Purification and Properties of the Inducible Nicotinamide Adenine Dinucleotide Phosphate-Specific Glutamate Dehydrogenase from *Chlorella sorokiniana*

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The nicotinamide adenine dinucleotide phosphate-specific glutamate dehydrogenase (NADP-GDH) of *Chlorella sorokiniana* was purified 260-fold to electrophoretic homogeneity in six steps. Depending on the technique used, the native enzyme appeared to have a molecular weight of 290,000 or 410,000 and to be composed of five to seven identical subunits with a molecular weight of 58,000. The amino acid composition of this enzyme was shown to differ considerably from that of the NAD-GDH in this organism. The NH₂-terminal amino acid was unavailable to dansylation. All six cysteines in the native enzyme were in the free sulfhydryl form. The pH optima for the aminating and deaminating reactions were 7.2 and 9.2, respectively. The $K_m$ values for NH₄⁺, $\alpha$-ketoglutarate, NADPH, L-glutamate, and NADP⁺ were 68, 12, 0.13, 32, and 0.038 mM, respectively. At low substrate concentrations, no cooperativity was seen; however, severe inhibition of enzyme activity was observed at high $\alpha$-ketoglutarate concentrations. Nucleotides did not affect enzyme activity. Antiserum produced in rabbits to the subunits of the enzyme yielded a single precipitin band with the purified enzyme in Ouchterlony double-diffusion analysis. Immunelectrophoresis was used to confirm the purity of the enzyme and also to quantify the amount of enzyme antigen. These studies indicate that the NADP-GDH and NAD-GDH isozymes are distinct molecular species in this organism.

A number of inducible enzymes in eucaryotic microorganisms (6, 7, 23) are being used as models to study the mechanism of enzyme induction in eucaryotic cells. One such model system under study in this laboratory, is the ammonium-inducible nicotinamide adenine dinucleotide phosphate-specific glutamate dehydrogenase (NADP-GDH) in *Chlorella* (24, 29). This organism also contains a constitutive NAD-GDH isozyme (24, 29) that is synthesized in either ammonium- or nitrate-containing medium.

In this laboratory, Talley et al. (29) and Israel et al. (14) have observed that the activities of both GDH isozymes change dramatically during the cell cycle and during shifts in the nitrogen nutritional status of cells of *Chlorella sorokiniana*. As initial steps for the elucidation of the molecular mechanisms responsible for the changes in activity of these isozymes, it will be necessary to determine whether these isozymes are distinct molecular species in primary structure or are readily interconvertible forms (i.e.,

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A covalent modification or sharing of subunits, etc.), and to measure directly by immunological procedures (21, 22) the rates of synthesis and turnover of these isozymes independent of activity measurements.

Because the inducible NADP-GDH has not been purified completely from any species of *Chlorella*, the purpose of the research was to accomplish its purification from *C. sorokiniana* for (i) comparison of its physical and kinetic properties with the NAD-GDH recently purified from this organism (19) and from *C. pyrenoidosa* (24) and (ii) preparation of antibody against it for use in future immunological studies. (This paper is part of the thesis of R. M. G. submitted in partial fulfillment of the requirements for the M.S. degree.)

**MATERIALS AND METHODS**

**Organism and growth conditions.** *C. sorokiniana* (25) was cultured in ammonium-containing medium (14) in a 35-liter Plexiglas chamber as previously described (2).

**Harvest procedure for cells.** When the cell number reached approximately $200 \times 10^6$ cells per ml, the cells in 30-liter suspension were harvested with a Sharples centrifuge at 50,000 rpm, resuspended in 2 vol-
umes of 0.01 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride buffer (pH 8.25), and then pelleted at 5,000 × g for 10 min. The cells were washed twice with the buffer, resuspended in an equal volume of the buffer (0.1 M), and frozen and stored at −20°C.

**Enzyme and total protein assays.** The assays for the *Chlorella* NADP- and NAD-GDH isozymes were essentially those described by Israel et al. (14).

