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ACTIVITY OF YEAST ALCOHOL DEHYDROGENASE AGAINST SOME SELECTED PRIMARY ALCOHOLS

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Abstract

The enzyme, yeast alcohol dehydrogenase (ADH) is responsible for the reversible oxidative breakdown of alcohols to their corresponding aldehydes, coupled with the reduction of the coenzyme nicotinamide adenine dinucleotide NAD^+ to NADH. In this work, alcohol dehydrogenase (ADH) from yeast was optimized and characterized for substrate specificity successfully. The value of the initial rate of enzyme reaction was found to be 0.299, and using the Beers Law, the rate of change in concentration of NADH was found to be 4.7 X 10^{-5} mol^{-1}/l/min, rate of ADH reaction was calculated to be 23.73 nanokatals. The values of V_{max} and K_m obtained from Michaelis-Menten were 3.2 mMs^{-1} and 0.4mM, and from Lineweaver Burk double reciprocal curve are 0.33 mMs^{-1} and 0.4 mM for ethanol. Although ADH possess a broad spectrum of activity towards a wide range of substrates, however, results showed that ethanol, 2-buten-1-ol and 2-propen-1-ol presented the highest specificity to yeast ADH, while activity of ADH declined on propanol. The variation in substrate specificity may be the result of configuration of the active site of ADH which exhibits geometric specificity allowing binding to different substrates that are geometrically isomeric. It may also be related to the ease with which different alcohol structures fit into active site of the enzyme.

Key words; *Yeast Alcohol dehydrogenase (YADH), substrate, concentration, ethanol, active site*

Introduction

Alcohol dehydrogenases (ADH) are oxidoreductases that catalyse the reversible oxidation of alcohols to aldehydes or ketones, with the concomitant reduction of coenzyme nicotinamide adenine dinucleotide NAD^+ and $NADP^+$ to NADH and $NADPH_2$ (De Smidt *et al*., 2008). ADH is a tetramer composed of four identical subunits each of which is made up of single polypeptide

chain of 36kDa with 347 amino acid residues, each subunit has one binding site for coenzyme and a tightly bound zinc ion (Raj *et al*., 2014). Investigation on the kinetic parameters of ADH has been done extensively using different buffers, for instance in 0.5 M pyrophosphate buffer at pH 8.7 and 25 °C, pure ADH presented specific activity of 380 – 430 µmol/min while commercial ADH has 200 – 250 µmol/min (Schopp & Aurich, 1975). Using tris buffer, ADH presented highest stability at pH 8.5 and 35 °C (Zanon *et al*., 2007), and in pyrophosphate buffer the optimum pH and temperature are found to be 8.8 and 25 °C respectively (Madhusudhan *et al*., 2008). The enzyme occur virtually in all organisms. Base on the structures and catalysis of ADH from different sources, the enzyme has been grouped into three (3) basic types. This include medium-chain zinc dependent type which consist of 327-376 amino acid residues per subunit (horse liver & *S. cerevisiae* ADH); short-chain zinc independent ADH found in Lactobacillus brevis which consist of 250 amino acid per subunit and the long-chain Iron (Fe) activated ADH which consist of 385 aa per subunit, common in saccharomyces cerevisiae (Neto *et al*., 2011). Study reveals that the molecular weight of ADH from yeast doubles that of the mammalian by two folds, and about a hundred times more active (Hayes & Velick, 1954), and ADH in higher eukaryotic (plants and animals) are usually dimeric where as those in prokaryotic (bacteria) and lower eukaryotic (yeasts) are tetrameric (Raj *et al*., 2014). ADH specificity is restricted to primary alcohols with linear aliphatic chains with ethanol being the best substrate, also alcohols increasingly become less effective as substrate to ADH as their chain length increases and on moving from primary to secondary alcohols (Kiledu *et al*., 2024; Dickinson & Monger, 1973). Also, ADH from Baker's yeast in heptane solution have been found to oxidize unsaturated alcohols, 3-methyl-2-buten-1-ol to the corresponding unsaturated aldehyde, 3-methyl-2-butenal (Kiledu *et al*., 2024). Yeast ADH does not measurably oxidize the secondary alcohols (Raj *et al*., 2014). Due to its wide distribution in animal tissues, plants, microorganisms, broad substrate and stereo selectivity, ADH has attracted major scientific attention (Neto *et al*., 2011), and has found an important application research and biochemical industries, forensic science for estimating concentration of primary alcohols, NAD^+, ethylene glycol, numerous aldehydes enzymatic breakdown of organic solvents, and in biosensor (Madhusudhan *et al*., 2008).

