Lactic Dehydrogenase

V. INHIBITION BY OXAMATE AND BY OXALATE*

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Several years ago one of us (A. J. G.) observed that lactic dehydrogenase is inhibited by oxamate, the salt of the half-amide of oxalic acid, and found that this inhibition is essentially competitive with respect to pyruvate (1). Since that time oxamate has been studied as a potential inhibitor of glycolysis in tumors (2, 3) but extension of the kinetic measurements of the phenomenon in this laboratory was deferred until the mechanism of the action of the enzyme was better understood and until more sensitive analytical methods were available. Since a working hypothesis of the mechanism of action of the enzyme has been proposed in the preceding paper in this series (4), it is now possible to formulate a tentative explanation of the phenomena which are observed in oxamate inhibition.

Measurements of the effects of oxalate as an inhibitor of lactic dehydrogenase are also reported since, as detailed in paper VII of this series (5), the effects of oxamate and of oxalate upon the fluorescent emission of the LDH-DPN complex are opposite in sign. Although oxalate has been known for a considerable time to be an inhibitor of lactic acid oxidation in preparations of bacterial cells (6), and while it has been shown that oxalate also inhibits heart muscle lactic dehydrogenase (7), the nature of the inhibition has been previously investigated only with rat liver mitochondria (8) and, more recently, with beef heart muscle lactic dehydrogenase (9, 10). Sodium oxalate was a Merck reagent grade product.

Materials—The preparations of beef heart lactic dehydrogenase, and of DPN, DPNH, potassium pyruvate, and calcium-L-lactate used in the present measurements have been described (9, 10). Sodium oxalate was a Merck reagent grade product.

Ethyl oxamate was prepared from diethyl oxalate by the procedure of Oelbers (12), which involves hydrolysis of the alkyl oxamate with ammonium hydroxide in boiling solution and isolation of oxamic acid following acidification, was followed. In later preparations a suggestion of Dr. S. P. Colowick* was adopted and this hydrolysis was carried out by boiling ethyl oxamate with slightly less than one equivalent of potassium hydroxide. This modification minimized the hydrolysis of ethyl oxamate to oxalate, an impurity which is difficult to separate from the desired product.

The free acid was precipitated by the addition of hydrochloric acid, washed with a small amount of water, neutralized with potassium hydroxide and brought to crystallization as well formed needles by the addition of ethanol. The nitrogen content of the free acid and of the potassium salt agreed with theory (semi-micro-Kjeldahl).

Methods—Measurements of reaction rate in 0.05 M phosphate buffer, pH 6.80, were carried out as described by Hakala et al. (9). Kinetic measurements in 0.2 ionic strength Tris-Tris-HCl-KCl buffers were performed by the procedures described by Winer and Schwert (4).

Kinetic Considerations and Inhibition by Oxamate—It has been proposed previously (10) that the reaction pathway for the reversible reaction catalyzed by lactic dehydrogenase can be represented by:

\[ E + O \xrightarrow{1/2} EO \]

\[ EO + L \xrightarrow{3/4} EXY \xrightarrow{5/6} ERP + P \]

\[ ER \xrightarrow{7/8} E + R \]  

(1)

where \( E \) is the enzyme, \( O \) and \( R \) are DPN and DPNH, respectively, and \( L \) and \( P \) are lactate and pyruvate respectively. Since measurements of the kinetics of the over-all reaction yield no information concerning the interconversion of the two possible ternary complexes, \( EXL \) and \( ERP \), the scheme has been simplified by including only a single ternary complex, \( EXY \), in the scheme. Alberty has shown (13) that the initial reaction velocity for the forward direction of the reversible reaction is given by:

\[ V = \frac{V_f}{1 + K_0/O + K_L/L + K_{DL}/OL} \]  

(2)

where \( V_f \) is the maximal reaction velocity in the forward direction of the reaction when the enzyme is saturated with respect to both DPN and lactate, \( K_0 \) and \( K_L \) are the Michaelis constants for DPN and lactate, respectively, and \( O \) and \( L \) are the initial

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1 The abbreviations used are: LDH, lactic dehydrogenase; and Tris, tris(hydroxymethyl)aminomethane.
concentrations of these reactants. $K_{OL}$ is a constant which has the same operational significance with respect to the product of concentrations, $OL$, as ordinary Michaelis constants do to single concentrations of reactants. The definitions of these constants have been given by Alberty (13). The expression for the initial reaction velocity in the reverse direction of the reaction is wholly symmetrical with Equation 2.

