Partial Purification and Properties of d-Glucosamine 6-Phosphate N-Acetyltransferase from *Phaseolus aureus*

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ABSTRACT

d-Glucosamine-6-P N-acetyltransferase (EC 2.3.1.4) from mung bean seeds (*Phaseolus aureus*) was purified 313-fold by protamine sulfate and isoelectric precipitation, ammonium sulfate and acetone fractionation, and CM Sephadex column chromatography. The partially purified enzyme was highly specific for d-glucosamine-6-P. Neither d-glucosamine nor D-galactosamine could replace this substrate. The partially purified enzyme preparation was inhibited up to 50% by 2 × 10⁻⁵ M EDTA, indicating the requirement of a divalent cation. Among divalent metal ions tested, Mg²⁺ was required for maximum activity of the enzyme. Mn²⁺ and Zn²⁺ were inhibitory, while Co²⁺ had no effect on the enzyme activity. The pH optimum of the enzyme in sodium acetate and sodium citrate buffers was found to be 5.2. The effect of Mg²⁺ on the enzyme in sodium acetate and sodium citrate buffers was particularly noticeable in the range of optimum pH. *Km* values of 15.1 × 10⁻⁴ M and 7.1 × 10⁻⁴ M were obtained for d-glucosamine-6-P and acetyl CoA, respectively. The enzyme was completely inhibited by 1 × 10⁻⁴ M *p*-hydroxymercuribenzoate, and this inhibition was partially reversed by L-cysteine, indicating the presence of sulfhydryl groups at or near the active site of the enzyme.

The presence of this enzyme together with all the other enzymes involved in the pathway for the biosynthesis of uridine diphosphate-N-acetyl-d-glucosamine in higher plants was demonstrated by Mayer et al. (8). Using acetyl-1-¹⁴C coenzyme A, these investigators were able to synthesize radioactive AcGm-6-P by crude extracts of mung bean seeds in the presence of Gm-6-P. The identity of the labeled product as AcGm-6-P was established by paper electrophoresis.

The present paper will deal with the partial purification of d-glucosamine-6-P N-acetyltransferase from *Phaseolus aureus* seeds by protamine sulfate and isoelectric precipitation, ammonium sulfate and acetone fractionation, and CM Sephadex column chromatography. A study of some of the kinetic properties of the partially purified enzyme will be made and compared with the data reported for mammalian and fungal enzymes.

MATERIALS AND METHODS

Materials. The following chemical compounds were obtained from Sigma Chemical Co.: sodium salt of Gm-6-P (98% pure); trilithium salt of CoA (85% pure); protamine sulfate (Grade I); PHMB (sodium salt); and Gm hydrochloride. Ellman's reagent [5, 5'-dithiobis-(2-nitrobenzoic acid)] for the determination of sulfhydryl groups was purchased from Aldrich Chemical Co. All other chemicals were obtained through other commercial sources.

Methods. Acetyl-CoA was prepared from CoA by a combination of the procedures described by Ochoa (9) and Stadtman (14). To 10 mg of CoA, 0.4 ml of water was added, and 1 µl of the solution was analyzed for —SH groups by the procedure of Ellman (4). To the rest of the above CoA solution, 0.10 ml of 1.0 M KHCO₃ was added, and the mixture was cooled to 0 C. To this solution, 0.15 ml of freshly prepared ice-cold 0.10 M acetic anhydride was added, stirred, and left at 0 C for 10 min. The pH of the solution was then brought to 6.0 by dropwise addition of 1 N HCl, and the excess acetic acid was removed from the solution by extraction with 2-ml aliquots of ether five times. The traces of ether were removed by bubbling nitrogen gas through the solution (14). Completion of the acetylation reaction was checked by estimating sulfhydryl groups on 10-µl aliquots of this solution (4). Measurement of acetyl-CoA in this preparation was performed by the procedure of Lipmann and Tuttle (6) as described by Stadtman (13), using acetyl-CoA reagent as a standard.