Yeast alcohol dehydrogenase (YADH) is a member of a large family of zinc-containing alcohol dehydrogenases (Leskovac *et al*., 2002). YADH has three isozymes; YADH-1, YADH-2 and YADH-3. YADH-1 is the constitutive form of YADH that is present in the cytoplasm, it is expressed during anaerobic fermentation of glucose, where it catalyse the reduction of

acetaldehyde to ethanol (Raj *et al*., 2014). YADH-2 is also found in the cytoplasm and is repressed by glucose, whereas YADH-3 is mitochondria bound (Leskovac *et al*., 2002). Routine optimization and characterization of enzyme assays provides mathematical relationships that describe the rate at which chemical reaction catalysed by the enzyme takes place (Juárez-Enríquez *et al*., 2022), it is also essential in studying and validating the behaviour of that enzyme, ensuring high enzyme yield and performance, enzyme sensitivity and specificity (Juárez-Enríquez *et al*., 2022).

Yeast ADH specificity to different substrates has been studied substantially, especially the primary alcohols, however, additional data is required on the actions of yeast ADH with unsaturated aliphatic alcohols. The unsaturated alcohol has form important industrial raw material in fine chemical synthesis, especially in the cosmetic industries where oxidation of fatty alcohols into aldehydes is a key reaction in the production of flavours and fragrances (Patocka *et al*., 2016; Ribeaucourt *et al*., 2022). This work was aimed at characterizing Yeast ADH reactivity against some selected primary alcohols which include ethanol, propanol, 2-propen-1-ol and butenol. The oxidoreductase potentials of yeast ADH in industrial processes, especially in the flavours and fragrance industries can be of economic and environmental importance, since the chemical synthesis processes are energy consuming, requires the use of metallic or toxic catalysts, and produces racemic mixtures due to poor regio/enantio-selectivity of the catalysts. This lead to additional requirements of further downstream processing (Ribeaucourt *et al*., 2022). Chemical synthesis also often generates large amount of wastes, which eventually form environmental pollution.

2.0 Materials and methods

2.1 Optimization of Enzyme Concentration required to determine the Kinetics of Yeast ADH

A pyrophosphate buffer (pH 9.0) containing 0.01M sodium pyrophosphate decahydrate, 0.34 mM EDTA disodium salt dehydrate was prepared. Spectrophotometer cuvettes were set up as shown in this table I.

Table I: Spectrophotometer Cuvettes Setup

Reaction Mixture	Volume
Pyrophosphate buffer (0.001M, PH 9)	1Ml
Fresh deionized water	1Ml
NAD+ (0.0030M)	200µl
Cysteine	200µl

ADH solution (17 units per mL)	100µl
Total volume	2.5Ml

Yeast alcohol Dehydrogenase (ADH) activity was determined by measuring the rate at which the coenzyme NAD^+ was reduced to NADH as ethanol was oxidized to acetaldehyde in the reaction mixture as shown in the reaction below:

$CH_3CH_2OH + NAD^+ \leftrightarrow CH_3CHO + NADH + H^+$

A Thermo Genesys 10S spectrophotometer was calibrated with deionized water and the wavelength set at 340nm. The assay mixtures were mixed thoroughly and the cuvettes were placed in the spectrophotometer to begin the kinetics. 500µl of 2M ethanol was added to make a total volume of 3ml to start the reaction after a kinetic of 15 seconds, and the change in absorbance per minute (ΔA/min) was as 0.299 at 340nm, this represents the initial velocity of ADH reaction. The level of conversion of ethanol to acetaldehyde was measured indirectly by the increase in absorbance of the reaction mixture, due to the reduction of NAD^+ to NADH which absorbs strongly in the UV region of the spectrum at 340nm.

Estimation of Michaelis constant (K_m) and maximum velocity (V_{max}) of YADH for ethanol

Background reaction was eliminated using two (2) blanks. The assay was set up using the initial protocol, under same pH and temperature, a serial dilution of the 2M ethanol to different concentrations of 1.5 M, 1.0 M, 0.5 M and 0.25 M was done and corresponding values of the change in absorbance at 340nm per minute were recorded.

Investigation on ADH substrate specificity

Yeast ADH was assayed for activity against different substrates including 1-propenol, 2-buten-1-ol, butanol and ethanol, change in absorbance per minute for all the substrates under same conditions were measured at 450nm and recorded.

3.0 Results

Alcohol dehydrogenase (ADH) assay was optimized, value of the initial velocity of the enzyme reaction (absorbance change per minute) was calculated as 4.7×10^{-5} $mol^{-1}l$.

Estimation of Michaelis Menten's constant (K_m) and maximum velocity (V_{max}) for ethanol

3.2 The Estimation K_m and V_{max} of ADH

Table 2 shows the values of absorbance for different working concentrations of ethanol. The values generated were used to draw Michaelis-Menten and Lineweaver-Burk double reciprocal plots on a graph sheet and with a computer software (Enzfiller).

Table 2: The Working Concentrations and Their Corresponding Absorbance Values

Working Concentration (mM)	Absorbance
8	0.130
20	0.216
25	0.220
33	0.250
83	0.308
166	0.344
250	0.400
330	0.425

The graphs were used to estimate the values of the K_m and V_{max} of ADH for ethanol. Table 3 shows a comparison of these values for the two plots drawn by hand and with a computer. The average K_m for both plots was 21.5mM while the average V_{max} was 0.426.

