THE OXALACETATE DECARBOXYLASE OF AZOTOBACTER VINELANDII*

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In 1941 Krampitz and Werkman (1) discovered an enzyme in Micrococcus lysodeikticus which catalyzed the decarboxylation of oxalacetate. By means of an exchange reaction they were able to demonstrate the incorporation of isotopic carbon dioxide into the β-carboxyl group of oxalacetate in the presence of this enzyme (2). Similar results were obtained by Evans, Vennesland, and Slotin (3) with an enzyme system from pigeon liver. Further studies on the C₅–C₁ condensation have been reviewed by Wood (4) and will, therefore, not be discussed here.

The apparent importance of oxalacetate decarboxylase as one of the gateway enzymes for the fixation of carbon dioxide into compounds of the Krebs isocitric acid cycle made it of interest to attempt to purify this enzyme and to study some of its properties. The biotin content of the enzyme fractions at various stages of purity was determined in view of the recent implication of biotin in the metabolism of carbon dioxide (5–11).

Lee, Burris, and Wilson (12) demonstrated that cell-free preparations of Azotobacter vinelandii have oxalacetate decarboxylase activity. Since this organism is easily grown on a large scale (13), cell-free extracts of this bacterium were used as the source material for this study.

EXPERIMENTAL

Purification of Enzyme

Azotobacter vinelandii was grown on the nitrogen-free medium of Lee and Burris (13) in 250 liter quantities with vigorous agitation and aeration at 30°C in 100 gallon iron tanks. After 24 hours the cells were harvested with a Sharples centrifuge. Usually 400 to 600 gm. of cell paste were obtained. The bacterial centrifugate was suspended with vigorous agitation in 6 liters of acetone at 5°C. The suspension was filtered with suction. The filter cake, still wet with acetone, was resuspended in 6 liters of cold acetone with vigorous agitation. After filtration with suction the residue was placed in a vacuum desiccator containing calcium chloride and shaved.

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paraffin and was dried at 10° in vacuo for 10 to 15 hours. In various runs 100 to 150 gm. of dried cell powder were thus obtained.

The progress of the purification was followed with the method subsequently described for measuring enzymatic activity. Nitrogen was determined by a micro modification of the direct nesslerization procedure of Koch and McMeekin. All specific enzyme activities were expressed as microliters of CO₂ per 15 minutes per microgram of N at 30°.

In a typical experiment the following procedure was used. 20 gm. of acetone-dried cells were ground to a fine powder and then stirred vigorously for 2 hours in 400 ml. of distilled water at 5°. The suspension was centrifuged in an angle head centrifuge at high speed at 5° until the supernatant was only faintly turbid. The supernatant enzyme solution had a specific activity of 1 μl. of CO₂ per microgram of N. The residue was discarded and the solution was chilled in an ice bath and acidified with 1 N hydrochloric acid to pH 4.8. The heavy precipitate thus formed was centrifuged off at 5°. 300 ml. of a clear orange solution with a specific activity of 7 μl. of CO₂ per microgram of N was obtained with a yield of 80 per cent of the enzyme activity.

The acid solution was next treated at 5° with 240 ml. of approximately 1 M copper hydroxide gel. After very gentle agitation for 5 minutes the suspension was centrifuged in the cold for 5 minutes. The supernatant, pH 5.8, contained practically no activity and was discarded. The copper precipitate was immediately suspended in 240 ml. of cold 0.1 M phosphate buffer of pH 6.8 and stirred for 10 minutes. The suspension was immediately centrifuged in the cold. The light orange supernatant solution had a specific enzyme activity of 14 μl. of CO₂ per microgram of N. The recovery for the copper step was 50 per cent. However, the contact time with copper hydroxide during adsorption and elution had a profound effect on enzyme recovery and the purity of the final product. Longer adsorption times led to loss of activity, while shorter contact with the copper gel was less effective in removing impurities.

The eluate was dialyzed with rapid agitation against three changes of distilled water at 10° for 150 minutes and was subsequently lyophilized.

