A Note on the Substrate Specificity of Horse Liver Alcohol Dehydrogenase

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During the course of a kinetic study of various aldehydes on the DPNH – ADH** compound with the aid of a fluorescence recorder [1], it was found that several hitherto unreported aldehydes and alcohols would function as substrates for horse liver ADH. The substrate specificity of ADH has been previously studied by several investigators [2-4]. Lutwak-Mann using impure ADH found that the relative order of reactivity was ethanol>propanol>amyl alcohol>methanol. Zatman with ADH prepared according to Lutwak-Mann found methanol to be oxidized at about one ninth of the rate for ethanol. Theorell and Bonnichsen using pure ADH found that the Michaelis constants of some higher aliphatic alcohols (allyl, *n*-propanol and *n*-butanol) were lower than for ethanol and stated that higher homologues of ethanol are substrates if they contain the group $-C-CH_2OH$. Bliss [5] has reported that Vitamin A alcohol is converted to retinene using an enzyme prepared according to Bonnichsen and Wassén [6], and Bonnichsen and Hubbard [7] confirmed this observation with pure ADH + DPNH + retinene. In the present study it was found that not only do a number of aliphatic aldehydes and alcohols show greater initial reaction velocities than the physiological substrates acetaldehyde and ethanol under the experimental conditions employed but also that benzyl alcohol, furfuryl alcohol and *cyclo*hexanol are reversibly oxidized by pure ADH.

Experimental part. Pure crystalline ADH was prepared by a modification procedure of Bonnichsen and Brink [8] as described by Dalziel [9]. All final determinations were made with the main component of the resolved enzyme as obtained by chromatography on carboxymethylcellulose. DPN and DPNH were commercial products from Sigma Chemical Company. Yeast ADH was a C. F. Boehringer Company product. All substrates were reagent grade chemicals further purified by distillation under suitable pressures. Rate determinations of the oxido-reduction of DPN(H) were made either in phosphate buffer, ionic strength 0.1, pH 6.95 or in glycine-NaOH buffer, 0.1 M, pH 9.50, at 23.5°, and initial velocities are expressed as moles per liter per min per mole of ADH ***. Details of the fluorescence characteristics of DPNH have been previously described [11,12].

Discussion. ADH appears to have a very broad specificity toward aliphatic carbinol compounds and acts as well on some aromatic and cyclic alcohols and aldehydes (see Table 1). As previously reported by Theorell and Bonnichsen [4], and confirmed in the present studies, pure ADH does not react with methanol + DPN to any observable extent, nor do the alcohols *tert*-butanol, *tert*-amyl or *iso*propyl. Methanol, furthermore, does not effect the activity toward ethanol or other alcohols. That Merritt [13], employing a rat liver acetone powder preparation, found *cyclo*hexanol to be reversibly oxidized to *cyclo*hexanone is probably due to the ADH present in the preparation. The K_m for *cyclo*hexanol was determined to be 1.3×10^{-3} M and for furfuryl alcohol 1.4×10^{-4} M (K_m for ethanol 5.3×10^{-4} M). Furfural was found to have a K_m of 7.7×10^{-6} M and *cyclo*hexanone of 1.9×10^{-3} M (K_m for acetaldehyde 2.1×10^{-4} M) ****. It is easily possible in the case of some alcohols and aldehydes listed in Table 1 to determine quantitative amounts of these substrates in concentrations of 10^{-7} M.

The reactivity of these substrates on yeast ADH are, however, quite different. *Cyclo*hexanol and *cyclo*hexanone will not react with the yeast enzyme and furfural acts at about one sixth of the rate for acetaldehyde while furfuryl alcohol reacts at about one-two hundredth of the rate for ethanol.

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** Abbreviations. DPNH: reduced, and DPN: oxidized diphosphopyridine nucleotide; ADH: 1/2 molecule of liver alcohol dehydrogenase.

*** The molecular weight was taken as 84 000 and the absorbancy index at 280 mμ as 0.42×10^5 cm per mole per liter [10].

**** See Table 1 for the coenzyme concentrations used to determine these constants.

Table 1. The influence of chain length and substitution on the ability of alcohols and aldehydes to serve as substrates for liver ADH.

Alcohol *	initial velocity (M/L/min/mole ADH)	Aldehyde **	initial velocity (M/L/min/mole ADH)
n-Butanol	215	*n*-Butyraldehyde	510
Allyl	192	Cinnamaldehyde	350
2-Phenylethanol	184	Furfural	236
n-Hexanol	170	*Iso*valeraldehyde	208
*Iso*amyl	167	Benzaldehyde	55
Amyl	160	Acetaldehyde	30
n-Propanol	146	Formaldehyde	7
Ethanol	135	*Cyclo*hexanone	5
*Cyclo*hexanol	135	DL-Glyceraldehyde	2
n-Octanol	135	Glyoxal	0
Benzyl	118	Methylethylketone	0
Methyl*cyclo*hexanol	108	Acetone	0
Furfuryl	108		
3-Phenyl-1-propanol	46		
3-Hexanol	35		
Methanol	0		
tert-Butanol	0		
tert-Amyl	0		
*Iso*propyl	0		

* The alcohols were tested at a concentration of 1.0×10^{-3} M; DPN, 1.20×10^{-4} M; ADH, 1.44×10^{-8} M. Glycine-NaOH buffer, 0.1 M, pH 9.50.

** The aldehydes (or ketones) were tested at a concentration of 5.0×10^{-5} M; DPNH, 3.21×10^{-6} M; ADH, 4.84×10^{-9} M. Phosphate buffer, ionic strength 0.1, pH 6.95.

The complete data will be given in a forthcoming paper in this journal.

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1. Theorell, H. and Nygaard, A. P. *Acta Chem. Scand.* **8** (1954) 877.
2. Lutwak-Mann, C. *Biochem. J.* **32** (1938) 1364.
3. Zatman, L. J. *Biochem. J.* **40** (1946) LXVII.
4. Theorell, H. and Bonnichsen, R. *Acta Chem. Scand.* **5** (1951) 1105.
5. Bliss, A. *Biol. Bull.* **97** (1949) 221.
6. Bonnichsen, R. and Wassén, A. M. *Arch. Biochem.* **18** (1948) 361.
7. *Personal communication.*
8. Bonnichsen, R. and Brink, N. G. in Colowick, S. P. and Kaplan, N. O. *Methods in Enzymology*, Vol. I, p. 495, Academic Press, New York 1955.
9. Dalziel, K. *Acta Chem. Scand.* **12** (1958) 275.
10. Ehrenberg, A. and Dalziel, K. *Acta Chem. Scand.* **12** (1958) 465.
11. Theorell, H., Nygaard, A. P. and Bonnichsen, R. *Acta Chem. Scand.* **9** (1955) 1148.
12. Winer, A. D. and Schwert, G. W. *Biochim. et Biophys. Acta* **29** (1958) 424.
13. Merritt, A. D. *Federation Proc.* **17** (1958) 275.

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