Kinetics of the potassium-activated aldehyde dehydrogenase of yeast

The mechanism by which dehydrogenases react is a subject of considerable interest. Studies with alcohol and lactic dehydrogenases have been carried out[1-3] in order to explain their action in terms of well defined kinetic models[4-7]. On the other hand, the kinetics of enzyme activation by metal ions has also been investigated but with non-dehydrogenating enzymes such as the pyruvic phosphopherase from muscle[8]. The possibility of a simultaneous study of dehydrogenating mechanisms and metal activation is offered by the potassium-activated aldehyde dehydrogenase from yeast, since the activity of this enzyme depends on the concentration of substrate (acetaldehyde), coenzyme (DPN, diphosphopyridine nucleotide) and a metal ion (K^+ or Rb^+)[9]. According to the reactant chosen as a first variable, three different possibilities must be considered.

(1) *Experiments with acetaldehyde as first variable*

In this case the activity of the aldehyde dehydrogenase preparations (obtained and measured as described previously[10]) increased with acetaldehyde concentration up to $9-10 \cdot 10^{-5}$ M; above this concentration acetaldehyde inhibited the reaction. Plots of the reciprocal of the initial velocity against the reciprocal of the acetaldehyde concentration did not yield straight lines but the experimental values fitted equations (1) and (2) where the symbols have the meaning given by LINEWEAVER AND BURK[11].

$$n \log [S] - \log K_2 \cdot V_{max} = \log ([S]/v - K_s/V_{max} - [S]/V_{max}); \quad (1)$$

$$[S]^n/v = K_s/V_{max} + ([S]^n + [S]^n/K_2)/V_{max} \quad (2)$$

After several trials the average values of n, K_s and K_2 were 2.0, $1.5 \cdot 10^{-5}$ M and $0.9 \cdot 10^{-3}$ M, respectively. This shows that two enzyme-acetaldehyde compounds are possible, the first (catalytically active) with one acetaldehyde equivalent per enzyme active site (Michaelis constant, K_s) and the second compound, which is inactive, with two acetaldehyde equivalents per active site (dissociation constant, K_2). Variation of DPN concentration from 0.027 to 0.440 mM did not alter significantly the value of n, K_s or K_2 and variation of the concentration of K^+ from 0.5 mM to 50 mM or of Rb^+ from 1.0 mM to 100 mM did not affect the value of K_s.

(2) *Experiments with DPN as first variable*

In these experiments the rate of the reaction was directly related to DPN concentration which was varied in the range from 0.027 mM to 0.440 mM. By plotting the reciprocal of the initial velocity against the reciprocal of the DPN concentration, straight lines were obtained and K_{DPN} was calculated according to the equation $1/v = 1/V_{max} + K_{DPN}/[S] \cdot V_{max}$. The average K_{DPN} value was $1.4 \cdot 10^{-4}$ M and was independent of acetaldehyde concentration from 1.8 to $53 \cdot 10^{-5}$ M and of the concentration of the activator (Rb^+) from 1.0 mM to 100 mM.

(3) *Experiments with the activator ions as first variable*

The rate of the reaction increased when the activator concentration was increased to 0.05 M KCl or 0.025 M RbCl. Above this concentrations, the metal ions slightly inhibited the reaction. Nevertheless, plots of the reciprocal of the initial velocity against the reciprocal of the activator concentration (concentrations near to or below those producing maximal activity) yielded straight lines and K_A (dissociation constant of the enzyme-activator compound) could be calculated from the ratio of the slope to intercept at $1/[A] = 0$. K_A was equal to $2.1 \cdot 10^{-3}$ M (with K^+) and $5.5 \cdot 10^{-3}$ M (with Rb^+). Variation of the concentration level of DPN or acetaldehyde did not affect K_A values.