The tan-colored powder was dissolved in 30 ml. of cold 0.01 M ammonium hydroxide. The turbidity which consisted mainly of residual copper hydroxide was centrifuged off and discarded.

27 ml. of saturated ammonium sulfate¹ were added to 30 ml. of solution. Upon centrifugation in the cold the residue was discarded, since it had a specific activity of only 3 to 4 μl. of CO₂ per microgram of N and constituted only 10 per cent of the eluate activity. The supernatant solution

¹Saturated at room temperature (ca. 23°) and containing 0.02 M ammonium hydroxide.
was more completely saturated by the dropwise addition, with stirring, of 43 ml. of saturated ammonium sulfate solution. After centrifugation the supernatant solution was discarded, since it contained practically no activity. The residue was dissolved in 20 ml. of cold succinate-borate buffer, pH 6.8, and dialyzed for 150 minutes with strong agitation against two changes of 8 liters of distilled water at 10°. The enzyme solution was lyophilized. The pink powder usually had a specific activity of 30 to 35 μl. of CO₂ per microgram of N and contained 50 to 60 per cent of the activity present in the phosphate eluate of the copper hydroxide gel.

The powder was dissolved in 20 ml. of cold 0.01 M ammonium hydroxide and 17 ml. of saturated ammonium sulfate were added. The low potency precipitate was discarded and the supernatant solution was more completely saturated with 30 ml. of ammonium sulfate solution. The residue obtained upon centrifugation dissolved completely in 20 ml. of succinate borate buffer, pH 6.8. After dialyzing for 150 minutes against two changes of 8 liters of distilled water at 10°, the solution was lyophilized. A pink powder with a specific activity of 40 to 80 μl. of CO₂ per microgram of N was obtained. The yield in this step was 60 to 70 per cent.

**Method of Determination**

The procedure employed to test for oxalacetate decarboxylase activity was essentially a modification of that used by Straub (14) and Krampitz and Werkman (1).

The reaction was carried out in Warburg vessels with two side arms. The enzyme solution, 0.01 ml. of 0.2 M MnSO₄, and sufficient 0.1 M buffer at pH 6.8 to bring the final fluid volume to 3.0 ml. were placed in the main compartment of the vessels. The substrate (0.2 ml. of 0.1 M oxalacetic acid) was placed in one side arm and 0.2 ml. of 10 N H₂SO₄ in the other. Deviations from this mixture will be so indicated in the text. The oxalacetic acid was always dissolved in sufficient 0.134 N NaOH to give a final concentration of 0.1 M oxalacetate. These solutions were prepared immediately before use and added to the flasks just before attaching them to the manometers. The flasks were incubated for 5 minutes at 30° with the stop-cocks open, the stop-cocks were closed, and the equilibration was continued for 10 minutes. At the end of this time the initial reading was taken. The substrate was then dumped into the main compartment. The sulfuric acid was dumped from the other side arm 15

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2 Succinate-borate buffer was prepared by mixing 500 ml. of 0.1 M sodium succinate and 500 ml. of 0.1 M boric acid; the solution was adjusted to pH 6.8 by the addition of 0.1 N HCl.

3 All enzyme preparations were analyzed for free ammonia nitrogen and, when present, this was subtracted from the total nitrogen of the preparation.
minutes later to stop the enzymatic reaction and to liberate the carbon dioxide retained as bicarbonate by the buffer. The final readings were then taken. A boiled enzyme blank was run with all determinations to account for the chemical decomposition of the substrate. This blank value (identical with no enzyme blank) was subtracted from the total gas evolved.

Previous investigators (14–20), particularly those working with animal tissues, have reported the presence of a "heat-stable" substance which, even after boiling, catalyzed the decomposition of oxalacetate. Such a material was never found in Azotobacter preparations. Crude cell extracts contained a trace of pyruvic decarboxylase activity which disappeared after the first purification step.