The assay mixture for the measurement of the amating reaction of the NADP-GDH was modified slightly to contain 50 mM Tris-hydrochloride buffer, 300 mM (NH₄)₂SO₄, 25 mM α-ketoglutarate, 0.35 mM NADPH, and 20 μl of enzyme solution to give a final assay volume of 1.02 ml and a pH of 7.2 at 38.5°C. The assay mixture for the deaminating reaction of the NADP-GDH was modified to contain 50 mM Tris-hydrochloride buffer, 200 mM L-glutamate, 1 mM NADP⁺, and 20 μl of enzyme solution to give a final assay volume of 1.02 ml and a pH of 8.7 at 38.5°C. One unit of GDH is defined as the amount of enzyme activity required to oxidize or reduce 1 μmol of coenzyme per min at 38.5°C.

Malate dehydrogenase and catalase were assayed by the procedures of Gregory et al. (12) and of Beers and Sizer (3), respectively.

Total protein was measured by the method of Lowry et al. (18). Thiols and other substances that would interfere with the protein analysis were removed by ultrafiltration in an Amicon model 12 cell with a PM-10 membrane.

**Analytical electrophoresis.** At the different steps in the subsequent purification procedure, the homogeneity of the NADP-GDH was examined by disc electrophoresis in polyacrylamide gels (9). The bands of NADP-GDH activity and protein were located by a tetrazoilum assay system and by Coomassie brilliant blue staining, respectively, as previously described (19, 29).

**Purification of NADP-GDH.** Unless stated otherwise, all purification steps were performed at 4°C. Approximately 200 g (fresh weight) of frozen cells, from 60 liters of culture, was thawed and then ruptured by passage through a mechanically driven French pressure cell (model 5-598A, American Instrument Co.) at a pressure of 18,000 to 20,000 lb/in². The cell homogenate was centrifuged at 100,000 × g for 1 h in a Beckman L2-65B ultracentrifuge (SW-27 rotor). The approximately 350 ml of supernatant (100,000 × g) was used as the starting material for purification of the NADP-GDH. The resulting supernatant (100,000 × g) was refrozen twice at −20°C and thawed at 22°C, and the precipitate was removed by centrifugation. The frozen-thawed supernatant was brought to 40% saturation with (NH₄)₂SO₄. The precipitate was removed by centrifugation, and the supernatant was brought to 70% (NH₄)₂SO₄ saturation. The resulting precipitate, containing most of the NADP-GDH activity, was dissolved in 60 ml of 0.01 M Tris-hydrochloride buffer (pH 7.4). This solution of proteins was allowed to stand for 3 days, and then a large precipitate was discarded after removal by centrifugation. The protein solution from the previous step was applied to a column (5 by 39 cm) of Sephadex G-200, which was equilibrated and eluted with 0.01 M Tris-hydrochloride buffer (pH 7.4). The fractions with NADP-GDH activity were combined and applied to a column (2.5 by 24 cm) of Whatman DE-52 that was equilibrated with 0.01 M Tris-hydrochloride buffer (pH 7.4). After this sample was applied, the column was washed with buffer and then eluted with a linear gradient from 0 to 0.4 M NaCl in the buffer. The enzyme solution from the previous step was adjusted to 0.1 M (NH₄)₂SO₄ and 2 mM dithiothreitol. The enzyme was bound to Ca₃(PO₄)₂ (80 mg/ml) suspended in 0.01 M Tris buffer, pH 7.4), pelleted by centrifugation, resuspended, and washed four times in 0.1 M Tris buffer (pH 8.2), containing 0.4 M (NH₄)₂SO₄ and 2 mM dithiothreitol, and then subjected to ultrafiltration to concentrate the enzyme and to change to the electrophoresis buffer (0.19 M glycine–2 mM dithiothreitol–0.025 M Tris-hydrochloride buffer, pH 8.75). Before preparative electrophoresis, the enzyme solution was adjusted to 10 mM dithiothreitol and then allowed to stand at 22°C for 1 to 3 h. Electrophoresis was performed at 20 mA with a 7% polyacrylamide separating gel (1 cm) and a 2.5% stacking gel (3 cm) in a Channelo Prep-Disc apparatus in the P2-320 column.

**Determination of molecular weight of NADP-GDH and its subunit.** Sedimentation equilibrium was performed by the meniscus depletion method of Yphantis (34). Centrifugation of the enzyme (0.2 mg/ml) was performed at 8,786 rpm at 4.2°C in a Beckman Spinco model E ultracentrifuge equipped with interference optics.

