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The Theorell-Chance Mechanism

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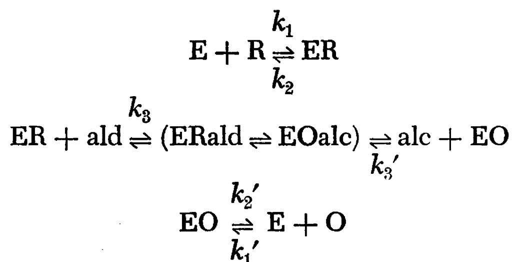
The Theorell-Chance compulsory order mechanism applies to liver alcohol dehydrogenase. This appears dependent on the coenzyme first attaching and inducing an appropriate conformational change in the enzyme before the substrate can attach.

The Theorell-Chance mechanism[1] was put forward for the liver alcohol dehydrogenase-coenzyme system* in 1951. It is to the credit of the authors that our thinking about the mechanism of action of the dehydrogenases is to-day dominated by this mechanism. It has been considered to also apply to yeast alcohol dehydrogenase[2], muscle lactic dehydrogenase[3], malic dehydrogenase[4], and possibly ribitol dehydrogenase[5]. The mechanism implies that only the binary coenzyme complexes are rate limiting.



$$E + R \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ER$$

$$ER + ald \overset{k_3}{\rightleftharpoons} (ERald \rightleftharpoons EOalc) \underset{k_3'}{\rightleftharpoons} alc + EO$$

$$EO \underset{k_1'}{\overset{k_2'}{\rightleftharpoons}} E + O$$

For LADH after first receiving support[6], the mechanism was questioned on the basis that the ternary complexes were also rate limiting[7–9]. This was later shown not to be the case, as sensitive kinetic tests for the mechanism[10], were fulfilled[11]. The accuracy of these experiments is exemplified by the fact that $K_m(DPNH) \times K_m(DPN)/K_{E,R} \times K_{E,O}$ was 0.8. Theoretically it should be $k_2'/k_1 \times k_1/k_2 \times k_2/k_1' \times k_1'/k_2'$ or unity[11]. Experiments with inhibitors[12] also showed

* Abbreviations: LADH: Liver alcohol dehydrogenase; E: μN LADH, since each enzyme molecule has two independent binding sites; R and O: Reduced and oxidized diphosphopyridine nucleotide, DPNH and DPN.

Theorell-Chance kinetics. However, they did not rule out the possibility that the general two substrate mechanism applied with certain restrictions and that the binary enzyme-substrate complexes Eald and Ealc with dissociation constants of 10 μM and 6100 μM might also exist[11]. The existence of these complexes with stabilities as indicated has now been tested.

EXPERIMENTAL AND RESULTS

(1) At pH 7 and 9, alcohol or aldehyde concentrations of up to 0.2 M do not protect LADH against inactivation by iodoacetamide while small coenzyme concentrations do[13].

(2) Experiments using a recording spectrophotofluorometer. When (a) 1 μM enzyme was excited at 280 mμ and the protein fluorescence at 350 mμ measured and (b) 1 μM LADH-DPNH complex was excited at 280 mμ and 330 mμ and the resultant protein fluorescence at 350 mμ and coenzyme fluorescence at 460 mμ examined, in no case was the fluorescence appreciably affected by alcohol concentrations of up to 0.61 M. While the protein fluorescence might not be expected to reflect $E \rightleftharpoons$ Ealc or ER $\rightleftharpoons$ E $\rightleftharpoons$ Ealc competition, the coenzyme fluorescence certainly would. Acetaldehyde has an absorption maximum at 277 mμ, and experiments which had to use the protein fluorescence were therefore more difficult to carry out. However, there was no evidence for an enzyme acetaldehyde complex with a dissociation constant of 10 μM.

(3) In optical rotatory dispersion experiments the Cotton effect at 325 mμ due to the LADH-DPNH complex[14] was also not affected by high alcohol concentrations. A Bellingham and Stanley automatic spectropolarimeter[15] was used with enzyme, coenzyme and alcohol concentrations of 20 μN, 40 μM and 140 mM, respectively, in 0.2 μ phosphate, pH 7.1.