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3. Abbreviations: Gm-6-P: d-glucosamine-6-P; Gm: d-glucosamine; Gm-6-P: d-galactosamine; AcGm-6-P: N-acetyl-d-glucosamine-6-P; AcGm: N-acetyl-d-glucosamine; CM: carboxymethyl; PHMB: 

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[5, 5'-dithiobis-(2-nitrobenzoic acid)]
The pH of this acetyl-CoA preparation was adjusted to the desired level by dropwise addition of 0.1 N HCl or 0.1 N NaOH, and the solution was immediately used as substrate for the enzyme assay.

AcGm-6-P was determined by a modified procedure of Reissig et al. (11). A 0.15-ml aliquot of the supernatant solution was withdrawn from the incubation mixture after inactivation in the presence of potassium tetraborate and boiling for 3 min followed by centrifugation. To this aliquot, 0.38 ml of freshly prepared Ehrlich’s aldehyde reagent (15) was added, and the mixture was incubated at 37°C for 20 min. At the end of this period, the colorimeter was set to zero absorbance using control mixtures prepared in the same manner as the incubation mixtures with the exception that the enzyme was boiled, and the absorbances were read at 585 nm. The amount of AcGm-6-P produced was extrapolated from standard curves established by treating various concentrations of AcGm under the same conditions. Protein was estimated by the procedure of Lowry et al. (7).

Enzyme Assay. Two types of standard incubation mixtures were used for the assay of d-glucosamine-6-P N-acetyl transferase enzyme. The first standard incubation mixture used during the purification of the enzyme was based on that employed by Davidson et al. (3) for the purification of the enzyme from Neurospora. The mixture contained the following components in μmoles in a final volume of 0.25 ml: Gm-6-P, 4.0; acetyl-CoA, 1.12; potassium phosphate buffer (pH 6.6), 18.75; EDTA (disodium salt), 0.65; and a certain amount of the enzyme protein. The mixtures were incubated at 37°C for 20 min. At the end of this incubation period, 0.05 ml of 0.8 M potassium tetraborate, pH 9.0, was added, and the reaction was terminated upon heating for 3 min in a boiling water bath. Control tubes, in which the enzyme had been inactivated by boiling for 3 min in the presence of potassium tetraborate prior to their incubation with the substrates, were included. Occasionally, control tubes prepared in the same manner as the incubation mixtures, except for the omission of acetyl-CoA, were included. After inactivation of the enzymatic reaction and removal of denatured proteins by centrifugation, 0.15-ml aliquots of the incubation mixtures were used for the determination of AcGm-6-P formed, as described before.

The second standard incubation mixture, mainly employed in kinetic studies, was prepared based on the properties of this enzyme; it contained the following compounds in μmoles in a final volume of 0.25 ml: Gm-6-P (pH 5.2), 4.0; acetyl-CoA (pH 5.2), 1.12; sodium acetate buffer (pH 5.2), 15; MgCl₂, 10; and a known amount of the partially purified enzyme protein. The enzyme preparation contributed 0.05 μmole of EDTA, 2.5 μmoles of KCl, and 0.42 μmole of potassium phosphate buffer, pH 7.3, to the incubation mixture. The pH of the incubation mixture was measured, making sure that it stayed in the range of 5.15 to 5.25. The mixtures were incubated for 30 min at 37°C, and after terminating the reactions, the products were analyzed as described under the first standard incubation mixture.