Inspection of Fig. 1B, which is a Lineweaver-Burk plot (14) of reciprocal initial reaction velocity against reciprocal pyruvate concentration at a single initial concentration of DPNH in the presence of various concentrations of oxamate, indicates that the slopes of the lines increase much more rapidly with oxamate concentration than do the intercepts. For measurements made at higher pH values, the change in intercepts is much less than that shown in Fig. 1B. This observation is most simply interpreted by assuming that one action of oxamate as an inhibitor is competition with pyruvate.

Fig. 1A indicates that oxamate is essentially uncompetitive in its action toward DPNH. Fig. 1C and 1D, which include measurements made at higher oxamate concentrations than were used for Fig. 1A and 1B, are qualitatively similar to Fig. 1A with the exception that, at higher oxamate concentrations, there is an increase in the slope of the lines. These increases in slope can be accounted for on the assumption that at sufficiently high oxamate concentrations a competition of oxamate with both DPN and lactate becomes apparent. These actions of oxamate as an inhibitor can be represented by:

$$E + I \rightleftharpoons EI$$  \( (3) \)

$$ER + I \rightleftharpoons ERI$$  \( (4) \)

$$E + I \rightleftharpoons EI$$  \( (5) \)

The steady state kinetic treatment of Equations 1, 3, 4, and 5 yields the following expression for the initial reaction velocity in the forward direction in the presence of oxamate:

$$v = \frac{V_f}{1 + \frac{K_o}{O} + \frac{K_l}{L} + \frac{K_{ol}}{OL} + \frac{k_d}{(b_0 + b_1)K_l} + \frac{K_{ol}}{K_l O} + \frac{K_{ol} I}{K_l L} + \frac{K_{ol} I}{K_l OL}}$$  \( (6) \)

The corresponding equation for the reverse reaction is:

$$v = \frac{V_r}{1 + \frac{K_r}{R} + \frac{K_p}{P} + \frac{K_{rp}}{RP} + \frac{k_d}{(b_2 + b_3)K_l} + \frac{K_r I}{K_l R} + \frac{K_p I}{K_l P} + \frac{K_{rp} I}{K_l RP}}$$  \( (7) \)
where \( I \) is the concentration of inhibitor and \( K_I, K_I', \) and \( K_I'' \) are the dissociation constants for the dissociation of inhibitor from the EOI, ERI, and EI complexes, respectively.

It is clear from Equation 6 that the slope of a plot of reciprocal initial reaction velocity against reciprocal initial lactate concentration in the presence of inhibitor is:

\[
\text{Slope}_I = \frac{1}{V_I(K_L + K_{OL})} \left( \frac{K_L O}{K_{OL} I} \right) \frac{1}{K_I} + \left( \frac{K_{OL}}{K_{OL} I} \right) \frac{1}{K_I'}
\]

From Equation 2 the corresponding slope in the absence of inhibitor is:

\[
\text{Slope}_0 = \frac{1}{V_I(K_L + K_{OL})}
\]

The ratio of these slopes is:

\[
\frac{\text{Slope}_I}{\text{Slope}_0} = \frac{1}{I} + \left( \frac{K_L O}{K_{OL} O} \right) \frac{1}{K_I} + \left( \frac{K_{OL}}{K_{OL} O} \right) \frac{1}{K_I'}
\]

Thus \([\text{Slope}_I/\text{Slope}_0] - 1/I\) contains as unknown terms only the two inhibitor dissociation constants, \( K_I \) and \( K_I'' \).

In practice, the slopes of \( 1/v \) versus \( 1/I \) plots were measured and plotted against inhibitor concentration. The best straight line was drawn through the resulting points and the value of the ratio, \( \text{Slope}_I/\text{Slope}_0 \), was taken from this line.

The relationships between slopes and intercepts of the various plots are summarized in Table I.

Since the slopes of both \( 1/v \) versus \( 1/I \) and \( 1/v \) versus \( 1/P \) plots yield values for \( K_I'' \) which are independent of the values assigned to the Michaelis constants, the average value of this constant was established first and was used in further calculations.

The estimation of numerical values for the other inhibitor dissociation constants clearly depends upon the values assigned to the Michaelis constants. Values for the various Michaelis constants were interpolated from plots of the Michaelis and inhibitor dissociation constants back into Equations 6 and 7. Similar experiments were carried out at pH 6.40, 8.45, and 9.70. The values used for the Michaelis constants, the initial concentrations of reactants and the values found for the inhibitor dissociation constants are summarized in Table II.

