Kinetics of ADH - Catalyzes Metabolism of Ethanol

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Introduction

Alcohol dehydrogenase (ADH) is a class of enzymes that are responsible for the catalysis of the oxidation of primary and secondary alcohols to their corresponding ketone or aldehyde. These enzymes work through the transfer of a hydride anion to NAD+ and release a proton. Individuals with decreased levels of ADH become intoxicated faster and remove ethanol slower when compared to individuals with higher levels of ADH. The main objectives of this experiment is to characterize ADH using Michaelis-Menten kinetics, characterization of an alternative substrate of ADH being methanol, and perform a pH profile of ADH.

Results

$[ethanol]_{stock}$ (M)	**Absorbance @ 635nm**	**[½ ethanol ½ methanol] (M)**	**Absorbance @ 635 nm**
1.2	0.216	1.2	0.204
6.0	0.187	6.0	0.154
3.0	0.113	3.0	0.116
1.5	0.110	1.5	0.080
0.75	0.070	0.75	0.044
0.30	0.023	0.30	0.015

Table 1. Absorbance of ADH. This table shows the absorbance of ADH with different levels of ethanol and a mixture of ethanol and methanol added.

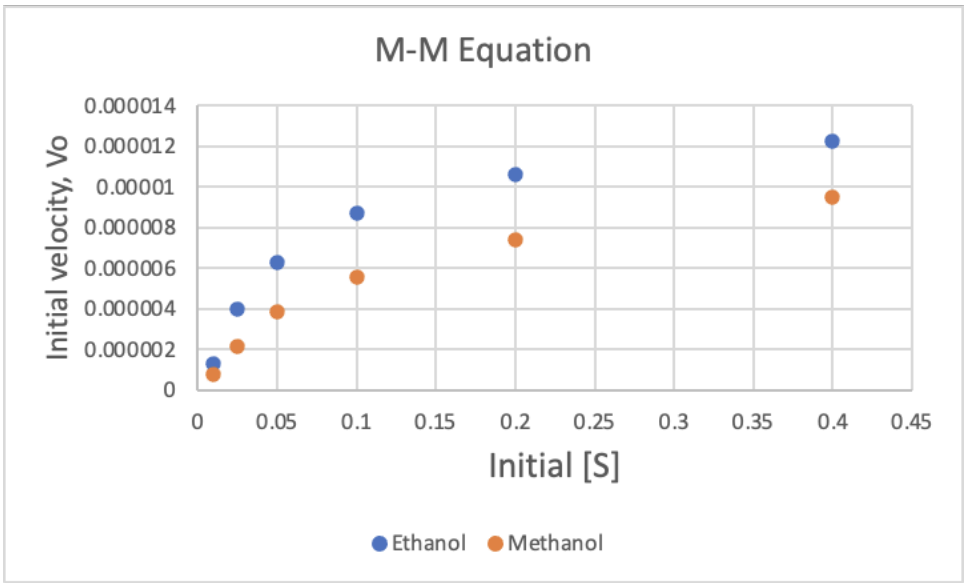

Figure 1. Michaelis-Menten Plot. This figure shows the M-M Plot of ADH with ethanol and with the ethanol/methanol mixture.

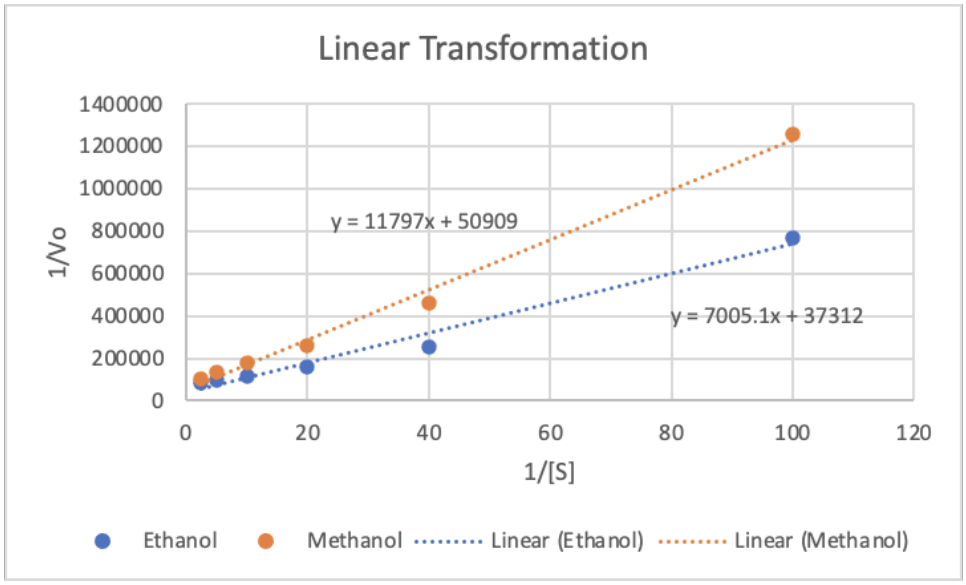

Figure 2. Lineweaver-Burk Plot. This figure shows the linear transformation of ADH with ethanol and with the ethanol/methanol mixture.

