

## Case 39

# Inhibition of Alcohol Dehydrogenase

---

*Last modified 18 October 2004*

### Focus concept

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

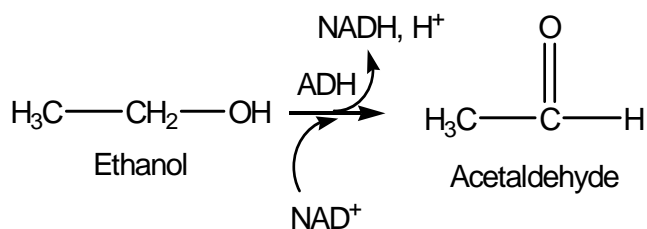
### Prerequisites

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

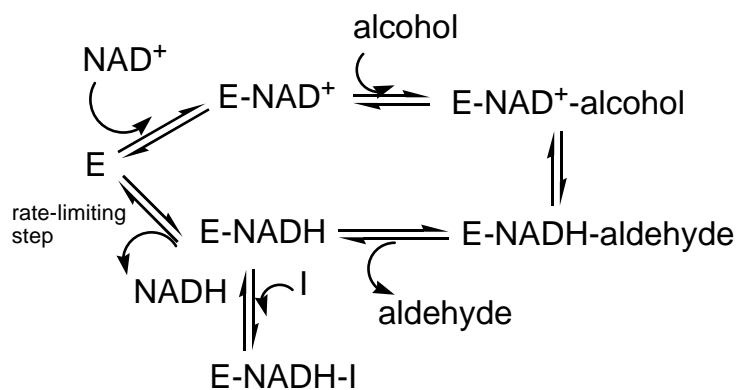
### Background

Alcohol dehydrogenase (ADH) is the enzyme that is responsible for converting ethanol to acetaldehyde (the reaction is shown in Figure 39.1). It is the enzyme 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 alcohol (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.

In this study, the authors investigated the ability of formamide compounds to inhibit alcohol dehydrogenase. Only a portion of their data is presented here. The authors 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 39.2.



**Figure 39.1:** ADH-catalyzed oxidation of ethanol.



**Figure 39.2:** Mechanism of ADH1. The inhibitor binds as an aldehyde analog.

## Questions

1. Certain individuals are more sensitive to alcohol than others. For example, women are more sensitive to alcohol than men—even when body weight and % body fat are taken into account, women become more intoxicated than men consuming an identical amount of alcohol. Using what we have learned in the enzyme chapters, give biochemical reasons that would explain why women become more intoxicated than men when consuming an equal amount of alcohol.
2. A treatment for methanol poisoning is to have the victim drink large amounts of ethanol. Why might this be an effective treatment?
3. The authors of this study 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  $\mu$ M inhibitor. The data are presented in Table 39.1.

**Table 39.1:** Inhibition of mouse ADH1 by *N*,1-5-dimethylhexylformamide

Ethanol Concentration, mM	ADH1 velocity, $\Delta$ NADH absorbance/min (without inhibitor)	ADH1 velocity, $\Delta$ NADH absorbance/min (with inhibitor)
0.20	0.036	0.022
0.25	0.042	0.024
0.36	0.048	0.027
0.60	0.065	0.029
2.00	0.075	0.033

- a. What are the  $K_M$  and  $V_{max}$  values for ADH in the absence of inhibitor? in the presence of the inhibitor?
  - b. What type of inhibitor is *N*-1,5-dimethylhexylformamide? Explain.
  - c. Calculate the values of  $\alpha$  and/or  $\alpha'$ , if they are significantly different from 1. What kind of inhibitor is *N*-1,5-dimethylhexylformamide? Explain.
  - d. Calculate the  $K_i$  and/or  $K_i'$  (whichever is appropriate) for *N*-1,5-dimethylhexylformamide (Hint: You can obtain these values from  $\alpha$  and  $\alpha'$ ).
4. The authors describe the mechanism of ADH as an “ordered bi-bi” mechanism. Give a written description of the mechanism, as shown in Figure 39.2. How does *N*-1,5-dimethylhexylformamide inhibit the activity of the ADH enzyme? How does *N*-1,5-dimethylhexylformamide differ from the “classic” inhibitors of this type that are described in our textbook?
  5. The authors found that a class of compounds called pyrazoles were also inhibitors of ADH. These inhibitors bind to the E-NAD<sup>+</sup> complex. What kind of inhibitor are pyrazoles? Are these inhibitors the same or different as the formamides?
  6.
    - a. Would *N*-1,5-dimethylhexylformamide be an effective alternative for the treatment of methanol and ethylene glycol poisoning, assuming that it is non-toxic itself (and as an alternative to getting the patient drunk, as described in Question 2)? Would *N*-1,5-dimethylhexylformamide be effective even if the concentrations of methanol or ethylene glycol were very high? (Hint: Compare the values of  $K_i$  or  $K_i'$ , whichever is appropriate, and  $K_M$ ).
    - b. The compound 4-methyl pyrazole is currently being used as a treatment for methanol poisoning. How would the effectiveness of 4-methyl pyrazole compare with the effectiveness of a formamide treatment?

## Reference

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