DIFFERENTIAL EFFECTS ON THE ALCOHOL DEHYDROGENASE AND ISOMERASE ACTIVITIES OF LIVER ALCOHOL DEHYDROGENASE*

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If an enzyme inhibitor is sterically unrelated to the substrate of the reaction and is thought to react at some locus removed from the active site of the enzyme, the proposed site at which the inhibitor acts has been designated the allosteric site and the inhibition has been referred to as an allosteric inhibition (Monod and Jacob, 1961). These inhibitions may be of significance in regulating enzymic activities. In one type of allosteric inhibition, the initial enzymic step of a metabolic sequence is inhibited by the final product of the sequence, e.g. the inhibition of threonine deaminase by isoleucine (Umbarger, 1956) and of aspartic transcarbamylase by cytidine triphosphate (Yates and Pardee, 1956). Another type of allosteric inhibition is illustrated by the action of diethylstilbesterol on glutamic dehydrogenase. Glutamic dehydrogenase has two enzymic activities, the dehydrogenation of glutamic acid and of alanine; the former activity is inhibited by diethylstilbesterol while the latter is stimulated by the same substance (Tomkins and Yielding, 1961).

Liver alcohol dehydrogenase (LADH) is a protein that catalyzes the oxidation of alcohols to aldehydes as well as the isomerization of several aldehydes to ketones (van Eys, 1961). It has not been ascertained whether these two enzymic activities of LADH occur at the same enzymic locus, or whether any relationship exists between the two activities. This report presents evidence

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that the alcohol dehydrogenase and isomerase activities are affected differentially by selected enzyme inhibitors; this evidence suggests that two types of sites may exist on the enzyme surface. Folic acid and aminopterin, which completely inhibited the alcohol dehydrogenase activity of LADH, stimulated the isomerase activity of the enzyme 400 per cent. 1,10-Phenanthroline neither stimulated nor inhibited the isomerase activity of LADH, but inhibited the alcohol dehydrogenase activity of LADH. Differential action on the two enzymic activities was also obtained with the sulfhydryl inhibitors, iodoacetate and p-chloromercuribenzoate (PCMB). Iodoacetate inhibited the isomerase activity of LADH; the alcohol dehydrogenase activity of LADH was unaffected unless the enzyme was preincubated with the inhibitor. The reverse situation was found with PCMB; while isomerase activity was only slightly inhibited by the mercurial compound, alcohol dehydrogenase activity was completely lost.

Isomerase activity of LADH was determined according to the method of van Eys (1961) using 3-phosphoglyceraldehyde as the substrate. For the determination of alcohol dehydrogenase activity, the same concentration of enzyme was used as in the isomerase assay; the concentrations of ethanol and NAD were 8×10^{-3}M and 2×10^{-4}M, respectively. The assay of alcohol dehydrogenase was based on the increase in optical density at 340 mμ due to the accumulation of NADH. LADH was a crystalline preparation obtained from Worthington Biochemical Corporation, N. J.

A comparison of several inhibitors acting on the alcohol dehydrogenase and isomerase activities of LADH is presented in Table 1. Folic acid and aminopterin stimulated isomerase as much as 400 per cent but completely inhibited the alcohol dehydrogenase activity of the same protein. As the concentration of folic acid was increased tenfold, there was a corresponding increase in isomerase activity. Complete inhibition of alcohol dehydrogenase occurred at 2×10^{-4}M folic acid (Snyder et al., 1963). Like folic acid, aminopterin inhibited alcohol dehydrogenase activity but stimulated isomerase. The differentia effects on the two activities of LADH are similar to those of diethylstilbesterol on glutamic dehydrogenase as reported by Tompkins and Yielding (1961).

Table 1

The effects of several compounds on the alcohol dehydrogenase and isomerase activities of LADH

Addition	Concentration (M)	Alcohol dehydrogenase activity (v_i/v_c)*	Isomerase activity (umoles dihydroxyacetone phosphate formed in 15 minutes
None		1.00	2.5
Folic Acid	4×10^{-4}	0	3.6
	1×10^{-3}	0	6.1
	3×10^{-3}	0	7.2
	5×10^{-3}	0	9.9
Aminopterin	1×10^{-3}	0	4.5
	3×10^{-3}	0	6.0
	5×10^{-3}	0	8.7
1,10-Phenanthroline	1×10^{-3}	0.34	2.5
	5×10^{-3}	0.07	2.3
Iodoacetate	1×10^{-2}	1.00	2.5
	1×10^{-1}	1.00	0.2
p-Chloromercuribenzoate	6×10^{-6}	1.00	2.0
	6×10^{-5}	0.28	1.8
	6×10^{-4}	0	1.7

*v_i/v_c represents partial specific activity calculated by dividing velocity of inhibited (v_i) reaction by that of control (v_c).

In agreement with the results of Vallee and Hoch (1957), the classical metal chelator, 1,10-phenanthroline, inhibited alcohol dehydrogenase activity; isomerase activity, however, was neither stimulated nor inhibited by 1,10-phenanthroline. Despite the complete inhibition of alcohol dehydrogenase activity at 5×10^{-3}M 1,10-phenanthroline isomerase activity equalled the control value. Vallee *et al.* (1957, 1958) and Plane and Theorell (1961) have presented evidence suggesting that 1,10-phenanthroline inhibits alcohol dehydrogenase by chelating zinc at the active site. Assuming reaction of 1,10-phenanthroline with zinc, it is doubtful that the same zinc ions are involved in the isomerase reaction or that the two enzymic activities are situated at the same site of the protein.

Other differential effects were obtained with the sulfhydryl inhibitors PCMB and iodoacetate. At 6 X 10^{-6}M PCMB, alcohol dehydrogenase activity was unaltered while isomerase was 80 per cent of the control value. At 6 X 10^{-4}M PCMB, however, LADH activity was completely inhibited while there was little additional effect on isomerase. The action of PCMB on alcohol dehydrogenase has been studied by Li et al. (1962), and the present results agree with those findings.

Iodoacetate inactivated alcohol dehydrogenase when it was preincubated with the enzyme but caused no immediate inhibition at concentrations as high as 0.01 M (Li and Vallee, 1963). In agreement, no inhibition of alcohol dehydrogenase activity was noted at concentrations as high as 0.1 M. Isomerase was almost completely inactivated at 0.1 M iodoacetate (Table 1). At 0.01 M iodoacetate, however, neither activity of the enzyme was altered.

Although specific metabolic pathways may be controlled by the allosteric enzymes threonine deaminase and aspartic transcarbamylase, it is not clear how the alterations of two enzymic activities of glutamic dehydrogenase may be related to a control mechanism. It is equally difficult at present to attach biological significance to the alterations of the two activities of alcohol dehydrogenase. The evidence presented in the report demonstrates that the two activities of LADH can be selectively altered. The inhibition of one enzymic activity by folic acid and the stimulation of the other activity by the same compound is reminiscent of the action of diethylstilbesterol on glutamic dehydrogenase.

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