PRELIMINARY NOTES

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Equilibrium kinetic study of enzyme modifier action

The measurement of reaction rates at equilibrium by means of isotopic exchange of reactants has proven to be a powerful tool for determination of aspects of enzyme mechanism which are not readily discernible by other means These include the determination of the presence and sequence of compulsory binding orders, the determination of fast and slow dissociation steps and the indication of whether chemical transformation is rate-limiting[1-5] One of the most interesting aspects of the study of equilibrium reaction rates of enzyme systems is the ability to follow simultaneously non-rate-limiting as well as rate-limiting steps in catalysis[2] Study of the action of a modifier of enzyme activity on the several isotopic exchange rates at equilibrium in a multi-substrate system thus offers an opportunity to study details of the mechanism of modifier action on enzymic catalysis which are not readily obtainable by following the initial rate of reaction to equilibrium

Heretofore equilibrium kinetic studies have been limited to the effect of substrate concentration on rate These studies have been extended in this laboratory to explore the effect of small molecular enzyme modifiers on enzyme reaction rates at equilibrium in order to obtain broader insight into the mechanism of action of modifiers of enzymic catalysis[6,7] The usefulness of this approach was investigated with the kinetically well studied enzyme horse-liver alcohol dehydrogenase[8] Initial

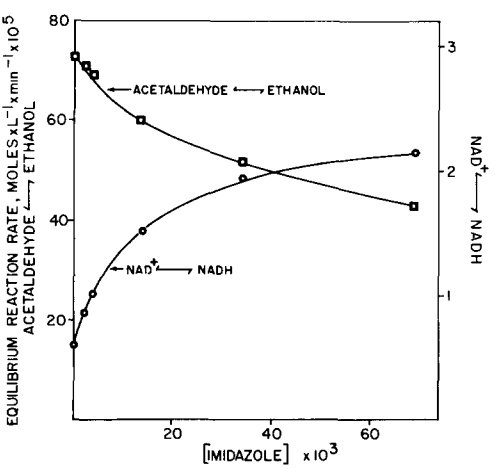

Fig 1 Effect of imidazole on reaction rates of horse-liver alcohol dehydrogenase at equilibrium Reaction mixture contained 2 79 mM NAD^+, 405 μM NADH, 303 mM ethanol, 1 93 mM acetaldehyde and 16 2 μg/ml of horse-liver alcohol dehydrogenase in 40 mM veronal buffer (pH 7 9), 25 $NAD^+ \rightleftarrows$ NADH was measured with [^{14}C]NADH (ref 9) and acetaldehyde $\rightleftarrows$ ethanol (ref 5) with [1,2-$^{14}C_2$]-acetaldehyde (Volk Radiochemical Corp)

observations of the effects of imidazole on horse-liver alcohol dehydrogenase are reported here

Isotopic exchange measurements at equilibrium with horse-liver alcohol dehydrogenase (Worthington Biochemical Corp., recrystallized) was performed as described previously[5,9] Results obtained with imidazole–HCl or imidazole–HNO_3 (pH 7.9) were similar Determinations were done in duplicate

In the absence of imidazole the acetaldehyde $\rightleftarrows$ ethanol rate was more than 100 times faster than the $NAD^+ \rightleftarrows$ NADH rate[5] (Fig. 1), indicating overall rate limitation of coenzyme dissociation, which is greatly exceeded by the rates of chemical transformation and substrate dissociation With increasing concentrations of imidazole a 4–6-fold acceleration in the $NAD^+ \rightleftarrows$ NADH rate was observed Half maximal acceleration occurred at about 10 mM imidazole This increase in the $NAD^+ \rightleftarrows$ NADH velocity is in agreement with the finding by THEORELL *et al.*[10,11] that imidazole stimulates the velocity of the initial net reaction at elevated levels of substrate This has been explained as due to a 5–10-fold acceleration of rate-limiting dissociation of coenzyme from enzyme–coenzyme complex by the formation of the ternary enzyme–coenzyme–imidazole complex from which coenzyme dissociation is faster[10,11]

An hitherto unobserved phenomenon is the simultaneous inhibition in the acetaldehyde $\rightleftarrows$ ethanol rate, which is not limiting and therefore not observed in net reaction studies Thus, while the reaction appears to be simply accelerated when investigated by initial rate kinetics, study of kinetics at equilibrium indicates that, while the slowest step is indeed accelerated, another step is simultaneously inhibited

The fall in the acetaldehyde $\rightleftarrows$ ethanol rate may be due to decrease in a rate-limiting substrate dissociation step (1) or to decrease in the rate of chemical transformation, either as a result of lowered active site saturation (increased non-rate-limiting substrate dissociation rate (1) or replacement by inhibitor (2)) or to diminished intrinsic enzyme catalytic rate by binding at an effector site (3)

$$\begin{array}{ccc} \text{Enzyme–NAD}^+\text{–ethanol} & \rightleftarrows & \text{enzyme–NAD}^+ + \text{ethanol} \\ | & & | \\ \text{imidazole} & & \text{imidazole} \end{array} \qquad (1)$$

$$\text{Enzyme–NAD}^+\text{–ethanol} + \text{imidazole} \rightleftarrows \text{enzyme–NAD}^+\text{–imidazole} + \text{ethanol} \qquad (2)$$

$$\begin{array}{ccc} \text{Enzyme–NAD}^+\text{–ethanol} & \rightleftarrows & \text{enzyme–NADH–acetaldehyde} \\ | & & | \\ \text{imidazole} & & \text{imidazole} \end{array} \qquad (3)$$

The imidazole effect on the acetaldehyde $\rightleftarrows$ ethanol rate is competitive with substrate[7,12] which rules out suppression of substrate dissociation or intrinsic catalytic rate by imidazole binding non-competitively at a non-active site as the mechanism for the decrease in the acetaldehyde $\rightleftharpoons$ ethanol rate Experiments are in progress to attempt to elucidate the mechanism further

These studies suggest that investigation of enzyme reaction rate at equilibrium is particularly useful for observation of non-rate-limiting steps in enzymic action and may yield new insight into the mechanism of alteration of enzymic catalysis by modifiers Such investigation of glutamate dehydrogenase[13,14] and other allosteric enzymes is in progress

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