Acid phosphatase activity in the partially purified enzyme preparation was assayed by the procedure of Berger and Rupple (1). The incubation mixture contained the following in μmoles in a final volume of 1.2 ml: p-nitrophenylphosphate, 7.6; sodium acetate buffer (pH 5.2), 150; MgCl₂, 100; and 164 μg of the partially purified enzyme preparation from the second CM-Sephadex column. The mixture was incubated at 37°C for 30 min. At the end of the incubation period, the reaction was stopped by the addition of 5 ml of 0.1 N NaOH. The denatured proteins were removed by centrifugation, and the absorbance of p-nitrophenol produced was measured at 400 nm. Control tubes, prepared in the same manner as the incubation mixture except for the inactivation of the enzyme by addition of 0.1 N NaOH prior to their incubation with the substrate, were used to set the colorimeter to zero absorbance.

d-Glucosamine-6-P deaminase activity in the partially purified enzyme preparation was assayed upon incubation of the enzyme with Gm-6-P followed by the measurement of d-fructose-6-P produced by the procedure of Roe and Papadopoulos (12).

Preparation of the Crude Enzyme. Mung bean seeds were soaked overnight in distilled water; they were rinsed with distilled water several times, blotted with paper towels, and dehydrated. Two hundred twenty-four grams of the dehydrated beans were chilled at 4°C for 2 hr, ground in a mortar with acid-washed sand in 70 ml of 0.05 m phosphate buffer (pH 6.6) made 0.002 m in EDTA, and filtered through two layers of cheesecloth. The extract was then centrifuged at 12,000 g for 15 min, and the supernatant fluid was used as the source of crude enzyme.

Protease Sulfate Precipitation. To 180 ml of the crude extract prepared as above, 68.4 ml of a 2% aqueous solution of protease sulfate were added dropwise while the mixture was being stirred on ice (3). After additional stirring of the suspension for another 10 min at 0°C, the suspension was centrifuged at 34,800 g for 15 min. The supernatant solution is referred to as the “protease sulfate fraction.”

pH Adjustment. The pH of the protease sulfate fraction was adjusted to 4.9 by dropwise addition of ice-cold 6 M acetic acid at 0°C. The precipitate formed was removed by centrifugation at 27,000 g for 15 min. The supernatant fluid was readjusted to pH 6.0 by dropwise addition of 1 N NaOH to 0°C. This fraction is referred to as the “pH 4.9 supernatant fluid.”

Ammonium Sulfate Fractionation. To 175 ml of the pH 4.9 supernatant solution previously adjusted to pH 6.0, solid ammonium sulfate was added to a saturation of 40%. The suspension formed was centrifuged at 27,000 g for 10 min, and the precipitate was discarded. The supernatant solution (190 ml) was brought to 70% saturation with respect to ammonium sulfate by gradual addition of solid ammonium sulfate. After centrifugation as above, the precipitate, 40 to 70% saturation fraction, was suspended in 0.05 M phosphate buffer, pH 6.6, made 0.002 m in EDTA to a final volume of 180 ml. This fraction is referred to as the “ammonium sulfate fraction.”

Acetone Fractionation. To 180 ml of the ammonium sulfate fraction, 162.5 ml of cold—20°C acetone were added gradually, while the enzyme solution was constantly being stirred. The turbid solution formed was centrifuged at 4,080 g for 20 min at —10°C. To the entire resulting supernatant solution, 167.1 ml of cold—20°C acetone were added gradually, and the turbid solution was centrifuged as before. The precipitate formed was suspended in 0.0167 M potassium phosphate buffer, pH 7.3, made 0.10 m in KCl and 0.002 m in EDTA, respectively, to a final volume of 15 ml. This fraction was referred to as the “acetone fraction.”

First CM-Sephadex Column Chromatography. CM-Sephadex C-50 (Pharmacia Fine Chemicals), with a particle size of 40 to 120 μ and a capacity of 4.5 ± 0.5 meq/g, was suspended in 0.0167 M potassium phosphate buffer, pH 7.3, made 0.10 m in KCl and 0.002 m in EDTA and left overnight at 4°C. The fine particles were removed by washing in the same buffer for several times in a large graduated cylinder. A column (37 × 2.5 cm) was packed with the CM-Sephadex slurry and equilibrated with the same buffer mixture. Five milliliters of the acetone fraction, 20 to 26 mg protein/ml, were applied to the column, and the column was eluted with the same buffer KCl mixture. Fractions (2.5 ml) were collected every 2.5 min. The protein elution pattern was established by reading the absorbance at 280 nm against the buffer. The solution from every
peak tube was assayed for the enzyme activity. The solution from the protein peak having enzyme activity was concentrated to a final volume of 1.5 to 1.6 ml by ultrafiltration over a UM-2 Diaflo membrane (Amicon Corporation) at a nitrogen pressure of 55 p.s.i. This fraction was referred to as the “first CM-Sephadex fraction.” This fraction was used either immediately in the next step or stored over liquid nitrogen for later use.