The validity of the method as an assay procedure is demonstrated by the results in Fig. 1. There is a linear relationship between enzyme concentration and amount of gas evolved and likewise between time and gas evolved. Hereafter all activities quoted in this paper are expressed as microliters of CO₂ per 15 minutes at 30°.
Determination of the pH optimum revealed a wide range of maximum activity of the enzyme between pH 6.5 and pH 8. The chemical decomposition of oxalacetate increases in a linear fashion from pH 6 to pH 8.4 (Fig. 2).

![Graph showing the effect of pH on oxalacetate decarboxylation at 30°. Curve 1, chemical decomposition; Curve 2, net enzymatic decomposition. Specific enzyme activity, 28 μl. of CO₂ per microgram of N per 15 minutes; 0.00067 M Mn⁺⁺ present.]

**Activators**

Krampitz and Werkman (1) reported that magnesium and manganese were activators for the enzyme from *Micrococcus lysodeikticus*, while Evans et al. (3) found Mn⁺⁺ to be the activator of choice for the pigeon liver enzyme. Vennesland and Felsher (19) obtained an increase of parsley root enzyme activity by the addition of Mn⁺⁺ or Mg⁺⁺.
In much of our earlier work 0.00067 M Mn\(^{++}\) was used to activate the *Azotobacter* enzyme after it was found that this level allowed maximum enzyme activity (Table I). Several other metals were subsequently tested. Co\(^{++}\) (Tables I and II) and Zn\(^{++}\) were found to be active, Mg\(^{++}\) had slight activity, while Ni\(^{++}\) and Fe\(^{++}\) were inert (Table II). Zn\(^{++}\), Ni\(^{++}\), and,

**Table I**

*Activation of Azotobacter Oxalacetate Decarboxylase by Co\(^{++}\) and Mn\(^{++}\)*

Specific activity of enzyme, 14 \(\mu l\) of CO\(_2\) per microgram of N per 15 minutes. The data represent average values of two similar experiments.

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Concentration of activator</th>
<th>Activity</th>
<th>(\mu l) CO(_2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MnSO(_4)</td>
<td>CoCl(_2)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.00067</td>
<td></td>
<td>156</td>
</tr>
<tr>
<td></td>
<td>0.00201</td>
<td></td>
<td>156</td>
</tr>
<tr>
<td></td>
<td>0.00335</td>
<td></td>
<td>167</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.00067</td>
<td></td>
<td>39</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.00017</td>
<td>222</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.00034</td>
<td>204</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.00067</td>
<td>200</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.00067</td>
<td>196</td>
</tr>
<tr>
<td></td>
<td>0.00067</td>
<td>0.00008</td>
<td>198</td>
</tr>
<tr>
<td></td>
<td>0.00067</td>
<td>0.00017</td>
<td>212</td>
</tr>
</tbody>
</table>

**Table II**

*Metal Activation of Oxalacetate Decarboxylase*

Incubated 15 minutes at 30°. The enzyme preparation was dialyzed against distilled water. Specific enzyme activity, 2 \(\mu l\) of CO\(_2\), per microgram of N per 15 minutes.

<table>
<thead>
<tr>
<th>Activator</th>
<th>Chemical decomposition</th>
<th>Net enzymatic decomposition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\mu l) CO(_2)</td>
<td>(\mu l) CO(_2)</td>
</tr>
<tr>
<td>None</td>
<td>62</td>
<td>21</td>
</tr>
<tr>
<td>0.00067 M Mn(^{++})</td>
<td>62</td>
<td>203</td>
</tr>
<tr>
<td>0.00017 &quot; Co(^{++})&quot;</td>
<td>60</td>
<td>225</td>
</tr>
<tr>
<td>0.00067 &quot; Zn(^{++})&quot;</td>
<td>152</td>
<td>158</td>
</tr>
<tr>
<td>0.00067 &quot; Mg(^{++})&quot;</td>
<td>63</td>
<td>56</td>
</tr>
<tr>
<td>0.00017 &quot; Ni(^{++})&quot;</td>
<td>101</td>
<td>27</td>
</tr>
<tr>
<td>0.00067 &quot; Fe(^{++})&quot;</td>
<td>63</td>
<td>22</td>
</tr>
</tbody>
</table>

In higher concentrations, Co\(^{++}\) increased the rate of chemical decomposition.