Gel filtration was performed with a Sephadex G-200 column (1.5 by 84 cm) equilibrated with 0.2 M Tris-hydrochloride buffer (pH 8.2) at 4°C. The column was calibrated by the method of Andrews (1). Elution of the protein standards was monitored at 280 nm, with the exception of catalase. The data were plotted by a modification of the method of Laurent and Killander (17).

Gradient gel electrophoresis was performed in 2.5 to 7% polyacrylamide slab gels (Gradiopore) in 0.01 M Tris-hydrochloride, 0.08 M borate, and 0.003 M ethylene diamine tetraacetic acid, by use of a Pharmacia GE-4 electrophoresis apparatus.

From the migration distance of the native NADP-GDH during analytical gel electrophoresis in 5, 7, and 9% polyacrylamide gels, a Ferguson plot (10) was used, as discussed by Rodbard and Chrambach (20), to estimate the molecular weight of the enzyme.

The molecular weight of the subunit of the dissociated NADP-GDH was estimated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis as described by Laemmli (15).

**Amino acid analysis.** The amino acid composition of the purified NADP-GDH was measured with a Beckman model 121 automatic amino acid analyzer by the procedure of Spackman et al. (28). The enzyme was hydrolyzed in 6 N HCl under an N₂ atmosphere for 24 h and dried in vacuo.

The tryptophan and tyrosine content of the enzyme was measured by the method of Benzce and Schmid (4). By titration with 5,5'-dithiobis (2-nitrobenzoic acid) in 1% SDS, the total number of sulfhydryl groups in the enzyme was measured as described by Habeeb (13). With the exception that the enzyme was not
oxidized, the dansylation method for NH₂-terminal amino acids was performed exactly as recommended by Gray (11). For this latter analysis, standard dansylated amino acids were separated by polyamide thin-layer chromatography with the solvent systems described by Woods and Wang (32).

Immunological techniques. Preimmune serum and NADP-GDH antiserum were prepared from blood obtained from a female New Zealand White rabbit. Before immunization of the rabbit with the purified NADP-GDH, blood was collected for preparation of preimmune serum. For the initial injection, 1 mg of NADP-GDH was dissolved in 0.5 ml of 0.04% SDS in water, mixed with 0.5 ml of Freund complete adjuvant, and then sonically treated (Sonifier cell disruptor, model W185D, Heat Systems-Ultrasonics, Inc.; microprobe) for approximately 1 min or until a stable emulsion was obtained. A 0.5-ml amount of this emulsion was injected intramuscularly into each thigh. After 3 weeks, the rabbit was injected with 0.5 mg of NADP-GDH prepared and administered as with the initial injection. Equivalent booster injections were given at 3-week intervals thereafter. Beginning with the second injection, blood was collected 2 weeks after each booster injection.

Svendsen buffer (Bio-Rad Buffer III) was used in all immunological procedures. Ouchterlony immunodiffusion (8) was performed in 1% agarose layered onto microscope slides (2.5 by 7.5 cm). The immunodiffusion from 4-mm-diameter wells was performed at 22°C in a water-saturated atmosphere for at least 18 h.

For “rocket” immunoelectrophoresis (16), 1% agarose was mixed with sufficient serum to give a final serum concentration of 1 or 3% (vol/vol) and was layered onto microscope slides. Immunoelectrophoresis was performed in a Bio-Rad electrophoresis cell (model 1400) at 15 to 25 V/cm for 3 to 5 h at 12°C. The agarose gels were then pressed and stained as described by Weeke (31).

Reagents. The dansyl amino acids, ceruloplasmin, and apoferritin, were from Sigma Chemical Co.; catalase was from Calbiochem, Inc.; chymotrypsinogen, ovalbumin, and aldolase were from Pharmacia Fine Chemicals, Inc.; immunoelectrophoresis buffer III and Ca₃(PO₄)₂ were from Bio-Rad Laboratories; polyamide layers were from Pierce; Freund complete adjuvant was from Grand Island Biological Co.; and gradient gels were from Isolab, Inc.