Second CM Sephadex Column Chromatography. To a column of CM-Sephadex (26 × 1.5 cm) prepared as above, 1.5 to 1.6 ml of the enzyme solution from the first CM-Sephadex chromatography was applied, and the column was eluted with the same buffer as in the first CM-Sephadex chromatography. Fractions (2.5 ml) were collected every 5 min. The solution from the major protein peak containing the enzyme was concentrated to a final volume of 5 to 6 ml over UM-2 Diaflo membrane as above. This enzyme preparation was stored over liquid nitrogen and used in kinetic studies of the enzyme.

RESULTS AND DISCUSSION

Enzyme Purification. A typical CM-Sephadex chromatogram of the mung bean proteins is shown in Figure 1. As noticed, three protein peaks were obtained with the acetyltransferase activity present in peak II. Recchromatography of the concentrated protein solution from this peak on CM-Sephadex column resulted in the formation of two protein peaks as shown in Figure 2 with the acetyltransferase activity being present in peak II. A summary of the data on the partial purification of the enzyme is presented in Table I. A 313-fold purification of the enzyme with 65% yield was obtained. Column chromatography on diethylaminoethyl cellulose, on Sephadex G-100, or adsorption chromatography on calcium phosphate gel did not result in any further purification of the enzyme. As seen in Table I, adjustment of pH to 4.9 did not result in an increase in specific activity compared to the protamine sulfate fraction. However, this step was necessary for clearing the supernatant solution for its use in the latter steps. Heating of the protamine sulfate fraction to 60 C at pH 4.9 or pH 6.0 resulted in complete inactivation of the enzyme and could not be used as a step for further purification of the enzyme as employed by others (3, 10). Ammonium sulfate fractionation resulted in an increase of the enzyme yield (Table I), most probably due to the removal of certain inhibitors.

The partially purified enzyme preparation was free from acid phosphatase and Gm-6-P deaminase, assayed according to the procedures. The enzyme activity was observed in peak II.

Effect of Time and Enzyme Concentration on Enzyme Activity. The effect of time on enzyme activity is shown in Figure 3. The enzyme activity was linear with the time of incubation up to 20 min in the absence of Mg²⁺, then it leveled off with a further increase in the time of incubation (Fig. 3). Inclusion of Mg²⁺ ion in the incubation mixture (Fig. 3) resulted in linearity between the enzyme activity and the time of incubation up to 40 min studied.

Figure 4 shows that the enzyme activity was linear with the concentration of the protein from the second CM-Sephadex fraction up to 61.5 µg of protein included in 0.25 ml of the incubation mixture.

Table I. Purification of d-Glucosamine-6-P N-Acetyltransferase from Phaseolus aureus seeds