**Inhibitors**

Utter and Wood (21) demonstrated that adenosine triphosphate (ATP) increased the fixation of CO\(_2\) into oxalacetate by pigeon liver extracts.
Vennesland et al. (20) purified this enzyme of pigeon liver. In agreement with Utter and Wood they obtained a stimulation of carbon dioxide fixation in the presence of ATP and also observed an inhibition by triphosphopyridine nucleotide. The decarboxylating activity of pigeon liver was found to be inhibited by ATP and stimulated by triphosphopyridine nucleotide.

**Table III**

*Effect of Adenylic Acid upon Azotobacter Oxalacetate Decarboxylase*

<table>
<thead>
<tr>
<th>Buffer system</th>
<th>Additions</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 M phosphate, pH 6.8</td>
<td>None</td>
<td>108</td>
</tr>
<tr>
<td>0.1 &quot; &quot; &quot; 6.8</td>
<td>0.002 M muscle adenylic acid</td>
<td>110</td>
</tr>
<tr>
<td>0.1 &quot; succinate-borate, pH 6.8</td>
<td>None</td>
<td>1650</td>
</tr>
<tr>
<td>0.1 &quot; &quot; &quot; 6.8</td>
<td>0.002 M muscle adenylic acid</td>
<td>1620</td>
</tr>
</tbody>
</table>

**Figure 3.** Inhibition of oxalacetate decarboxylase. 0.1 M succinate-borate buffer pH 6.8, and 0.00067 M manganous sulfate. ●, pyrophosphate; △ adenosine triphosphate; ○, orthophosphate; ▲, L-malate.
It seemed possible from the foregoing that adenylic acid, acting as phosphate acceptor, might stimulate the rate of decarboxylation of the *Azotobacter* enzyme. This was found not to be the case. The possibility was next considered that the stimulating activity of adenylic acid could be demonstrated in the presence of low phosphate concentrations. The 0.1 M phosphate buffer of pH 6.8 which had been used in the studies up to that time was, therefore, replaced by a 0.1 M succinate-borate buffer of pH 6.8. No increase in activity due to adenylic acid was observed. These experiments are summarized in Table III.

When the activity of the same enzyme preparation was compared in the two buffers, it was 15-fold higher in the succinate-borate buffer. This observation pointed to the possible inhibition of the enzyme by phosphate. Increasing quantities of phosphate were added to the system in succinate-borate buffer. Fig. 3 shows the inhibiting effect of varying levels of phosphate. That succinate-borate is not stimulating was shown by the fact that its addition, at a level of 0.017 M, to a predominantly phosphate buffer system caused no increase in decarboxylating activity.

It seemed possible that the inhibition by phosphate was caused by a binding of the activator, manganese. Increasing quantities of manganous sulfate, up to 0.00335 M, were added to a system containing a constant small concentration of phosphate and a constant quantity of enzyme. No enhancement of activity resulted when the manganese level was increased.

It had been found in the meantime that Co++, activated the enzyme in lower concentrations than did Mn++. When Co++ was used as an activator, phosphate was found not to inhibit the enzymatic decarboxylation of oxalacetate (Table IV).

Upon the addition of increasing amounts of Co++ to a system containing constant amounts of Mn++ and phosphate, a progressive reversal of inhib-
Inhibition was found (Fig. 4). When these data were plotted in the manner customary for a competitive inhibition, a straight line was obtained (Fig. 5).

If the phosphate inhibition in the presence of Mn$^{++}$ were merely caused by a binding of manganese by phosphate, one would have expected a complete reversal of inhibition upon the addition of a small yet maximally activating quantity of Co$^{++}$. The progressive nature of the reversal by increasing amounts of Co$^{++}$ points to the formation of an inactive phosphate-manganese-enzyme complex. Apparently Co$^{++}$ competes with the manganese-phosphate complex for the same place of attachment on the enzyme.