Substitution of the values shown in Table II into the expression relating slopes of \( 1/v \) versus \( 1/P \) plots to oxamate concentration (Table I) indicates that the contribution of the term involving \( K_I'' \) amounts to only a few per cent of the total value of the expression. Thus, if the term involving \( K_I'' \) is neglected, an error is introduced into the value assigned to \( K_I' \) which is certainly much less than the experimental error of the measurements.

Measurements made in phosphate buffer at pH 6.80 are technically much more difficult than those made in Tris at the same pH because the values of the Michaelis constants are somewhat

### Table I

**Relationships between slopes and intercepts of Lineweaver-Burk plots obtained in presence and in absence of inhibitor**

<table>
<thead>
<tr>
<th>Plot</th>
<th>( \frac{\text{Int}_I}{\text{Int}_0} - 1 )</th>
<th>( \frac{\text{Slope}_I}{\text{Slope}_0} - 1 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( 1/v \ vs. 1/P )</td>
<td>( \left( \frac{K_L}{K_L + L} \right) \frac{1}{K_L} + \left( \frac{k_5}{k_5 + k_8} \right) \left( \frac{L}{K_L + L} \right) \frac{1}{K_I'} )</td>
<td>( \frac{K_L O}{K_{OL} O} \frac{1}{K_I} + \left( \frac{K_{OL}}{K_{OL} O} \right) \frac{1}{K_I''} )</td>
</tr>
<tr>
<td>( 1/v \ vs. 1/L )</td>
<td>( \left( \frac{k_3}{k_3 + k_8} \right) \left( \frac{O}{K_O + O} \right) \frac{1}{K_I} + \left( \frac{K_O}{K_O + O} \right) \frac{1}{K_I'} )</td>
<td>( \frac{K_L O}{K_{OL} O} \frac{1}{K_I} + \left( \frac{K_{OL}}{K_{OL} O} \right) \frac{1}{K_I''} )</td>
</tr>
<tr>
<td>( 1/v \ vs. 1/R )</td>
<td>( \left( \frac{k_4}{k_4 + k_8} \right) \left( \frac{P}{K_R + P} \right) \frac{1}{K_I} + \left( \frac{K_R}{K_R + P} \right) \frac{1}{K_I'} )</td>
<td>( \frac{K_P R}{K_P R + K_{RP}} \frac{1}{K_I} + \left( \frac{K_{RP}}{K_P R + K_{RP}} \right) \frac{1}{K_I''} )</td>
</tr>
</tbody>
</table>

* \( \text{Int}_I \) and \( \text{Slope}_I \) indicate the intercept and slope obtained in the presence of a concentration of inhibitor \( I \). \( \text{Int}_0 \) and \( \text{Slope}_0 \) are the intercepts and slopes found in the absence of inhibitor.

### Table II

**Values used for Michaelis constants and for initial substrate concentrations and values found for dissociation constants for dissociation of oxamate from EOI, ERI, and EI complexes at various pH values in 0.2 ionic strength Tris buffers**

