A NOTE ON THE MECHANISM OF ACTION OF RABBIT MUSCLE LACTATE DEHYDROGENASE*

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In the course of a series of experiments on the effect of deuterium on rabbit muscle lactate dehydrogenase, we have obtained some data that have a bearing on the reaction mechanism of this enzyme. When the transferable hydrogen of DPNH is replaced by deuterium to form α-DPND, appreciable changes are produced in all four constants of the rate equation (Dalziel, 1957):

$$\frac{E_o}{v} = \phi_0 + \frac{\phi_1}{A} + \frac{\phi_2}{B} + \frac{\phi_{12}}{AB}$$

where v is the initial velocity, E_o, A, and B are the initial concentrations of the enzyme, coenzyme, and substrate, respectively, and the ϕ's represent various combinations of rate constants, the significances of which depend on the reaction mechanism. The effects observed with α-DPND are shown in Table 1; particular attention is called to the ratio ϕ_{0_H}/ϕ_{0_D}.

Zewe and Fromm (1962) on the basis of product-inhibition studies have suggested that rabbit muscle lactate dehydrogenase obeys the Theorell-Chance mechanism, an ordered binding of coenzyme and substrate without

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formation of kinetically significant ternary complexes:

$$E + A \underset{k_2}{\overset{k_1}{\rightleftharpoons}} EA$$

$$EA + B \underset{k_4}{\overset{k_3}{\rightleftharpoons}} EC + D$$

$$EC \underset{k_6}{\overset{k_5}{\rightleftharpoons}} E + C$$

where C and D represent the coenzyme and substrate products.

For the Theorell-Chance mechanism, ϕ_0 is equal to $1/k_5$. Baker and Mahler (1962) have pointed out that the ratio of ϕ_0 (DPNH)/ϕ_0 (α-DPND) would be unity; i.e., there would be no isotope effect, since the hydrogen (or deuterium) would have been previously transferred at step 2 of the reaction sequence. Since the value observed is significantly different from unity, it would appear that an important requirement for the mechanism proposed by Zewe and Fromm is not satisfied. A mechanism involving one or more ternary complexes, as postulated by Takenaka and Schwert (1957) for beef heart lactate

TABLE 1

Effect of Replacement of DPNH by α-DPND on Rate Constants for Rabbit Muscle Lactate Dehydrogenase

Constant	Ratio, ϕ_H/ϕ_D, ± S.D.
ϕ_0	0.71 ± 0.11
ϕ_1	2.50 ± 0.29
ϕ_2	0.58 ± 0.09
ϕ_{12}	0.33 ± 0.06

These data are based on results of experiments involving 181 combinations of DPNH and pyruvate concentrations, and 86 of α-DPND and pyruvate. Experimental methods are described elsewhere (Thomson, Bray, and Bummert, 1962).

dehydrogenase, or one of the modifications of the Theorell-Chance mechanism discussed by Mahler et al. (1962), would be more likely.

Although the ϕ_0 values were calculated by the method of Dalziel (1957) and thus represent an extrapolation from a series of values which themselves are extrapolations, the ratio can also be estimated by assaying the enzyme in the presence of near-saturation levels of coenzyme and substrate. Under these conditions, $v_{DPND}/v_{DPNH} \sim \phi_0$ (DPND)/ϕ_0 (DPNH), and the ratio observed by this direct comparison was 0.73.

Zewe and Fromm (1962) suggested that secondary reactions occurred, with the formation of inactive ternary complexes:

$$EC + B \underset{k_8}{\overset{k_7}{\rightleftharpoons}} ECB$$

$$EA + D \underset{k_{10}}{\overset{k_9}{\rightleftharpoons}} EAD$$

It may be pointed out that conventional steady-state treatment of the Zewe-Fromm scheme with C present does not yield the equation (number 8) that they presented, but rather one in which $1/k_5$ is multiplied by $(1+k_7B/k_8)$. Their equation can be derived by treating C not as a product, but as an inhibitor which forms a complex EC that is different from the EC produced from EA + B.

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