle Danielsson and Hans Jornvall; "Enzymogenesis": Classical liver alcohol dehydrogenase origin from the glutathione-dependent formaldehyde dehydrogenase line; *Proceeding of the National Academy of Sciences of the United States of America,*1992, Vol. 89, pp. 9247-9251

20. Jan-Olov Hoog, Margareta Brandt, Jesper J. Hedberg, Patrik Stromberg; Mammalian alcohol dehydrogenase of higher classes: analyses of human ADH5 and rat ADH6; *ELSEVIER*, 2001, pp 395-404

21. Natalia Y. Kedishvili et al.; cDNA Sequence and Catalytic Properties of a Chick Embryo Alcohol Dehydrogenase That Oxidizes Retinol and 3b,5a-Hydroxysteroids; *THE JOURNAL OF BIOLOGICAL CHEMISTRY*, Vol. 272, No. 11, pp. 7494–7500, 1997

22. M Estonius et al.; Avian alcohol dehydrogenase: the chicken liver enzyme - Primary structure, cDNA-cloning, and relationships to other alcohol dehydrogenases; *Eur. J. Biochem.* 194: 593-602, 1990