<table>
<thead>
<tr>
<th>Constant or substrate concentration</th>
<th>( \text{pH} 6.40 )</th>
<th>( \text{pH} 8.45 )</th>
<th>( \text{pH} 9.70 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( K_L )</td>
<td>( 1.2 \times 10^{-4} )</td>
<td>( 7.0 \times 10^{-4} )</td>
<td>( 1.5 \times 10^{-4} )</td>
</tr>
<tr>
<td>( K_{OL} )</td>
<td>( 2.4 \times 10^{-2} )</td>
<td>( 6.0 \times 10^{-4} )</td>
<td>( 6.13 \times 10^{-4} )</td>
</tr>
<tr>
<td>( O )</td>
<td>( 2.84 \times 10^{-4} )</td>
<td>( 1.21 \times 10^{-4} )</td>
<td>( 3.78 \times 10^{-4} )</td>
</tr>
<tr>
<td>( L )</td>
<td>( 2.50 \times 10^{-3} )</td>
<td>( 1.10 \times 10^{-3} )</td>
<td>( 2.19 \times 10^{-3} )</td>
</tr>
<tr>
<td>( K_R )</td>
<td>( 1.45 \times 10^{-6} )</td>
<td>( 2.5 \times 10^{-5} )</td>
<td>( 3.3 \times 10^{-5} )</td>
</tr>
<tr>
<td>( K_{RP} )</td>
<td>( 1.0 \times 10^{-4} )</td>
<td>( 5.0 \times 10^{-4} )</td>
<td>( 3.2 \times 10^{-4} )</td>
</tr>
<tr>
<td>( K_{EI} )</td>
<td>( 3.5 \times 10^{-10} )</td>
<td>( 5.0 \times 10^{-9} )</td>
<td>( 4.2 \times 10^{-9} )</td>
</tr>
<tr>
<td>( K_{EI} (EOI = E + I) )</td>
<td>( 8.86 \times 10^{-4} )</td>
<td>( 9.63 \times 10^{-4} )</td>
<td>( 9.08 \times 10^{-4} )</td>
</tr>
<tr>
<td>( K_{EI} (ERI = E + R) )</td>
<td>( 6.13 \times 10^{-4} )</td>
<td>( 5.85 \times 10^{-4} )</td>
<td>( 4.00 \times 10^{-4} )</td>
</tr>
<tr>
<td>( K_{EI} (EI = E + I) )</td>
<td>( 6.9 \times 10^{-4} )</td>
<td>( 5.7 \times 10^{-4} )</td>
<td>( 1.1 \times 10^{-3} )</td>
</tr>
<tr>
<td>( I )</td>
<td>( 2.6 \times 10^{-4} )</td>
<td>( 1.7 \times 10^{-4} )</td>
<td>( 1.6 \times 10^{-4} )</td>
</tr>
<tr>
<td>( k_5 )</td>
<td>( 1.0 \times 10^{-4} )</td>
<td>( 7.8 \times 10^{-4} )</td>
<td>( 2.0 \times 10^{-4} )</td>
</tr>
<tr>
<td>( k_3 \times k_8/k_3 + k_8 )</td>
<td>( 1.8 )</td>
<td>( 3.4 )</td>
<td>( 2.9 )</td>
</tr>
</tbody>
</table>
smaller in phosphate (A, 9). Clear results were obtained in phosphate buffer only when pyruvate concentration was the parameter varied. $K_r'$ was estimated from slope changes of plots of $1/v$ against $1/P$ in the absence of oxamate and in the presence of two concentrations of oxamate, neglecting the contribution of $K_r''$, and was found to be $9.4 \times 10^{-8}$ M at $18.9^\circ$, $1.2 \times 10^{-8}$ M at $26^\circ$, $1.7 \times 10^{-4}$ M at $31^\circ$, and $1.8 \times 10^{-4}$ M at $36.7^\circ$.

Inhibition by Oxalate—Typical results obtained in the presence of oxalate at $28^\circ$ in Tris buffer, pH 8.05, ionic strength 0.2, are shown in Fig. 2. It is evident from a comparison of Fig. 2B and 2D with Fig. 1B and 1D that oxalate and oxamate act reciprocally with respect to competition with pyruvate and lactate.

The points shown in Fig. 2 are experimental points and the lines were calculated from Equations 6 and 7 by substitution of the values for the various constants and substrate concentrations which are shown in the legend to Fig. 2. In this case, the contribution of the term involving $K_r'$ to the expression relating the intercepts of $1/v$ versus $1/O$ plots to oxalate concentration (Table I) is so small that the ratio $(k_3 + k_7)/k_5$ cannot be estimated.

Further, if the values assigned to the various constants are correct, the contribution of the term involving $K_r''$ to the change of slope of $1/v$ versus $1/L$ plots in the presence of oxalate is only a few per cent of the total slope change. This is clearly analogous to the negligible contribution of the term involving $K_r''$ to the slope changes of $1/v$ versus $1/P$ plots in the presence of oxamate. It follows that values of $K_r'$ can be estimated from the slopes of $1/v$ versus $1/L$ plots with negligible additional error if the term

![Diagram]

