THE EFFECT OF CATIONS ON THE DECARBOXYLATION OF OXALACETIC ACID*

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In connection with studies on the β-keto acid carboxylases of plants (1, 2), the effects of metal ions on the decarboxylation of oxalacetic acid, in the presence and absence of enzyme, were studied. In confirmation of Krebs (3) the decarboxylation was found to be accelerated by a variety of polyvalent cations. It was also observed that the enzyme oxalacetic carboxylase from parsley roots, which is nearly inactive in the absence of metal ions, is activated by a variety of divalent cations.

Methods

The experimental procedures used in studying keto acid decarboxylation are described in the preceding paper (2). Evolution of carbon dioxide from oxalacetic acid was measured in Warburg manometers, with a reaction mixture buffered at pH 5 to avoid retention. Since in most cases the decarboxylation reactions follow first order kinetics with respect to oxalacetic acid, the rates are conveniently expressed as first order reaction rate constants.

Results

Effect of Cations on Non-Enzymatic Decarboxylation of Oxalacetate—All the polyvalent cations tested, with the exception of Ba++, accelerate the decarboxylation of oxalacetate. Monovalent cations were not studied, but Krebs (3) found them to be ineffective. In agreement with Krebs, it was noted that the effect of the cation is independent of the nature of the anion added with it, provided the salt is ionized. Except in the case of Fe+++ and Al++++, which are described later, the decarboxylation reactions follow first order kinetics with respect to oxalacetic acid. When Cu++ is present in concentrations of 0.01 M or greater, the first order constants tend to decrease somewhat with time, and the average has been taken.

In Fig. 1 the first order rate constants are plotted against the logarithms

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of the molarities of the cations. The curves for the various ions are similar in shape, but the concentration giving a maximum effect and the magnitude of the effect vary with different cations. Cu^{++} and La^{+++} give maxima at concentrations of 0.001 M. The activity maximum shown for Pb^{++} may not be similar in nature, because in samples containing Pb^{++} at concentrations of 0.01 M or greater a precipitate (perhaps lead oxalacetate) appears on tipping in the oxalacetic acid. Other ions, such as Zn^{++}, Ni^{++}, Co^{++}, and Fe^{++}, tend to approach maxima at the highest concentrations studied.

It was necessary to employ anaerobic conditions in testing the action of Fe^{++} ions. When air was used as the gas phase, a yellow color (probably...
due to formation of basic ferric acetate) appeared at the moment when the oxalacetic acid was tipped in. Subsequently oxygen was taken up at the same time that carbon dioxide was liberated, and the kinetics of the reaction were complex. Under anaerobic conditions (nitrogen as the gas phase, yellow phosphorus in the center well), no yellow color appeared on tipping in oxalacetic acid, and the decarboxylation followed first order kinetics.

Fe$^{+++}$ and Al$^{+++}$ gave results different from those obtained with the other ions, and these effects are represented in Figs. 2 and 3. Decarboxylation of oxalacetate in the presence of these ions follows first order kinetics only at low cation concentrations (below 0.001 M). At higher concentrations the rates fall off more rapidly than expected for first order reactions, particularly in the case of Fe$^{+++}$. Both ions give maximum rates of decarboxy-
Decarboxylation at a concentration of about 0.001 M, and the maximum rates are lower than those observed with the most effective divalent cations, such as Zn\(^{++}\) and Ni\(^{++}\). The experiments with Fe\(^{+++}\) were performed under anaerobic conditions.

Effect of Cations on Enzymatic Decarboxylation of Oxalacetate—A preparation of oxalacetic carboxylase from parsley roots, made as described in the preceding paper, was used in studying the effect of cations on the enzymatic decarboxylation of oxalacetate. In the absence of added divalent cations, decarboxylation of oxalacetate by the enzyme is very slow. All the divalent cations studied activate the enzyme; i.e., the rate of decarboxylation of oxalacetate in the presence of enzyme plus cation is greater than in the presence of cation alone, of enzyme alone, or of cation plus heat-inactivated enzyme. In all cases of activation, the reactions follow first order kinetics.

