BMOL2201/6201 Tutorial 3 Case Study

Inhibition of Alcohol Dehydrogenase

Focus concept

The inhibition of the alcohol dehydrogenase by a formamide compound is examined.

Prerequisites

- Principles of enzyme kinetics
- Identification of inhibition via Lineweaver-Burk plots

Background

Alcohol dehydrogenase (ADH) is the enzyme that is responsible for converting ethanol to acetaldehyde, with NAD^+ as cofactor. The reaction is shown in Figure 1. This enzyme is responsible for the metabolism of ethanol in the alcoholic beverages we consume. Five different isozymes of ADH have been identified, and it has been shown that the enzyme has a rather broad substrate specificity and can oxidize aldehydes as well as primary and secondary alcohols. For example, ADH can also oxidize methanol (wood alcohol) and ethylene glycol (antifreeze). The poisonous nature of these compounds results from the ADH-catalyzed conversion of these compounds to toxic products. For example, ADH converts methanol to formaldehyde, which is toxic to the optic nerve and can produce blindness. In high doses, formaldehyde may be fatal.

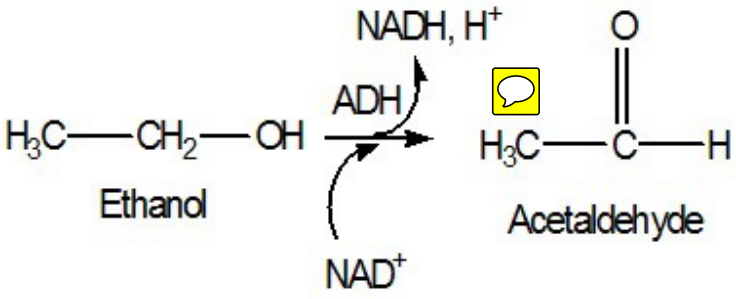

Figure 1: ADH-catalyzed oxidation of ethanol.

Q1. A treatment for methanol poisoning is to have the victim drink large amounts of ethanol. Why might this be an effective treatment?

Scientists investigated the ability of formamide compounds to inhibit alcohol dehydrogenase. Only a portion of their data is presented here. The researchers were able to propose a mechanism for the inhibition from the extensive data they collected using a wide variety of formamide compounds. The mechanism is shown in Figure 2.

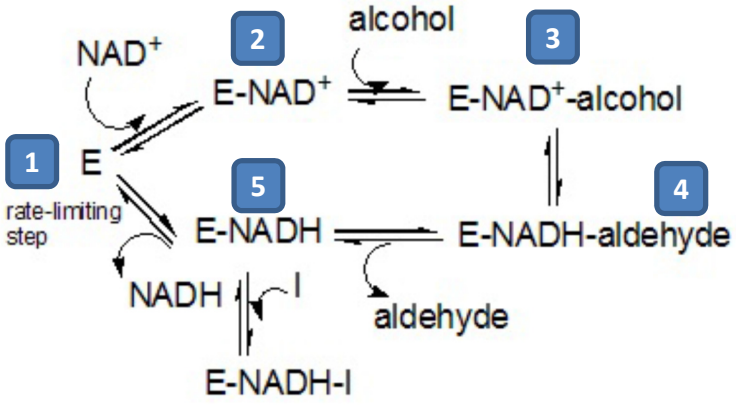

Figure 2: Mechanism of ADH1. The inhibitor, formamide, binds as an aldehyde analogue.