**Fig. 2.** A plot similar to Fig. 1 showing the effects of oxalate on initial reaction velocity when the concentration of each of the four reactants is varied. The points are experimental points for measurements made in 0.2 ionic strength Tris-Tris.HCl-KCl buffer, pH 8.05, at $28^\circ$. The lines were calculated from Equations 6 and 7 with the following values for the constants and for the initial constant concentrations of reactants: $K_o, 6.5 \times 10^{-4}$ M; $K_L, 7.0 \times 10^{-4}$ M; $K_{Ox}, 1.0 \times 10^{-4}$ M; $O, 3.84 \times 10^{-4}$ M; $L, 6.03 \times 10^{-4}$ M; $K_E, 2.3 \times 10^{-4}$ M; $K_E', 5.5 \times 10^{-4}$ M; $K_E''$, $2.6 \times 10^{-4}$ M; $P, 6.08 \times 10^{-4}$ M; $K_r$, $9.7 \times 10^{-4}$ M; $K_r', 1.9 \times 10^{-4}$ M; $K_r''$, $2.4 \times 10^{-4}$ M; and $(k_3 + k_7)/k_5, 4.6$.

### Table III

<table>
<thead>
<tr>
<th>pH 6.05</th>
<th>pH 6.33</th>
<th>pH 8.03*</th>
<th>pH 9.12</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_L$</td>
<td>$3.4 \times 10^{-4}$</td>
<td>$1.7 \times 10^{-4}$</td>
<td>$7.0 \times 10^{-4}$</td>
</tr>
<tr>
<td>$K_{Ox}$</td>
<td>$7.5 \times 10^{-4}$</td>
<td>$3.8 \times 10^{-4}$</td>
<td>$1.9 \times 10^{-4}$</td>
</tr>
<tr>
<td>$O$</td>
<td>$6.67 \times 10^{-4}$</td>
<td>$6.53 \times 10^{-4}$</td>
<td>$3.84 \times 10^{-4}$</td>
</tr>
<tr>
<td>$K_r$</td>
<td>$9.2 \times 10^{-4}$</td>
<td>$1.5 \times 10^{-4}$</td>
<td>$8.4 \times 10^{-4}$</td>
</tr>
</tbody>
</table>

* This is the experiment reported in detail in Fig. 2. The value of $K_r'$ shown here was calculated from the slope of the $1/v$ versus $1/L$ plot alone and the contribution of the $K_r''$ term was neglected.
involving $K_f^*$ is neglected. Table III lists the values found for $K_f$ at various pH values by the use of the slopes of $1/v$ versus $1/L$ plots alone and by neglecting the contribution of the $K_f^*$ term to these slopes. Four levels of oxalate concentration were used for each estimation of $K_f$.

**DISCUSSION**

In view of the sensitivity of Lineweaver-Burk plots with respect to both experimental and subjective errors, it is quite reasonable to question the proposal that oxamate and oxalate act as inhibitors by forming three different complexes, EI, ERI, and EOI. The plots shown in Fig. 1 and 2 do appear to indicate beyond reasonable doubt that oxamate and oxalate act by competing with pyruvate and lactate, respectively. If the arguments (10) that the reaction follows the compulsory pathway mechanism shown in Equation 1 are correct, this competition must result in the formation of an ERI complex with oxamate and of an EOI complex with oxalate.

Paper VII of this series (5) presents evidence that oxalate also forms a ternary $EKL$ complex. While there is no direct evidence for the existence of an EOI complex with oxamate, it seems probable that the ERI complex which is formed with oxalate has an analogue in an EOI complex with oxalate.

The argument for the existence of an E1 complex rests simply upon the increase in slope observed at high inhibitor concentrations in plots of the type shown in Fig. 2C.

At the outset of this investigation the oxamate and oxalate inhibition of the lactic dehydrogenase system appeared to provide a useful tool for the study of the effects of inhibitors on reaction rates from both sides of a reversible reaction. Grossly, as was expected, inhibitors which are essentially competitive with respect to one substrate are noncompetitive or uncompetitive with respect to the other substrate and to the oxidized and reduced coenzyme. A point which was not anticipated, but which is clear from theoretical considerations, is that the action of an inhibitor which competes with the second reactant in a compulsory reaction pathway cannot be characterized by the same constant for the forward and reverse reactions. Specifically, if it is assumed that in the present case the only action of an inhibitor is to form an EOI complex, Equation 6 reduces to:

$$v = \frac{V_f}{1 + K_o/O + K_L/L + K_{OL}/OL + K_{IL}/K_{IL}} \quad (11)$$

and Equation 7 reduces to:

$$v = \frac{V_o}{1 + K_R/R + K_{P}/P + K_{RP}/RP + k_I/(k_I + k_I)K_f} \quad (12)$$

In plots of experimental data the rate of increase of the intercepts of both $1/v$ versus $1/R$ and of $1/v$ versus $1/P$ plots with increasing inhibitor concentration will be characterized by the same constant, but this constant will not be identical with that which characterizes the rate of increase of slope of the $1/v$ versus $1/L$ plot. The exception to this generalization is obviously the case in which $k_t$ is much less than $k_i$ so that the ratio $k_t/(k_t + k_i)$ has a value approaching unity.