RESULTS AND DISCUSSION

Purification of the NADP-GDH. This inducible GDH was purified to electrophoretic homogeneity in six steps beginning with a supernatant (100,000 × g) from a cell homogenate (Table 1). Essentially all of the NADP-GDH activity in cell homogenates was recovered in the supernatant (100,000 × g). When analyzed by analytical (Fig. 1) and by gradient polyacrylamide gel electrophoresis, the purified NADP-GDH also gave only a single band of protein.

With the exception of the preparative gel electrophoresis step, the activity of the NADP-GDH was stable through all of the purification steps in the absence of added reducing agents. By pretreatment of the enzyme preparation with 10 mM dithiothreitol and the addition of 2 mM

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TABLE 1. Purification of NADP-GDH from C. sorokiniana

<table>
<thead>
<tr>
<th>Step</th>
<th>Total U*</th>
<th>Total protein (mg)</th>
<th>Sp act†</th>
<th>Recovery (%)</th>
<th>Fold purification*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supernatant (100,000 × g)</td>
<td>9,400</td>
<td>12,000</td>
<td>1.3</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>1. Freeze-thaw</td>
<td>8,000</td>
<td>3,300</td>
<td>2.5</td>
<td>85</td>
<td>2</td>
</tr>
<tr>
<td>2. Ammonium sulfate precipitation (40–70%)</td>
<td>6,400</td>
<td>910</td>
<td>7.0</td>
<td>68</td>
<td>5</td>
</tr>
<tr>
<td>3. G-200 gel filtration</td>
<td>5,500</td>
<td>850</td>
<td>16</td>
<td>58</td>
<td>12</td>
</tr>
<tr>
<td>4. DE-52 ion-exchange chromatography</td>
<td>3,400</td>
<td>82</td>
<td>42</td>
<td>36</td>
<td>32</td>
</tr>
<tr>
<td>5. Ca₃(PO₄)₂ gel</td>
<td>2,900</td>
<td>23</td>
<td>130</td>
<td>31</td>
<td>100</td>
</tr>
<tr>
<td>6. Preparative electrophoresis</td>
<td>2,400</td>
<td>7</td>
<td>340†</td>
<td>25</td>
<td>260</td>
</tr>
</tbody>
</table>

* One unit is defined as the enzyme necessary to oxidize 1 μmol of NADPH per min at 38.5°C.
† Specific activity is defined as units per milligram of protein.
* Fold purification is defined as the specific activity of step x per specific activity 100,000 × g.
† Specific activity decreases with storage due to loss of catalytic activity.
dithiothreitol to the buffer reservoirs, 100% of the NADP-GDH activity could be stabilized during preparative gel electrophoresis.

Although dithiothreitol stabilized the activity of the NADP-GDH during preparative electrophoresis, this reducing agent (10 mM) caused the loss in activity of the enzyme in the purified state or in crude cell extracts during storage at −20°C. In the absence of dithiothreitol, the activity of the enzyme in crude cell extracts was stable at −20°C. In the absence of added substrates, the activity of the purified enzyme was stable in degassed 0.1 M Tris-hydrochloride buffer under argon at 4°C; however, at −20°C for 1 week, 50% of its activity was lost. NADPH (1 mM) was the only substrate that enhanced the loss in enzyme activity at −20°C.

As observed for the NAD- and NADP-GDH isozymes in *C. pyrenoidosa* (24), the thermal stability characteristics of these isozymes are also quite different in *C. sorokiniana*. In the present study, whereas the NAD-GDH was observed to be stable for at least 25 min at 60°C, the NADP-GDH activity decayed rapidly (i.e., $t_{1/2} = 3$ to 4 min) at this temperature.

**Molecular weight of NADP-GDH and its subunit.** From the elution volumes of protein standards and of the purified NADP-GDH from a Sephadex G-200 column, the molecular weight of the native enzymes was estimated to be 400,000 (Fig. 2). Gradient polyacrylamide gel electrophoresis (Fig. 3) and polyacrylamide gel electrophoresis by the method of Ferguson (10) gave molecular weight estimates of 420,000 and 400,000, respectively.