Table 3: The K_m and V_{max} Values Estimated from a Graph Sheet and a Computer

	Michaelis-Menten (Hand Drawn)	Michaelis-Menten (Computer Generated)	Lineweaver-Burk (Hand Drawn)
K_m (Mm)	22.00	21.70	22.20
V_{max} (min^{-1})	0.42	0.42	0.41

Results of yeast ADH assay for different substrates are presented as bar chart below, yeast ADH showed the highest activity against ethanol, 2-propen-1-ol and 2-buten-1-ol while reactivity with propanol decreased substantially.

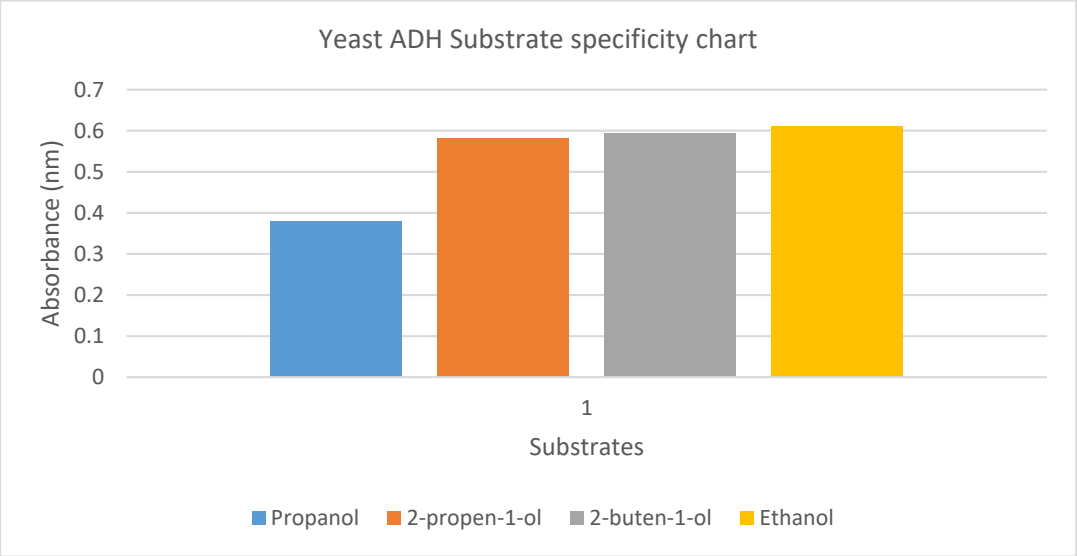

Figure 1 Showing yeast ADH reactivity with different substrates

4.0 Discussion

From the results in figure 1, ethanol, 2-buten-1-ol and 2-propen-1-ol presented the highest activity with yeast ADH, while activity of ADH declined with propanol. This result concur with the report that ADH specificity is restricted to primary alcohols having linear carbon chains, with ethanol as the best substrate, substrate specificity of alcohols to ADH decrease with increase in chain length (Dickinson & Monger, 1973). The ability of yeast ADH to oxidize unsaturated alcohol in this case 2-propen-1-ol was reported by Yang & Russell (1993).

Findings have suggested that a more likely candidate than ethanol for the physiological substrates of ADH in mammalian systems are unsaturated alcohols (Pietruszko *et al*., 1973).

The broad range of substrate specificity may be the result of configuration of the active site of ADH which exhibits geometric specificity allowing binding to different substrates that are geometrically isomeric. It may also be related to the ease with which different alcohol structures fit into active site of the enzyme. Biochemical studies of the mechanisms and substrate specificities of ADH can provide fundamental knowledge about catalysis and useful information for the commercial application, such as diagnostic reagents and production of alcohols (Jacobi *et al*., 2024).

Conclusion

Alcohol dehydrogenases (ADH) are oxidoreductases that catalyse the reversible oxidation of alcohols to aldehydes or ketones, with the concomitant reduction of coenzyme nicotinamide

adenine dinucleotide NAD^+ and $NADP^+$ to NADH and $NADPH_2$ (De Smidt *et al*., 2008). In this work, yeast alcohol dehydrogenase (ADH) was optimized and characterized for substrate specificity successfully. The value of the initial rate of enzyme reaction was found to be 0.299, and using the Beers Law, the values of V_{max} and K_m obtained from Michaelis-Menten were $3.2mMs^{-1}$ and 0.4mM, and from Lineweaver Burk double reciprocal curve are $0.33mMs^{-1}$ and 0.4mM respectively for ethanol. Data from many experiments showed ADH possess a broad spectrum of activity towards a wide range of substrates, however, in this report, ethanol, 2-buten-1-ol and 2-propen-1-ol presented the highest specificity to yeast ADH, while activity of ADH declined on propanol. The variation in substrate specificity may be due to configuration of the active site of ADH which exhibits geometric specificity allowing binding to different substrates that are geometrically isomeric.

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