**Fig. 4.** The reversal of orthophosphate inhibition by Co$^{++}$. Curve 1, no orthophosphate or Mn$^{++}$; Curve 2, 0.0067 M orthophosphate and 0.00067 M Mn$^{++}$. Aqueous extract of bacterial acetone powder. The data are averages of two experiments.
An attempt was made next to ascertain whether this inhibition was also of the substrate competitive type. Oxalacetate concentrations from 0.0017 M to 0.0303 M were employed in assays with a constant phosphate concentration (Table V). Although the data are variable, there is no consistent trend which would indicate a competitive type of inhibition. The enzymatic rate of decarboxylation was constant over the substrate concentration employed, while the chemical decomposition increased with rising oxalacetate. The latter is in keeping with observations of Straub (14) and

![Graph showing competitive reversal of orthophosphate inhibition by Co++.](image)

**Table V**

*Effect of Varying Concentrations of Oxalacetate upon Phosphate Inhibition*

Specific activity of enzyme, 5.5 μl. of CO₂ per microgram of N per 15 minutes. The data are averages of six experiments.

<table>
<thead>
<tr>
<th>Oxalacetate</th>
<th>Inhibition by 0.0067 M phosphate</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>per cent</td>
</tr>
<tr>
<td>0.0017</td>
<td>46.0</td>
</tr>
<tr>
<td>0.0025</td>
<td>52.0</td>
</tr>
<tr>
<td>0.0034</td>
<td>55.4</td>
</tr>
<tr>
<td>0.0067</td>
<td>46.0</td>
</tr>
<tr>
<td>0.0101</td>
<td>40.5</td>
</tr>
<tr>
<td>0.0303</td>
<td>52.0</td>
</tr>
</tbody>
</table>
Ochoa et al. (22) that the chemical decarboxylation follows first order kinetics. Concentrations lower than 0.0017 m and higher than 0.0303 m oxalacetate were not employed, since the former provided too little gas for accurate measurement and in the latter case the large chemical evolution of carbon dioxide made it difficult to determine the enzymatic reaction with accuracy.

ATP was found to be about twice as active an inhibitor of the Azotobacter enzyme as the same molar concentration of orthophosphate (Fig. 3). Of the various phosphorus compounds tested, pyrophosphate was the most effective inhibitor of the enzyme (Fig. 3). The inhibition by pyrophosphate is not the result of breakdown of this compound to orthophosphate, since the inhibition caused by the former is always more than twice as large as that caused by an equimolar concentration of orthophosphate. The enzyme does not exhibit apyrase activity.

**TABLE VI**

*Increasing Concentrations of Oxalacetate versus Malate Inhibition*

Specific enzyme activity, 7 μl. of CO₂ per microgram of N per 15 minutes. The data are averages of two experiments.

<table>
<thead>
<tr>
<th>Oxalacetate</th>
<th>Inhibition by 0.0101 m malate</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>per cent</td>
</tr>
<tr>
<td>0.0034</td>
<td>53.9</td>
</tr>
<tr>
<td>0.0067</td>
<td>41.0</td>
</tr>
<tr>
<td>0.0134</td>
<td>56.8</td>
</tr>
<tr>
<td>0.0268</td>
<td>54.5</td>
</tr>
</tbody>
</table>

Lwoff et al. (23, 24) observed that the oxalacetate decarboxylase activity of resting cell suspensions of Moraxella lwaffi is inhibited by malate. Ochoa and Weisz-Tabori (25) found malate to inhibit the decarboxylase of Micrococcus lysodeikticus and of pigeon liver. Malate retarded the activity of the enzyme from Azotobacter (Fig. 3). The inhibition could not be competitively reversed by the addition of increasing quantities of substrate (Table VI). The extent of inhibition was the same in the presence of Mn⁺⁺ or Co⁺⁺ (Table IV).