![Graph showing the effect of Al\(^{+++}\) on the non-enzymatic decarboxylation of oxalacetate.](http://www.jbc.org/)

Fig. 3. Effect of Al\(^{+++}\) on the non-enzymatic decarboxylation of oxalacetate. Samples contained 0.1 M acetate, pH 5.0, oxalacetic acid equivalent to 150 μl. of CO\(_2\), and Al\(_2\)(SO\(_4\))\(_3\) in the concentrations indicated, in a volume of 2.0 ml. Temperature 30°. Curve 1 was calculated for a first order rate constant of 0.292 min\(^{-1}\).
La$^{+++}$ ions do not activate the enzyme, and Fe$^{+++}$ and Al$^{+++}$ ions give complex results, which are described later.

In Fig. 4 the relative activity of the parsley root enzyme in the presence of different cations is plotted against the logarithm of the cation concentration. The various ions give curves of similar shape which differ from each other in the position and height of the maximum. All of the ions except Mg$^{++}$ and Ba$^{++}$ show maximum effects within the range of concentrations studied. The maximum for Ca$^{++}$ actually lies just at the highest concentration, 0.03 M; higher levels give lower enzyme activities. Of the ions tested, Mn$^{++}$ is most effective in activating the parsley root enzyme.

In most cases the first order rate constants for the decarboxylation of oxalacetate in the presence of cation plus heat-inactivated enzyme are the
same as in the presence of an equal concentration of cation alone. This indicates that the inactivated protein does not firmly bind these ions. However, the heat-inactivated protein reduces the rates obtained with Co\(^{++}\) and Ni\(^{++}\) at 0.03 M concentrations and with Zn\(^{++}\) at concentrations of 0.003 M and above. Decarboxylation of oxalacetate in the presence of heated enzyme and La\(^{+++}\) or Pb\(^{++}\) occurs at the same rate as that observed in the absence of added metal ions; the inactive protein abolishes the catalytic activity of these cations, although Pb\(^{++}\) activates the unheated enzyme. Fe\(^{+++}\) and Al\(^{+++}\) gave anomalous results, which are represented in Figs. 5 and 6. Decarboxylation of oxalacetate in the presence of these ions does not follow first order kinetics. At cation concentrations of 0.0001 and 0.0003 M the rates in the unheated samples are the same as or slightly lower than in the control. De
greater than in the heated samples; both rates are considerably lower than those observed with the ions in the absence of any protein (compare with Figs. 2 and 3). At cation concentrations of 0.001 and 0.003 M the rates in the heated samples are equal to or greater than those in the unheated samples; both rates are somewhat lower than those observed in the absence of protein. At cation concentrations of 0.01 and 0.03 M, the rates in the unheated samples are the same as or greater than in the heated samples; both rates are greater than those found in the absence of protein. An explanation for these results is not apparent. When Fe+++ was present in high concentrations, some tendency to form deposits of basic ferric acetate on protein particles was noted. In any case, it seems unlikely that either Fe+++ or Al+++ activates the parsley root carboxylase significantly.

The activity of the carboxylase with mixtures of two different cations,
each in suboptimum concentration, was studied. In the presence of heated enzyme the effects produced by Zn\textsuperscript{+2} and Cd\textsuperscript{+2} are partially additive. The following first order rate constants were observed: 0.012 min\textsuperscript{-1} with 0.001 M CdSO\textsubscript{4}, 0.030 min\textsuperscript{-1} with 0.0003 M ZnSO\textsubscript{4}, and 0.037 min\textsuperscript{-1} with both. However, these cations compete in activating the unheated enzyme, so that intermediate activity is observed with both present. The relative enzyme activities (activity with 0.01 M MnCl\textsubscript{2} taken as 100) were 39 with 0.001 M CdSO\textsubscript{4}, 23 with 0.0003 M ZnSO\textsubscript{4}, and 35 with both.

Experiments were carried out in which the acetate buffer was replaced by benzoate, phthalate, oxalate, succinate, tartrate, or citrate of the same pH and molar concentration. 0.001 M MnCl\textsubscript{2} was present, and the rate of decarboxylation of oxalacetate was measured with and without enzyme. All of the buffers except benzoate reduce the rate of non-enzymatic decarboxylation below the level observed with 0.001 M Mn\textsuperscript{+2} in acetate, and added enzyme is completely inactive. In the case of benzoate, the non-enzymatic rate is the same as with 0.001 M Mn\textsuperscript{+2} in acetate, but added enzyme is inactive. Probably phthalate, oxalate, succinate, tartrate, and citrate decrease the decarboxylation rate by forming complexes with Mn\textsuperscript{+2}, while benzoate more specifically inhibits the enzyme.