By sedimentation equilibrium, the molecular weight of the native enzyme was estimated to be 290,000. Because molecular weights measured by this latter method are not influenced greatly by the shape of native proteins, the molecular weight of the NADP-GDH estimated by this method is probably closer to the actual value. By use of sucrose density gradient centrifugation, Talley et al. (29) estimated the molecular weight of the same enzyme in crude cell extracts to be 270,000. Differences in molecular weight estimates of the magnitude observed with the NADP-GDH have been proposed to occur with "cigar-shaped" proteins (27).

By use of SDS-polyacrylamide gel electrophoresis by the method of Laemmli (15) and also Weber and Osborn (30), with two different gel concentrations and two different buffers, the subunit(s) of the dissociated NADP-GDH was shown to migrate as a single protein species (Fig. 4) with a molecular weight of approximately 58,000 (Fig. 5). Thus, the native NADP-GDH appears to be composed of five to seven identical subunits.

In contrast, Meredith et al. (19) estimated the native NADP-GDH from *C. sorokiniana* to have a molecular weight of 180,000 by all methods tested and to be composed of four identical subunits with a molecular weight of 45,000. The NAD-GDH from *Neurospora crassa* is the only other GDH reported to be composed of only four subunits (6).

**Amino acid composition of NADP-GDH.** The amino acid composition of the NADP-GDH was shown (Table 2) to be quite different from that reported by Meredith et al. (19) for the NAD-GDH in this same organism.

Because the cysteine content of the NADP-GDH was measured by two independent methods, the value of six cysteines per subunit was used to calculate a subunit molecular weight of 59,500. This molecular weight estimate is in good agreement with the value determined by sedimentation equilibrium and SDS-polyacrylamide gel electrophoresis.
agreement with the molecular weight (i.e., 58,000) estimated by SDS-polyacrylamide gel electrophoresis. In the native NADP-GDH, six sulfhydryl groups could be titrated with Ellman reagent (13), i.e., 5,5'-dithiobis (2-nitrobenzoic acid). Thus, all of the cysteine residues in the enzyme appear to exist in the free sulfhydryl form, indicating that the enzyme is similar to GDHs from other organisms in that it lacks disulfide bridges (26).

When the average hydrophobicity ($H_{\text{ave}}$) of the NADP-GDH from C. sorokiniana was calculated from its amino acid composition by the method of Bigelow (5), a value of 1,056 cal per residue was obtained which is 339 cal per residue higher than that reported (19) for the NAD-GDH from this same organism. The average

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Residues/subunit$^a$</th>
<th>NADP-GDH</th>
<th>NAD-GDH$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lys</td>
<td>38</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>His</td>
<td>6</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Arg</td>
<td>28</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Cys</td>
<td>6</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Asp (Asn)</td>
<td>53</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>Met</td>
<td>15</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Thr</td>
<td>24</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Ser</td>
<td>30</td>
<td>74</td>
<td></td>
</tr>
<tr>
<td>Glu (Gln)</td>
<td>55</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>Pro</td>
<td>23</td>
<td>38</td>
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</tr>
<tr>
<td>Gly</td>
<td>54</td>
<td>35</td>
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</tr>
<tr>
<td>Ala</td>
<td>50</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Val</td>
<td>38</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Ile</td>
<td>23</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Leu</td>
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<td>14</td>
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</tr>
<tr>
<td>Phe</td>
<td>15</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Tyr</td>
<td>21</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Trp</td>
<td>5</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Subunit molecular weights of NADP and NAD enzymes are 59,500 and 45,000, respectively.
$^b$ Data from reference 19.
hydrophobicity of most proteins, and those GDHs examined, is close to 1,000 cal per residue (5).

The NH₂-terminal amino acid of the NADP-GDH appears to have its α-amino group blocked. The identical dansylation procedure (11) used in this laboratory to show (19) that lysine is the NH₂-terminal amino acid of the NAD-GDH, indicated that the amino group of the NH₂-terminal amino acid of the NADP-GDH is unavailable for dansylation. The absence of a free NH₂-terminal group has also been observed for both the NADP- and NAD-GDH isozymes in N. crassa (6, 33).