Evans et al. (3) reported the complete inhibition of pigeon liver oxalacetate decarboxylase by 0.01 M malonate. The Azotobacter enzyme was not inhibited by this concentration of malonate.

**Carbon Dioxide Fixation**

Exchange reactions of NaHCl¹⁴O₃ and oxalacetate in the presence of the Azotobacter enzyme were carried out according to the technique described by Krampitz et al. (2).
In all cases the amount of carbon dioxide fixed in oxalacetate was exceedingly small; in no case did it constitute more than 0.113 per cent of the β-carboxyl carbon of the remaining oxalacetate. The fixation was not increased by 0.004 M ATP, 0.0067 M phosphate, 0.0067 M pyruvate, or 0.00067 M fumarate, either alone or in combination. The degree of fixation of the crude bacterial extract (specific activity, 1 μL of CO₂ per microgram of N) was of the same order of magnitude as that of a purified preparation (specific activity, 80 μL of CO₂ per microgram of N).

### Table VII

**Biotin Content versus Enzymatic Activity (Fractionation 32)**

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Description of sample</th>
<th>Specific enzyme activity μL CO₂ per mg N</th>
<th>Untreated hydrolysate μgmg. biotin per 100 μL CO₂</th>
<th>Phosgene-treated hydrolysate μgmg. biotin per 100 μL CO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Crude bacterial extract</td>
<td>0.5</td>
<td>1.94</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>Acid-treated bacterial extract</td>
<td>4.0</td>
<td>1.35</td>
<td>1.51</td>
</tr>
<tr>
<td>D</td>
<td>Phosphate eluate of copper hydroxide</td>
<td>8.2</td>
<td>1.05</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>Residue from 2.4 M ammonium sulfate</td>
<td>3.7</td>
<td>8.76</td>
<td>8.07</td>
</tr>
<tr>
<td>G</td>
<td>Residue from 3.4 M ammonium sulfate</td>
<td>14.0</td>
<td>0.79</td>
<td>1.03</td>
</tr>
<tr>
<td>I</td>
<td>Residue from 2.4 M ammonium sulfate (refractionation of Fraction G)</td>
<td>3.6</td>
<td>2.34</td>
<td>2.32</td>
</tr>
<tr>
<td>J</td>
<td>Residue from 3.4 M ammonium sulfate (refractionation of Fraction G)</td>
<td>35.3</td>
<td>0.73</td>
<td>0.78</td>
</tr>
</tbody>
</table>

### Enzyme Activity and Biotin Content

The biotin content of some of the samples obtained during the fractionation of the *Azotobacter* enzyme was determined. *Lactobacillus arabinosus* was used as the test organism according to the method of Wright (26). The fractions were hydrolyzed by autoclaving at 15 pounds pressure for 2 hours in 2 N sulfuric acid to liberate bound biotin from the protein.

The results obtained (Table VII) seem to indicate that there was no increase in biotin concentration with respect to activity as a result of an increase in enzyme purity. It was found that the purest fraction described in Table VII, 32J, contains 0.017 mole of biotin per 100,000 gm. of protein (assuming a nitrogen content of 16 per cent). There was no increase in biotin activity upon treatment of hydrolysates with phosgene under the conditions of the Schotten-Baumann reaction, indicating that the samples contained no biotin diaminocarboxylic acid (Table VII). Control experi-
ments with pure biotin dianinocarboxylic acid⁴ resulted in almost quantitative conversion to biotin following treatment with phosgene.