This behavior has been observed with the present data in that, if the best values estimated for the Michaelis constants in previous studies (4) are substituted into the relations shown in Table I, the same value of $K_f^*$ cannot be used for the slopes of $1/v$ versus $1/P$ plots and for the intercepts of $1/v$ versus $1/L$ plots in the presence of oxamate, nor will the same value of $K_f$ satisfy the relations for the slopes of $1/v$ versus $1/L$ plots and the intercepts of $1/v$ versus $1/P$ plots in the presence of oxalate. While this interpretation depends heavily upon the values previously assigned to the Michaelis constants, quite large changes in the values of the Michaelis constants would be required before the same values of $K_f$ and of $K_f'$ could be used for both sets of plots.

This interpretation results in values for the ratios $(k_s + k_i)/k_t$ and $(k_t + k_i)/k_s$ which are different than unity. In previous studies it was concluded that $k_t$, the rate of dissociation of DPNH from the LDH-DPNH complex was the limiting reaction velocity in the forward direction of the reversible reaction (4). Thus the present results indicate that $k_s$ and $k_t$ are of the same order of magnitude. In view of the fact that estimations of velocities of individual steps and of the magnitude of the ratio $(k_s + k_t)/k_s$ are derived from lengthy treatments of the primary data, this order of disagreement is not surprising.

The effects of pH upon $K_f$, $K_f'$, and $K_f^*$ for oxamate and upon $K_f$ for oxalate are essentially the same as the effect of pH upon $K_f$, the Michaelis constant for pyruvate. This point is illustrated graphically and is discussed in detail in paper VII of this series (5). The constant which characterizes inhibition by high concentrations of pyruvate also varies in a similar way with pH (4).

This concurrence of the effects of pH upon the inhibitor dissociation constants and upon $K_f$ might be taken to indicate that $K_f$ is essentially a simple dissociation constant. This conclusion is ruled out, however, by the observation that the value of $\Delta H$ for the dissociation of oxamate from the LDH-DPNH-oxamate complex, calculated from the values estimated for $K_f$ at various temperatures in pH 6.80 phosphate buffer, is 6.9 ± 0.3 kilocalories per mole. The apparent value of $\Delta H$ for the dissociation of pyruvate from the LDH-DPNH-pyruvate complex, calculated from values previously reported for $K_f$ in the same buffer (9), is 18.8 ± 0.9 kilocalories per mole. If $K_f$ were determined essentially by the reaction rates for the reversible dissociation of LDH-DPNH-pyruvate complex into LDH-DPNH complex and free pyruvate, these values of $\Delta H$ would be expected to be essentially identical.

**SUMMARY**

The inhibitory action of oxamate, the salt of the half-amide of oxalic acid, and of oxalate upon the reversible reaction catalyzed by beef heart muscle lactic dehydrogenase has been investigated. Oxamate acts principally by competing with pyruvate by combining with the enzyme-reduced diphosphopyridine nucleotide complex to form an inactive ternary complex, and oxalate acts...
principally by competing with lactate. There are indications, however, that each inhibitor also forms an inactive complex with the enzyme itself and with the enzyme-coenzyme complex containing coenzyme in the other oxidation state. All of the constants characterizing the dissociation of the inhibitor from its complexes increase markedly with pH in the range between pH 6 and pH 10.

**ADDENDUM**

Ottolenghi and Denstedt (15), with the use of an enzyme preparation from rabbit erythrocytes, have also reported recently that oxalate competes with lactate but not with pyruvate. To account for this finding, these workers propose that there are separate binding sites on the enzyme surface for lactate and for pyruvate. In our model this hypothesis is not necessary. The location of the binding site for lactate and for pyruvate is presumably the same as regards the geometry of the enzyme surface and in part in the bound coenzyme. Since DPN and DPNH differ in both steric and chemical properties, the configuration of the binding site for substrate or inhibitors on the enzyme-DPN complex is quite different from that on the enzyme-DPNH complex.

**REFERENCES**


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