Inhibition of Liver Alcohol Dehydrogenase by Amides

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Winer and Theorell [1] have reported that fatty acid amides form complexes of the type EI ** and EIR with horse liver alcohol dehydrogenase. The saturated straight chain hydrocarbon amides from C_1 to C_6, as well as the branched chain amides isobutyramide and isovaleramide, were found to form complexes. Isobutyramide was tested for inhibition and was found to be a competitive inhibitor for acetaldehyde. The present communication reports the results of kinectic experiments using a group of amides with widely different steric and polar properties as inhibitors for acetaldehyde.

Experimental. Alcohol dehydrogenase from horse liver was prepared essentially according to the method of Bonnichsen and Brink [2], and was recrystallized 3 times. β-DPNH was purchased from Sigma. It was found to be 90 % pure by weight when based on total absorbancy at 340 mμ, and 85 % pure by weight when based on enzymatic oxidation at pH 7 in the presence of a large excess of substrate. Reagent grade salts were used for the buffer solutions. The acetaldehyde and water were redistilled from a glass still.

Some amides were purchased from commercial sources and were recrystallized 2—3 times. The following reports the amide, melting point in °C, and solvent used for recrystallization: acetamide, 81—82, ethylacetate; propionamide, 81—82, ethylacetate; isobutyramide, 129—130, $CHCl_3$; acrylamide, 85.7—86, ether; α-methylacrylamide, 110—112, ether; thioacetamide, 114—114.5, benzene-ether; fluoroacetamide, 108—109, $CHCl_3$; chloroacetamide, 121, water; trichloroacetamide, 142—143, ether-petroleum ether; iodoacetamide, 94—95.7, water.

The other amides were synthesized by adding dry NH_3 gas to an ice-cold solution of the appropriate intermediate in anhydrous ether [3]. The starting materials were commercial products. The following gives the starting material, the synthesized amide, the melting point of the amide in °C, and the solvent used for recrystallization: difluroacetic acid, difluoroacetamide [4], 51—52, $CHCl_3$; trifluoroacetic acid, trifluoroacetamide [3], 75, ether; trimethylacetic acid, trimethylacetamide [5], 160—160.5, water; tiglic acid, tiglic amide [6], 69—71, benzene; dichloroacetyl chloride, dichloroacetamide, 100, $CHCl_3$; bromoacetyl bromide, bromoacetamide, 91, $CHCl_3$; ethyldibromoacetate, dibromoacetamide, 159, ethylacetate; α,α-diethylacetyl chloride, α,α-diethylacetamide, 113.5—114, water; and *trans*-crotonyl chloride, *trans*-crotonamide, 159.5—160, $CHCl_3$.

The reactions were performed in duplicate at 23.0±0.1° in 0.11 μ sodium phosphate buffer, pH 7.15±0.05. Four to five different aldehyde concentrations were used. The reaction was followed by measuring the rate of change of fluorescence when DPNH was oxidized [7]. The enzyme concentration in the stock solution was determined by a modification of the method of Dalziel [8], in which the enzyme concentration obtained by Dalziel's method is multiplied by 0.83 as described by Theorell [9]. The enzyme concentrations in this article refer to the normality of the enzyme, a 1 M solution being 2 N with respect to the number of active sites on the enzyme molecule at pH 7 [10]. The DPNH was determined by its absorbancy at 340 mμ using an absorption coefficient of 6.22×10^6 cm^2/mole [11], and the aldehyde concentrations were determined enzymatically.

Results and dicussion. The results of these experiments, presented in Table 1, show that in almost all cases mixed inhibition occurs. The competitive inhibitor constants were determined, as decribed by Theorell *et al.*[12,13] for similar cases, from the slopes of the Lineweaver-Burk plots according to eqn. 1:

$$K_I = [I] / \frac{\text{Slope with I}}{\text{Slope without I}} - 1 \qquad (1)$$

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** Abbreviations used: E = Free enzyme; I = Inhibitor; R = DPNH = Reduced diphosphopyridine nucleotide; O = DPN^+ = Oxidized diphosphopyridine nucleotide; EI, EIO, etc. = Enzyme-Inhibitor complex, Enzyme-Inhibitor-DPN^+ complex, etc.;

$K_{E,I}$, $K_{ER,I}$, etc. = [E][I]/[EI], [ER][I]/[EIR], etc.;

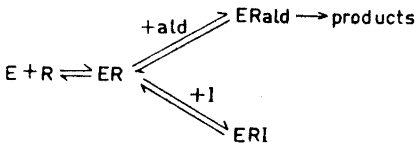
e = total concentration of enzyme including that combined in complexes.