**DISCUSSION**

In initial studies on the purification of this enzyme it was found that fractional precipitation with solvents or salts of the original or acid-treated aqueous bacterial extract did not yield fractions of increased purity. This behavior suggested that the enzyme may be attached to some inert material. Since the extracts gave a positive Molisch test, it seemed possible that the enzyme might be associated with a polysaccharide. Herriott (27), working on the purification of swine pepsinogen, removed the enzyme protein from accompanying carbohydrates by adsorption on copper hydroxide gel and by elution with phosphate buffer. When this method was applied to the decarboxylase, the eluate still gave a positive Molisch test but it was now possible to increase appreciably the purity of the enzyme in the eluate by salt fractionation. The purified decarboxylase preparation from Azotobacter which has an activity of 40 μl. of CO₂ per 15 minutes per microgram of N is approximately 30 times as active as the purest fraction obtained from Micrococcus lysodeikticus (28) and 83 times more active than the pigeon liver preparation (20).

The competitive reversal by Co⁺⁺ of the inhibition caused by phosphate in the presence of Mn⁺⁺ indicates the existence of an inactive phosphate-manganese-decarboxylase complex. It seems possible that the Co⁺⁺ competes with the phosphate-manganese complex for the same point of attachment on the enzyme. These observations are reminiscent of the inhibition of enolase by a magnesium-fluorophosphate complex (29). A similar inhibition of phosphoglucomutase has recently been reported by Najjar (30) in which the inhibitory agent is a complex of magnesium-fluoro-glucose-1-phosphate.

The apparent lack of carbon dioxide fixation into oxalacetate by the Azotobacter enzyme may be due to the fact that it only catalyzes the reaction

\[
\text{Oxalacetic acid} \rightleftharpoons \text{pyruvic acid} + \text{CO}_2
\]

The equilibrium of this reaction is so far in the direction of decarboxylation \((K = 4.9 \times 10^3 (3))\) that it is impossible to demonstrate the exchange of carbon dioxide. The demonstrated ability of extracts of Micrococcus lysodeikticus and pigeon liver to incorporate carbon dioxide into oxalacetate must be ascribed to the presence of coupling enzymes in these preparations. Even the purified pigeon liver enzyme of Vennesland et al. (20) is probably

⁴ We appreciate a gift of this compound from Dr. D. B. Melville and Dr. V. du Vigneaud.
still an enzyme system rather than a single carboxylase. In this connection it is of interest that Ochoa (22) has proposed that the "malic" enzyme is either a single enzyme or a "functional enzyme unit."

It is tempting to speculate that oxalacetate decarboxylase may act as the common gateway enzyme for the fixation of carbon dioxide into dicarboxylic acids in conjunction with different coupling enzymes, such as transaminase, citrogenase, etc. The coupling reaction may then supply the necessary energy for the endergonic fixation of CO₂ with pyruvate (9).

SUMMARY

1. The oxalacetate decarboxylase of Azotobacter vinelandii has been purified 40- to 50-fold from aqueous extracts of bacterial acetone powder. 
2. The enzyme was activated by Mn⁺⁺, Co⁺⁺, Zn⁺⁺, and slightly by Mg⁺⁺. Orthophosphate, pyrophosphate, and ATP were shown to inhibit the enzyme in the presence of Mn⁺⁺. Malate inhibited when Mn⁺⁺ or Co⁺⁺ was used as activator. Orthophosphate failed to slow the rate of enzymatic catalysis when Co⁺⁺ was used for activation. The inhibition by orthophosphate in the presence of Mn⁺⁺ was competitively reversed by the addition of increasing quantities of Co⁺⁺. 
3. Fixation of carbon dioxide in appreciable quantities into oxalacetate could not be shown with the Azotobacter enzyme. 
4. There was no increase in biotin concentration with respect to activity as a result of an increase in enzymatic purity. The most highly purified enzyme fraction which was assayed microbiologically for biotin activity was found to contain 0.017 mole of biotin per 100,000 gm. of protein.

We are indebted to Professor R. H. Burris, who first pointed out Azotobacter as a source of oxalacetate decarboxylase, for cultures of Azotobacter vinelandii. We also wish to thank Dr. R. L. Potter and Miss K. L. Armstrong for performing the microbiological assays for biotin.

BIBLIOGRAPHY

29. Warburg, O., and Christian, W., Biochem. Z., 310, 384 (1942).
G. W. E. Plaut and Henry A. Lardy

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