<table>
<thead>
<tr>
<th>Step</th>
<th>Volume</th>
<th>Enzyme Activity</th>
<th>Protein</th>
<th>Specific Activity</th>
<th>Purification</th>
<th>Total Activity</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ml</td>
<td>units/ml</td>
<td>mg/ml</td>
<td>units/mL</td>
<td>fold</td>
<td>units/mL</td>
<td>%</td>
</tr>
<tr>
<td>1. Crude</td>
<td>56.4</td>
<td>522</td>
<td>101.9</td>
<td>5.12</td>
<td>1.00</td>
<td>29,440.8</td>
<td>100</td>
</tr>
<tr>
<td>2. Protamine sulfate fraction</td>
<td>54.8</td>
<td>375</td>
<td>32.1</td>
<td>11.68</td>
<td>2.28</td>
<td>20,550.0</td>
<td>69.8</td>
</tr>
<tr>
<td>3. pH 4.9 supernatant fluid</td>
<td>54.8</td>
<td>330.8</td>
<td>27.1</td>
<td>12.20</td>
<td>2.38</td>
<td>18,127.8</td>
<td>67.6</td>
</tr>
<tr>
<td>4. Ammonium sulfate fraction</td>
<td>56.4</td>
<td>419.1</td>
<td>6.9</td>
<td>60.73</td>
<td>11.86</td>
<td>23,637.2</td>
<td>80.2</td>
</tr>
<tr>
<td>5. Acetone fraction</td>
<td>4.7</td>
<td>4,080.5</td>
<td>26.0</td>
<td>156.94</td>
<td>30.65</td>
<td>19,178.3</td>
<td>65.5</td>
</tr>
<tr>
<td>6. First CM-Sephadex (peak II)</td>
<td>1.6</td>
<td>12,044.1</td>
<td>10.9</td>
<td>1104.95</td>
<td>215.81</td>
<td>19,270.6</td>
<td>65.5</td>
</tr>
<tr>
<td>7. Second CM-Sephadex (peak II)</td>
<td>6.0</td>
<td>3,242.6</td>
<td>2.02</td>
<td>1605.24</td>
<td>313.52</td>
<td>19,455.5</td>
<td>65.5</td>
</tr>
</tbody>
</table>

1 Enzyme units expressed as nmoles of AcGm-6-P produced per hr under standard assay condition 1 at 37 C.
2 This step was mainly used to clarify the protamine sulfate fraction and obtain a clear solution for later fractionation steps.
Enzyme Activity as a function of time. Assays were done under standard conditions 1 (●) or 2 (○), except that reactions stopped at time intervals indicated. The enzyme sources were 44.8 μg (●) or 61.5 μg (○) of the second CM-Sephadex fraction per 0.25 ml of the incubation mixture. Results expressed as nmoles of AcGm-6-P formed per ml of the incubation mixture.

Effect of pH on Enzyme Activity. The effect of pH on the activity of mung bean acetyltransferase is shown in Figure 5. The enzyme showed an optimum pH of 5.2 both in citrate and in acetate buffer. The acetate buffer incubation mixtures contained Mg++. The higher activity of the enzyme in acetate buffer in the presence of Mg++ is especially noticeable in the range of the optimum pH. The pH optimum of 5.2 observed for mung bean enzyme is considerably lower than the values of 7.4 obtained by Pattabiraman and Bachhawat (10) for the enzyme from sheep brain, and also lower than the optimum pH range of 6 to 7.1 obtained for N. crassa enzyme by Davidson et al. (3). Apparently, these investigators have not considered the buffering capacity of Gm-6-P and the pH of this substrate has not been adjusted to the desired level. This may account for their optimum pH values of this enzyme. In fact, in experiments where the pH of the individual substrates were not adjusted to the indicated levels, similar curves were obtained to those of Davidson et al. (3).

Effect of EDTA and Divalent Cations on Enzyme Activity. Figure 6 shows that the acetyltransferase is inhibited up to 50% by 20 × 10^{-4} M EDTA. In this respect, this enzyme is different from N. crassa (3) and the sheep brain enzymes (10) which are actually activated by EDTA to some extent. The effect of divalent cations such as Mg++, Mn++, and Co++ on the activity of the mung bean enzyme in the presence of 8.6 × 10^{-4} M EDTA is shown in Figure 7. Mg++ ions had a slight inhibitory effect at lower concentrations, whereas a linear increase in enzyme activity was obtained with further increase in Mg++ concentration up to 4.0 × 10^{-2} M Mg++ ion concentration tested. From this point on, this concentration of Mg++ was included in the incubation mixtures used in kinetic studies of the enzyme. The optimum concentration of Mg++ ions required for maximum enzymatic activity has not been tested. Mn++ ion had exactly the opposite effect, and an increase in the concentration of this ion resulted in a linear decrease in the activity of the enzyme up to 4.0 × 10^{-2} M Mn++ tested. Co++ ion had practically no effect on the activity of the mung bean acetyltransferase. Patterson and Bachhawat (3) had observed a slight enhancing effect of Mg++ and Mn++ ions on the activity of the enzyme from sheep brain. They had also observed that Co++ ion had no effect on the activity of this enzyme from sheep brain.
The inhibition of the enzyme by PHMB and its partial reactivation by L-cysteine is an indication for the presence of sulfhydryl groups at or near the active site of the enzyme.