**pH optima and Michaelis constants for NADP-GDH.** The pH optima for the aminating and deaminating reactions of the NADP-GDH were 7.2 and 9.2, respectively. Because of instability of substrates above pH 9.0, the Km values for the substrates in the deaminating direction were measured at pH 8.7. At the pH optima for the enzyme, the activity with NAD⁺ or NADH was only approximately 0.1% of that with NADP⁺ and NADPH. The Km values for the substrates are given in Table 3. The Km values for glutamate and NADP⁺ were identical at pH 7.8 and 8.7.

From Hill plots of kinetic data, it was revealed that the substrates do not exert cooperative effects on NADP-GDH activity. However, above 25 mM α-ketoglutarate, enzyme activity was inhibited severely.

Although nucleotides have been reported to affect the activity of GDHs in other organisms (26), the activity of NADP-GDH in the aminating direction was unaffected by 1.0 mM AMP, ADP, ATP, CMP, CDP, CTP, GMP, GDP, or GTP.

**Immunological studies with NADP-GDH.** With antiserum developed in rabbits from purified NADP-GDH, Ouchterlony double-diffusion analysis showed that only a single precipitin band forms with either the purified NADP-GDH or with a crude enzyme preparation purified through the freeze-thaw purification step. Preimmune serum gave no precipitin bands with these enzyme preparations. At 0.25 μg of protein,

![Fig. 6. Rocket patterns produced by immunoelectrophoresis of 0.25 μg (A), 0.5 μg (B), and 1.0 μg (C) of purified NADP-GDH from C. sorokiniana in a 1% agarose gel containing 3% antiserum produced against the purified enzyme. After electrophoresis, the protein on the gel was stained with Coomassie brilliant blue.](http://jb.asm.org/)

TABLE 3. Comparison of physical and kinetic properties of GDH isozymes isolated from two species of Chlorella

<table>
<thead>
<tr>
<th>Source and nucleotide specificity</th>
<th>Mol wt (× 10⁴)</th>
<th>pH optimum (amination)</th>
<th>Km values for substrates (mM)</th>
<th>Pyridine nucleotide</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>NH⁺</td>
<td>α-Ketoglutarate</td>
</tr>
<tr>
<td>C. sorokiniana-NAD⁺</td>
<td>2.9-4.1</td>
<td>7.2</td>
<td>68</td>
<td>12</td>
</tr>
<tr>
<td>C. sorokiniana-NAD</td>
<td>1.8</td>
<td>8.0</td>
<td>40</td>
<td>2.0</td>
</tr>
<tr>
<td>C. pyrenoidosa-NAD⁺</td>
<td>3.0</td>
<td>8.1</td>
<td>18</td>
<td>0.35</td>
</tr>
<tr>
<td>C. pyrenoidosa-NAD</td>
<td>3.0-3.1</td>
<td>8.7</td>
<td>41</td>
<td>1.3</td>
</tr>
</tbody>
</table>

*a* Cooperativity was seen with several substrates with the C. pyrenoidosa enzymes.

*b* The Km stated is for the pyridine nucleotide of highest activity (i.e., NADP and NADPH for NADP-enzyme).

*c* Data from reference 19.

*d* Data from reference 24.
the NAD-GDH did not form a precipitin band with NADP-GDH antiserum. Therefore, it is tentatively concluded that NADP-GDH antibody does not cross-react with the NAD-GDH.

Because rocket immunoelectrophoresis is a more sensitive technique than Ouchterlony double diffusion, the homogeneity of the purified NADP-GDH was analyzed by this technique. From the rocket patterns, the purified NADP-GDH appears to be immunologically homogenous (Fig. 6). Moreover, the peak heights of the rockets are proportional to the amount of NADP-GDH antigen present and less than 0.25 μg of the enzyme could be detected (Fig. 7). This specific antibody will be useful in elucidation of the molecular events associated with the induction of the enzyme and also with the observed rapid in vivo loss in its activity upon NH₄⁺ removal from cells (14).

In conclusion, the NADP-GDH purified for the first time from C. sorokiniana (or any other species of Chlorella) appears to be physically, chemically, kinetically, and antigenically distinct from the NAD-GDH isozyme in the same organism. Moreover, these isozymes appear to have different properties from their counterparts in C. pyrenoidosa (Table 3).

ACKNOWLEDGMENTS

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