The maximal velocity of reaction (V_{max}) for each variable was calculated from the value of the intercept at $1/[S]$ or $1/[A] = 0$, in the plots of initial reaction velocity against the reciprocal of the substrate (or activator) concentration. The introduction of a second variable meant the appearance of several convergent straight lines which allowed the calculation of the effects of substrates and activators on the maximal velocity of the reaction. Plots of $1/V_{max}$ against $1/[S]$ or $1/[A]$ were linear and the ratio of slope to intercept gave the value of the dissociation (or Michaelis) constant corresponding to the second variable. In this way K_A, K_s and K_{DPN} were recalculated and the results compared well with the values quoted above.

The oxidation of acetaldehyde by the yeast aldehyde dehydrogenase is practically irreversible. Acetate did not oxidize DPNH nor inhibited the rate of the acetaldehyde oxidation which means that the enzyme–acetate compound is too dissociated to be of kinetic significance. On the other hand, DPNH inhibited the forward reaction. Assuming that oxidized and reduced DPN are bound at the same site of the enzyme, DPNH was considered as a competitive inhibitor and the dissociation constant of the enzyme–DPNH compound was calculated with the respective rate equation[5]. K_{DPNH} was found equal to $5.0 \cdot 10^{-5}$ M which means that the enzyme has a stronger affinity for DPNH than for DPN; this agrees with the comparatively higher ability of the former to protect the enzyme thiols[10].

Our experimental results fit an equation of the form (3), and, therefore, agree with the theory of ternary complexes consisting of enzyme–acetaldehyde–coenzyme or enzyme–substrate–activator, according to the variables chosen.

$$V_{max}/v = 1 + K_A/[A] + K_B/[B] + K_{AB}/[AB] \quad (3)$$

In equation (3), A and B are substrates, or substrate and activator, respectively. K_{AB}, the complex constant, could be measured directly and the value obtained agreed well with the value of the product $K_A \times K_B$ whichever were the pair of variables chosen. The dissociation constant of each enzyme–substrate or enzyme–activator compound is not affected by the presence of the other reactants and therefore random formation of the active complex could take place if the system were operating in equilibrium conditions. Nevertheless, the possibility of compulsory order of binding of substrates under steady-state conditions cannot be ruled out entirely. The point will be discussed in more detail elsewhere.

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The enzymic synthesis of δ-aminolevulinic acid*

δ-Aminolevulinic acid has been demonstrated to be an intermediate in the synthesis of porphyrins[1,2] and the porphyrin-like moiety of vitamin B_{12}[3]. This aminoketonic acid arises biologically from the condensation of "active" succinate[4] and glycine. However, the details of this condensation have not yet been elucidated. This communication reports our finding that the supernatant fluid obtained on centrifugation of an extract of the photosynthetic bacterium *Rhodopseudomonas spheroides* for 30 min at 100,000 × *g* is capable of catalyzing the net synthesis of δ-aminolevulinic acid from succinate and glycine on addition of cofactors or from succinyl-coenzyme A and glycine.

LASCELLES has demonstrated[5] that not only are porphyrins synthesized by *R. spheroides* by the route previously elaborated for other cells, but also that porphyrins and porphobilinogen accumulate in the medium. We were encouraged to investigate this bacterium for the enzymes responsible for the synthesis of δ-aminolevulinic acid, for we found that δ-aminolevulinic acid was also present in the medium after the cells were suspended in medium I of LASCELLES[5]. LAVER AND NEUBERGER[6] recently reported that a particulate fraction obtained from erythrocytes of phenylhydrazine-treated chicks was capable of synthesizing this compound from succinate and glycine and that the amount synthesized was increased 2.5 fold on addition of both coenzyme A (CoA) and pyridoxal phosphate.

The strain 241C of *R. spheroides*, obtained from Professor R. Y. STANIER, which originally came from Professor C. V. VAN NIEL's collection, was grown in medium S of LASCELLES. After about 36 h, the bacteria were harvested and washed several times with 0.9% saline. The cells were ground with alumina, extracted with 0.1 *M* phosphate buffer, pH 6.9, containing 0.9% NaCl, and centrifuged for 30 min at 5000 × *g*. The supernatant fluid was then centrifuged for 30 min at 100,000 × *g*, and this latter supernatant fluid was then used as the initial enzyme system.

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