The scientists studied the ability of *N*-1,5-dimethylhexylformamide to inhibit mouse ADH1. The activity of the enzyme was measured in the absence of inhibitor, and in the presence of 1.0 μM inhibitor. The Lineweaver-Burk plot is shown below (Figure 3), with the blue line for the reaction rate without the inhibitor and the yellow line for the enzyme rate with the inhibitor:

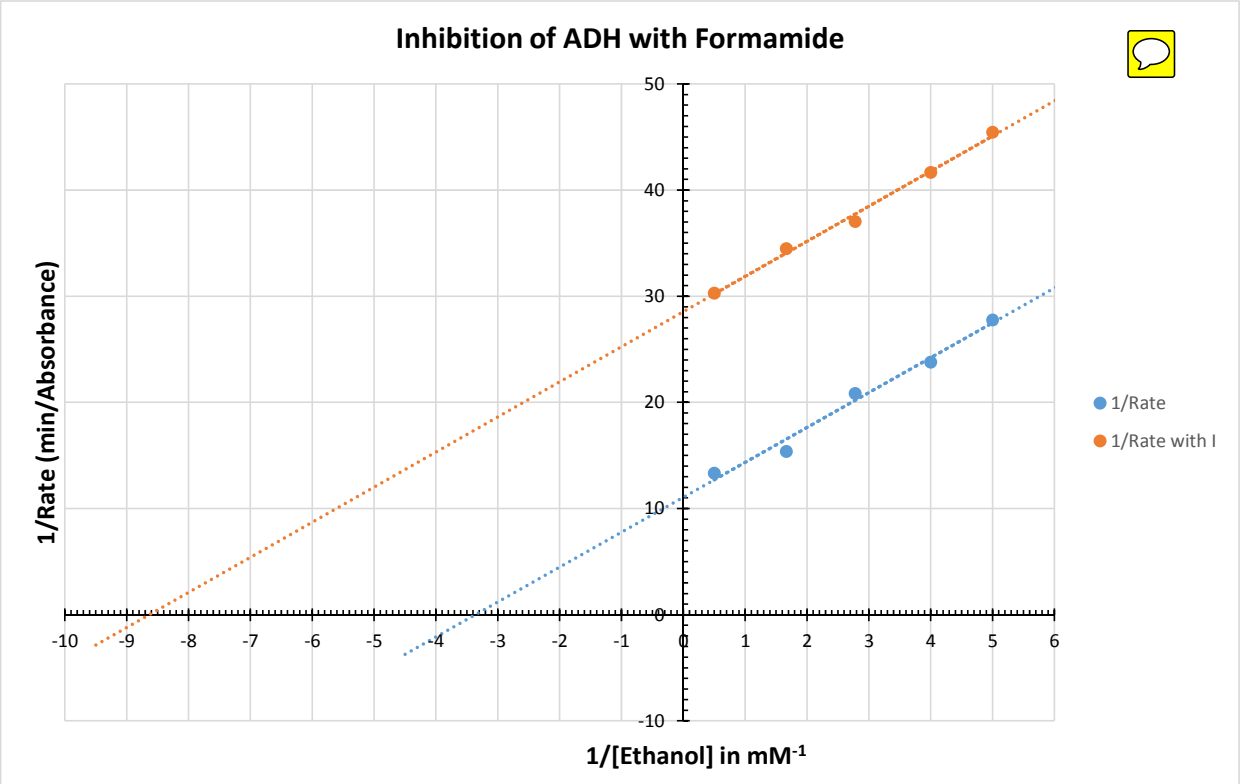

Figure 3: Kinetics of ADH1 with and without formamide inhibitor tested.

Q2. What are the K_M and V_{max} values for ADH in the absence of inhibitor and in the presence of the inhibitor?

Q3. What type of inhibitor is *N*-1,5-dimethylhexylformamide? Explain.
(Hint: use the mechanism proposed and the kinetic data to support your answer).

Q4. The scientists found that a class of compounds called pyrazoles were also inhibitors of ADH. These inhibitors bind to the E-NAD^+ complex, preventing the alcohol from binding. What kind of inhibitor are pyrazoles? Are these inhibitors the same or different to the formamides?

Reference

Venkataramaiah, T. H., and Plapp, B. V. (2003) *J. Biol. Chem*. **278**, pp. 36699-36706.