DISCUSSION

The experiments indicate that binary enzyme-substrate complexes with stabilities as indicated from inhibition experiments[12] do not exist. The competitive behaviour from which they were adduced must be considered to involve ternary complexes. Lack of agreement in the enzyme-alcohol dissociation constants obtained from the inhibition experiments with imidazole and caprate, had already caused their existence to be in doubt. Thus, there is now no longer any need to consider non coenzyme containing ternary complexes or whether the substrates affect the "on" velocity of the enzyme with the coenzyme[11]. The Theorell-Chance mechanism with rate limiting binary complexes and non rate limiting ternary complexes must be considered to apply in its entirety. The ternary complexes were of course manifest in the [illegible]riments with imidazole and inhibitors[12]. They can also be demonstrated [illegible]roduct inhibition experiments[16,17].

Recently on the basis of isotope experiments the [illegible]eorell-Chance mechanism has been questioned for the alcohol dehydrogenases [illegible],19 and for lactic dehydrogenase[20]. With A-DPND or CH_3CD_2OH, it is a consequence of the mechanism that k_2' $(1/\Phi_0)$ or k_1' $(1/\Phi_1')$ should show no isotope effects. For rabbit muscle lactic dehydrogenase Φ_0^H/Φ_0^D was 0.71[20]. However, this may be due to the fact that the reaction from the reduced coenzyme side is particularly susceptible to

inhibitors[21], and DPND preparations have been repeatedly found to be more impure than commercial DPNH. Impurities would result in k_2' for DPND being too low, Φ_0^D too high, and the ratio less than unity. This appears to also apply to the deuterium work with the alcohol dehydrogenases. With LADH although the results are somewhat invalidated by the type of buffer used, there was as would be expected no isotope effect on k_1', but Φ_0^H/Φ_0^D was 0.63[18]. For the yeast enzyme, where binary substrate complexes have been assumed, the latter value was 0.57[19].

The problem of purity may also arise with the substrates. On the basis of the Theorell-Chance mechanism different alcohols and aldehydes should not affect k_2' $(1/\Phi_0)$ or k_2 $(1/\Phi_0')$. With LADH for some twentyseven different alcohols and aldehydes, they have been stated to do so[22], although the three carbon homologues behave as expected, showing no such influence[21]. However, unless the substrates are carefully purified spurious effects could be caused by inhibitors present. These problems are currently being examined using 3,3-dimethylallyl alcohol and 1,1-dideutero-3,3-dimethylallyl alcohol purified by gas chromatography.

It is interesting to enquire why no binary substrate complexes are formed and why the maximum rate is the rate of breakdown of the binary coenzyme complexes. Experiments with the thiol reagent iodoacetamide designed to test the theory of conjugate catalysis[13,23] bear on this. Iodoacetamide reacts much more slowly than mercurials with thiol groups and so protection effects can be studied with greater precision. Protection against iodoacetamide by DPNH and DPNH + + isobutyramide was found to depend on the amount of ER formed. Isobutyramide appeared to exert its effect not by tightening the complex further but by increasing the amount of coenzyme complexed[24]. The experiments indicated that protection was by the coenzyme alone. A conformational change with a resultant change in the distribution of the polar-non polar groups in the enzyme must be involved when the coenzyme attaches[24], and the rate limiting step is the conformation change involved when ER or EO break down. It seems likely that the octahedral zinc in the coenzyme complexes, with three bonds to the protein, two to the coenzyme-adenine and one to water or the substrate[12,25], reverts to the tetrahedral configuration in the free enzyme.

It may be general for pyridine nucleotide coenzyme reactions that as for LADH a compulsory order mechanism is involved due to the coenzyme inducing a conformational change which is necessary before the substrate can attach. Also the stability of ternary substrate complexes may be determined by non aqueous properties of the enzyme, such that they dissociate as well as interconvert so rapidly that they do not affect the rate and give added significance to the Theorell-Chance mechanism.

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