DISCUSSION

Krampitz and Werkman (4) first described the acceleration of the non-enzymatic decarboxylation of oxalacetate by metal ions for the particular case of Mg\textsuperscript{+2}, and Krebs (3) investigated this effect of cations in greater detail. The results of his studies and of the experiments reported in the present paper may be summarized as follows: Many polyvalent cations accelerate the decarboxylation of oxalacetate. Of those tested, Zn\textsuperscript{+2} and Ni\textsuperscript{+2} are most effective. Anions and monovalent cations do not influence the reaction. This action of cations is general for the decarboxylation of \(\beta\)-keto dicarboxylic acids, such as oxalacetate, acetonedicarboxylate, and oxalosuccinate (5). Non-enzymatic decarboxylation of \(\alpha\)- or \(\beta\)-keto monocarboxylic acids is not affected by metal ions.

Enzymes which catalyze the decarboxylation of oxalacetate have been found in bacteria (4, 6), animal tissues (7), and plants (1). All these enzymes require metal ions for full activity, and Mn\textsuperscript{+2} has nearly always been employed. However, complete studies on the cation specificity of oxalacetic carboxylases from various sources have not been made. The carboxylase from parsley roots is activated by a considerable number of divalent positive ions, including Cu\textsuperscript{+2}, Pb\textsuperscript{+2}, Ba\textsuperscript{+2}, Mg\textsuperscript{+2}, Fe\textsuperscript{+2}, Ni\textsuperscript{+2}, Zn\textsuperscript{+2}, Ca\textsuperscript{+2}, Cd\textsuperscript{+2}, Co\textsuperscript{+2}, and Mn\textsuperscript{+2}; Mn\textsuperscript{+2} is most effective. There is no obvious correlation between the activities of the ions in the presence and absence of enzyme, but it appears that maximum activation of the enzyme
is achieved at lower concentrations than are required for maximum rates of decarboxylation in the absence of enzyme. A similar carboxylase prepared from the red radish is active in the presence of Pb++, Ni++, Zn++, Mg++, Cd++, Co++, and Mn++; Mn++ again gives the most rapid rates.¹ Oxalacetate acid carboxylase from pigeon liver is activated by Mn+++ (7) and less effectively by Co++.² The enzyme from Micrococcus lysodeikticus functions with Mg++ or Mn++ (4, 8) and that from Escherichia coli with Mn++ (6).

The concentrations of divalent cations in intact plant tissues are probably not sufficiently high to permit maximum activity of oxalacetic carboxylase unless the ions are localized at the site of enzyme action. For example, the Mn+++ content of parsley and parsnip roots is only about 0.075 mM per 1000 gm. of fresh weight.³ A number of the cations which activate the plant carboxylases are essential nutrients for plants; for example, Cu++, Mg++, Fe++, Zn++, Ca++, and Mn++ (10). These ions may function in part as cofactors for oxalacetic carboxylase or other enzymes of wide distribution and possible great importance in plant metabolism.

Kornberg, Ochoa, and Mehler (8) have recently presented evidence that the effect of cations such as Al+++ and Mn+++ on oxalacetate decarboxylation is due to formation of an unstable cation-oxalacetate complex, which decomposes to give pyruvate, carbon dioxide, and free cation. The carboxylase protein appears to accelerate the formation or breakdown of the complex. A number of cations seem capable of forming such complexes with oxalacetate; the affinity between metal ions and oxalacetate and the rate of decomposition of the complex vary with the different metal ions. The ability of enzymes to accelerate the formation or breakdown of certain of these complexes introduces a new element of specificity into the effects, since enzymes from different sources may vary in their activity with different ions.

**SUMMARY**

Decarboxylation of oxalacetic acid in the presence of polyvalent cations follows first order kinetics. The rates of decarboxylation with fourteen different ions over a range of concentrations are reported. The enzyme oxalacetic carboxylase from parsley roots is activated by a number of divalent cations, of which Mn+++ is most effective. The relative enzyme activities with different concentrations of these metal ions are given.

¹ These experiments were performed by Miss Miriam C. Gollub of this department.
² Personal communication from Dr. Birgit Vennesland.
³ The analyses were kindly carried out by Dr. Ernest Kun, using a procedure which he has recently described (9).
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