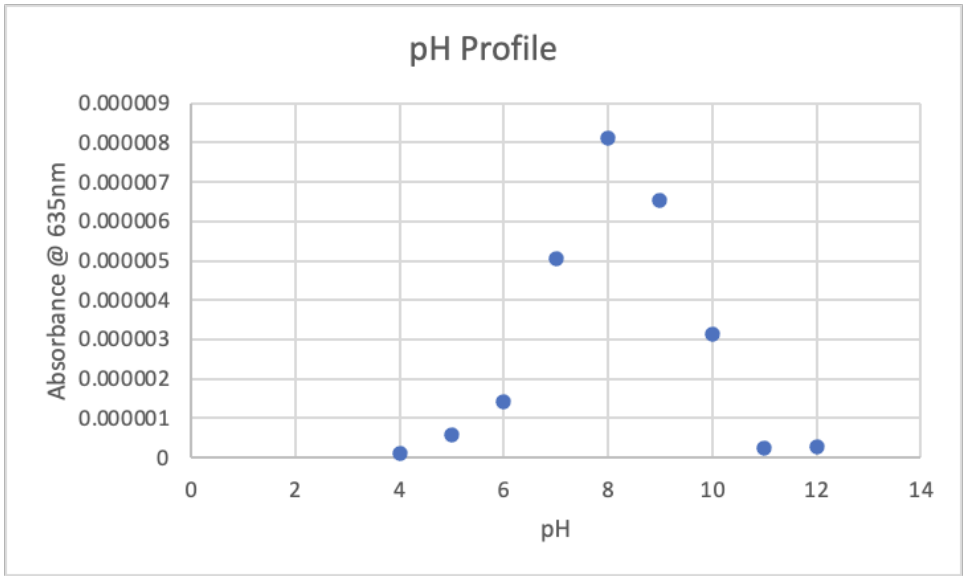

Figure 3. pH Profile of ADH. This figure shows the pH profile of alcohol dehydrogenase.

	Km	**Vmax**	**kcat**	**kcat/Km**
ADH w/ ethanol	0.4	0.0000268	0.00000564	0.00000301
ADH w/ ethanol & methanol mix	0.5	0.00001964	0.00001342	0.00005792

Table 2. ADH characteristics. This table shows the Km, Vmax, kcat, and kcat/Km values for ADH with ethanol and with the methanol/ethanol mix.

Discussion

The Km value of ADH with ethanol alone is lower than the Km value for ADH with ethanol and methanol mixture while the **kcat value of ADH with ethanol alone is lower than the kcat value of ADH with ethanol and methanol. Vmax is higher in ADH with ethanol compared to the Vmax of ADH with ethanol and methanol.** The BRENDA database states that the literature value of Km for ADH and ethanol is 0.022M, and 10.4M for ADH and methanol (BRENDA). The Km literature value is higher than the experimentally determined ethanol/methanol mixture because a mixture was used instead of using all methanol.

The ethanol substrate binds more tightly to ADH because the Km of the ethanol is less than the Km of the ethanol/methanol mixture **(Km 0.4 and 0.5, respectively).** This is seen in Figure 1 in the Michaelis-Menten Plot. **The Vmax of ethanol is higher than that of the methanol/ethanol mixture.** The Km is the amount of substrate required to reach half of the Vmax which correlates to the affinity to bind to the enzyme, being ADH. The lower the Km value, the tighter the binding will be. This is consistent with our data where we have the Km for ethanol and ADH being lower than the Km for methanol and ethanol. The methanol/ethanol mixture converts into products faster. This is seen in the linear conformation in Figure 2 as methanol has a higher rate (slope) than ethanol. The slope of the methanol/ethanol mixture is 11,797, while the slope of the ethanol alone is 7,005.1.

The activity of ADH starts to become affected at pH 7 and is the most affected at pH 8. This is seen in Figure 3 where the pH profile shows an increase in absorbance until it peaks at pH of 8 and decreases after that. This makes sense because ADH is a common enzyme in the human body where physiological pH is around 7. Changes in pH will ultimately change the shape of the active site. The results are consistent with the current understanding of the ADH mechanism considering the highest level of activity of the enzyme is near physiological pH.

References

"Text-Based Queries." *BRENDA*, https://www.brenda-enzymes.