CHICKEN LIVER GLUTAMIC DEHYDROGENASE*

By JOHN E. SNOKE

(From the Department of Physiological Chemistry, University of California, Los Angeles, California)

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During the course of fractionation of chicken liver extracts, it was observed that glutamic dehydrogenase could be obtained in the crystalline form after a few simple purification steps. The procedure used in the isolation of glutamic dehydrogenase of chicken liver as well as some of the properties of the crystalline enzyme are described in the present communication. Although the chicken liver enzyme appears to be similar to the crystalline glutamic dehydrogenase from beef liver (1-3) previously described, the present results are reported in view of the simplicity of the isolation procedure and the availability of the starting material.

Materials and Methods

The pyridine nucleotides were the products of the Sigma Chemical Company, and ribose nucleic acid, L-glutamic acid, and α-ketoglutaric acid were purchased from the Nutritional Biochemicals Corporation. The reagent solutions were prepared as described by Olson and Anfinsen (1).

Enzymatic activity was determined by measuring the change of optical density of the reaction mixture at 340 m\(\mu\) in a model DU Beckman spectrophotometer. The composition of the reaction mixtures is described under "Results," in all cases the reaction having been initiated by the addition of the enzyme. The amount of enzyme added to the reaction mixture was chosen so that the rate of the reaction was proportional to enzyme concentration and essentially linear for at least 5 minutes. Unless otherwise indicated, enzymatic experiments were carried out with three times crystallized glutamic dehydrogenase.

For comparative purposes, the activity of the enzyme was defined in the same manner as that described by Olson and Anfinsen (1). The activity (\(\Delta E\)) was termed the change in optical density in 5 minutes (light path = 1 cm.), while the specific activity was defined as the change in optical density in 5 minutes per mg. of protein in 1 ml. of reaction mixture. Protein concentrations were determined by the method of Bücher (4).

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Results

Purification of Enzyme—Unless otherwise specified, all fractionation procedures were carried out at 0°. An acetone powder, prepared in the conventional manner from frozen chicken liver, was stirred for 1 hour with 10 volumes of 0.05 M phosphate buffer, pH 7.4, and the mixture centrifuged to yield the crude extract. To each liter of extract were added 122 ml. of ribose nucleic acid solution (50 gm. of nucleic acid and 7.5 ml. of 1 N sodium hydroxide per 100 ml.) and 204 ml. of 95 per cent ethanol. During the dropwise addition of the ethanol, the solution was stirred and cooled gradually to -5°. The mixture was adjusted to pH 6.75 with 2 M acetic acid, stirred for 15 minutes, and centrifuged at -5°. The precipitate was discarded. At a temperature of -5°, the supernatant fluid was adjusted to pH 6.25, stirred 15 minutes, and the precipitate collected by centrifugation. The precipitate was dissolved in a volume of water approximately one-sixth that of the crude extract. To the resulting solution was added 0.6 volume of a saturated ammonium sulfate solution (saturated at 0°), and, after adjusting the pH to 8.1 with 3.0 M ammonium hydroxide, the mixture was stirred for 30 minutes. The precipitate was collected by centrifugation at 20,000 X g for 15 minutes and dissolved in a minimal volume of water. Within a few minutes, crystallization of glutamic dehydrogenase occurred, as indicated by a pronounced silky sheen of the mixture when stirred. Crystallization was allowed to continue overnight and the precipitate was collected by centrifugation. Recrystallization was carried out by dissolving the crystals in 0.1 M potassium phosphate buffer, pH 7.4, at room temperature, adding 0.1 volume of saturated ammonium sulfate, and allowing the mixture to stand overnight in the cold. The enzyme crystallized as thin hexagonal plates which were readily visible in a microscope.

Occasionally, the precipitation of the enzyme during the first crystallization was incomplete. In this case, 1.0 volume of saturated ammonium sulfate was added to the supernatant fluid, the precipitated protein collected by centrifugation, and the crystallization step repeated. A summary of a typical fractionation is given in Table I.

The enzyme was completely stable for at least 6 months when kept at -15° in 0.1 M phosphate buffer, pH 7.4. In the purified state, the enzyme became rapidly inactivated if diluted in the absence of salt, and was also inactivated at pH 8.0, under conditions used for the assay. This latter inactivation was prevented by carrying out enzymatic experiments in the presence of 0.05 per cent serum albumin.

Effect of Enzyme Concentration—In Fig. 1 is shown the proportionality

1 A number of different commercial products were used without any noticeable difference.
between the concentration of the crystalline enzyme and the oxidative deamination of glutamate by DPN as well as the reverse reaction. Under the conditions described in Fig. 1, legend, the specific activity of glutamic

**Table I**

**Purification of Glutamic Dehydrogenase**

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Volume (ml)</th>
<th>Protein (mg. per ml)</th>
<th>Specific activity</th>
<th>Total activity (per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extract</td>
<td>1300</td>
<td>13.0</td>
<td>3.30</td>
<td>100</td>
</tr>
<tr>
<td>Nucleic acid-ethanol ppt.</td>
<td>200</td>
<td>37.5</td>
<td>15.1</td>
<td>81</td>
</tr>
<tr>
<td>Ammonium sulfate ppt.</td>
<td>25</td>
<td>52.3</td>
<td>47.5</td>
<td>44</td>
</tr>
<tr>
<td>Crude crystals</td>
<td>12.0</td>
<td>25.0</td>
<td>111</td>
<td>24</td>
</tr>
<tr>
<td>Recrystallized once</td>
<td>10.0</td>
<td>13.6</td>
<td>155</td>
<td></td>
</tr>
<tr>
<td>“</td>
<td></td>
<td>3.5</td>
<td>183</td>
<td></td>
</tr>
</tbody>
</table>

The starting material was 155 gm. of acetone-dried chicken liver. The activities of the various fractions were determined at pH 8.0 and 23°. The reaction mixtures, 3.0 ml. in volume, contained 0.167 M Tris buffer, 0.0133 M l-glutamate, 2.0 × 10^{-4} M DPN, and 0.05 per cent serum albumin.