Table 1. Summary of Experiments.

Expt.	[β-DPNH] μM	Amide	[Amide] mM	K_m Aldehyde mM	Observed $V_{max}/[e]$ sec^{-1}	Competitive K_I mM
I	10.4	None	0.00	0.110	41.5	—
		Acetamide	12.35		37.7	10.5
		Propionamide	12.35		16.7	0.439
		Isobutyramide	12.35		19.3	0.169
		Trimethylacetamide	12.35		20.8	2.16
II	10.1	None	0.0	0.114	40.3	—
		Chloroacetamide	12.5		28.1	6.29
		Dichloroacetamide	12.5		18.3	4.69
		Dichloroacetamide	25.0		16.6	4.78
		Trichloroacetamide	10.0		32.4	12.8
III	9.4	None	0.0	0.105	37.0	—
		Acrylamide	12.5		37.3	5.32
		α-Methylacrylamide	12.5		23.4	0.814
		α,α-Diethylacetamide	12.5		16.3	0.290
IV	9.1	None	0.0	0.105	39.4	—
		Bromoacetamide	12.5		19.2	3.86
		Iodoacetamide	12.5		9.48	1.83
V	9.4	None	0.0	0.103	36.8	—
		Tiglic amide	12.5		19.4	0.501
		Dibromoacetamide	12.5		13.2	2.72
VI	9.3	None	0.0	0.106	38.2	—
		Crotonamide	12.5		29.2	1.01
		Fluoroacetamide	62.5		27.4	31.4
		Difluoroacetamide	62.5		30.1	59.2
		Trifluoroacetamide	62.5		27.3	39.6
		Thioacetamide	125		23.8	230

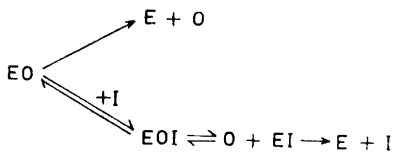
The observed values for V_{max}/e were obtained from the e/v intercepts of the Lineweaver-Burk plots, and are reported without extrapolation to infinite DPNH concentration. The competitive K_I values obtained in this way are independent of the inhibitor concentration, as can be seen in the case of dichloracetamide. The maximum velocity, on the other hand, depends on the inhibitor concentration. These results can be explained on the following basis:

The increases in the slopes of the Lineweaver-Burk plots represent competition with aldehyde in the first phase of the Theorell-Chance [14] mechanism:



This scheme is supported by the fact that the K_I values obtained under these conditions are found to be equal, within the limits of experimental error, to $K_{ER,I}$ [15].

The changes in the intercepts of the Lineweaver-Burk plots can be explained by assuming the formation of an EOI complex in the last phase of the reaction.



In the absence of I, the breakdown of EO is rate-limiting [14]. In the presence of I, part of the EO breaks down to E + O, and part of it forms EOI. If the breakdown of EOI in the forward direction is slower than the breakdown of EO, the observed maximum velocity will be reduced. A similar case involving an EO-imidazole complex, which breaks down at a faster rate, has been studied by Theorell [12].

The formation of an EOI complex containing an amide was not observed with the low amide concentrations employed by Winer and Theorell [1], although Theorell [12] later suggested that such a complex might form in the presence of a high concentration of amide. The data presented here indicate that EO-amide complexes do form, and that $K_{EO,I}$ is much larger than either $K_{EI,R}$ or $K_{ER,I}$ for most of the amides studied so far. In fact, $K_{EO,I}$ would appear to be of the same order of magnitude as $K_{E,I}$, thereby indicating that a mutual stabilization of I and O in the complex does not occur to any great extent. A more detailed report will be submitted for publication at a later date.

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Notiz über die Addition von Thioharnstoff an Citraconsäure

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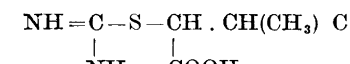
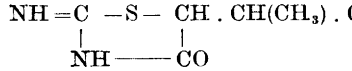
Nach Andreasch [1] wird Thioharnstoff in der Mercaptoform an Citraconsäure unter Bildung eines Zwischenproduktes Ia addiert, das unter Wasserabspaltung zu Ib cyclisiert wird:

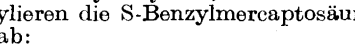


(Ia)



(Ib)

Die Verbindung Ib wurde zu der Mercaptosäure Ic hydrolysiert, die dann durch Benzylieren die S-Benzylmercaptosäure Id ergab:



(Ic)