The effect of enzyme concentration on the initial reaction rate. The experimental conditions for glutamate oxidation (●) were identical to those given in Table I. Experiments in which the reverse reaction was measured (○) were carried out at pH 7.6 and 23° in the presence of 0.167 M Tris buffer, 1.5 × 10^{-4} M DPNH, 0.15 M ammonium chloride, 1.11 × 10^{-4} M α-ketoglutarate, and 0.05 per cent serum albumin.

The specific activity of glutamic dehydrogenase was 183 when the oxidation of glutamic acid by DPN² was measured and 1350 when the reverse reaction was studied. When DPN

*The following abbreviations were used: Tris, tris(hydroxymethyl)aminomethane; DPN, diphosphopyridine nucleotide; DPNH, reduced diphosphopyridine nucleotide.
was replaced by an equimolar concentration of triphosphopyridine nucleotide, the rate of glutamic acid oxidation was one-third the rate observed with DPN.

Effect of pH—The effect of pH on the oxidation of glutamic acid by DPN and on the oxidation of DPNH by ammonia and α-ketoglutarate is seen in Fig. 2. The oxidation of glutamic acid proceeds maximally at pH 8.0 in both Tris and phosphate buffers, but the rate at pH 8.0 in phosphate buffer is only 50 per cent of that observed in Tris buffer. The rate of DPNH oxidation in Tris buffer is optimal at pH 7.6. Like the oxidation of glutamic acid by DPN, the reverse reaction is also inhibited by phos-

![Graph](http://www.jbc.org/)

**Fig. 2.** Effect of pH on glutamic dehydrogenase activity. The experimental conditions for the oxidation of DPNH by α-ketoglutarate and ammonium chloride (●) were identical to those given in Fig. 1. The experimental conditions for glutamate oxidation were identical to those given in Table I except for the buffer. Buffer used, (○) 0.167 M Tris; (▲) 0.167 M potassium phosphate.

### Table II

<table>
<thead>
<tr>
<th>Salt added</th>
<th>Relative activity (per cent)</th>
<th>Salt added</th>
<th>Relative activity (per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>100</td>
<td>0.1 M Na₂HPO₄</td>
<td>77</td>
</tr>
<tr>
<td>0.1 M K₂HPO₄</td>
<td>60</td>
<td>0.1 M KCl</td>
<td>92</td>
</tr>
<tr>
<td>0.1 M Na₂SO₄</td>
<td>100</td>
<td>0.2 M KCl</td>
<td>85</td>
</tr>
<tr>
<td>0.3 M NaCl</td>
<td>100</td>
<td>0.1 M NaNO₃</td>
<td>40</td>
</tr>
</tbody>
</table>

The oxidation of glutamic acid by DPN was determined in the presence of the above salts under the conditions given in Table I.
phate. At pH 7.6 in Tris buffer, the rate of DPNH oxidation was inhibited 34 per cent when 0.1 M potassium phosphate was added.

Salt Inhibition—The inhibition of glutamic dehydrogenase by a number of salts is shown in Table II. The lower activity of the enzyme in potassium phosphate buffer, as compared to that observed in Tris buffer (Fig. 2), is due to the inhibition by potassium as well as phosphate ions, since both sodium phosphate and potassium chloride are inhibitory. This inhibition is not related to ionic strength since both sodium chloride and sodium sulfate at an ionic strength of 0.3 had no effect on enzymatic activity. The inhibition of glutamic dehydrogenase by nitrate ions is even more effective than by phosphate.

It was of interest to determine whether the inhibition by phosphate was of a competitive nature. For this purpose the effect of glutamate and DPN concentration on enzymatic activity was determined in both the presence and the absence of potassium phosphate. The results obtained were plotted according to one of the equations of Lineweaver and Burk.
(5), as shown in Fig. 3. If the inhibition by phosphate was competitive, it would be expected that the maximal velocity which is given by the slope of the lines plotted in Fig. 3 should be independent of the presence of phosphate. The data in Fig. 3 show that increasing the concentration of either glutamate or DPN does not overcome the inhibition by potassium phosphate, and therefore the inhibition is of a non-competitive type. The $K_m$ values calculated from the data in Fig. 3 were $2.0 \times 10^{-3} \text{M}$ for glutamate, and $6.1 \times 10^{-4} \text{M}$ for DPN.

**DISCUSSION**

The present enzyme appears to be similar to the glutamic dehydrogenase of beef liver previously described (1-3). The specific activity and pH optimum of the enzymes are approximately the same when determined under identical conditions. Both enzymes are inhibited by salts, although, under the conditions used for studying the chicken liver enzyme, the inhibition is observed at lower salt concentrations and is not related to ionic strength. The nature of the salt inhibition is not clear.

**SUMMARY**

By means of a simple fractionation procedure, glutamic dehydrogenase has been isolated in the crystalline form from extracts of acetone-dried chicken liver. The procedure is based on precipitation with nucleic acid and ammonium sulfate fractionation. The pH optimum of glutamic acid oxidation by DPN was found to be 8.0, while the reverse reaction had a pH optimum of 7.6.

**BIBLIOGRAPHY**

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John E. Snoke

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