Effect of Gm-6-P and Acetyl-CoA Concentration on the Enzyme Activity. The plot for the velocity of the enzymatic reaction as a function of Gm-6-P concentration and its double reciprocal plot is shown in Figure 8, A and B. As calculated from the x-intercept of Figure 8B, a $K_m$ value of $1.51 \times 10^{-3}$

![Figure 7](image7.png)

**Fig. 7.** Effect of divalent cations on the activity of mung bean Gm-6-P N-acetyltransferase. Assays were done under standard condition 2 with the exclusion of Mg$^{2+}$ and inclusion of $8.6 \times 10^{-5}$ M EDTA. Divalent cations, Mg$^{2+}$ (●), Co$^{2+}$ (○), or Mn$^{2+}$ (△) in their chloride forms were included as indicated. Incubations were done for 20 min. The enzyme source was 50.47 μg of the enzyme protein from the second CM-Sephadex fraction in 0.25 ml of the incubation mixture. Results were expressed as in Figure 5.

**Table II. Effect of p-Hydroxymercuribenzoate and L-Cysteine on Activity of Mung Bean Acetyltransferase**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Additions</th>
<th>Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>None</td>
<td>0%</td>
</tr>
<tr>
<td>2</td>
<td>Plus 1 × 10^{-3} M cysteine</td>
<td>26.8%</td>
</tr>
<tr>
<td>3</td>
<td>Plus 1 × 10^{-3} M PHMB</td>
<td>23.3%</td>
</tr>
<tr>
<td>4</td>
<td>Plus 5 × 10^{-3} M PHMB</td>
<td>91.1%</td>
</tr>
<tr>
<td>5</td>
<td>Plus 5 × 10^{-3} M PHMB and 1 × 10^{-3} M cysteine</td>
<td>27.7%</td>
</tr>
<tr>
<td>6</td>
<td>Plus 1 × 10^{-4} M PHMB</td>
<td>100%</td>
</tr>
</tbody>
</table>

Zn$^{2+}$ ion completely inhibited the mung bean acetyltransferase activity at $1 \times 10^{-2}$ M ZnCl$_2$ concentration, and slightly higher concentration of Zn$^{2+}$ resulted in the precipitation of Gm-6-P substrate.

**Effect of p-Hydroxymercuribenzoate and L-Cysteine on Enzyme Activity.** The effect of PHMB and L-cysteine on the activity of the partially purified mung bean acetyltransferase is shown in Table II. The enzyme lost 23.3% of its activity in the presence of $1 \times 10^{-4}$ M PHMB; 91.1% of the activity was lost in the presence of $5 \times 10^{-3}$ M PHMB; and the enzyme was completely inactivated in the presence of $1 \times 10^{-4}$ M PHMB. Complete inhibition of the sheep enzyme had been observed by $1 \times 10^{-4}$ M p-chloromercuribenzoate (4). Inclusion of $1 \times 10^{-3}$ M L-cysteine hydrochloride resulted in the re-enhancement of the enzymatic activity. However, the re-enhancement was not complete and in fact when only L-cysteine hydrochloride was included in the incubation mixture, it inhibited the enzyme activity up to 26.8% (Table II). This observation may be due to the fact that the product of this enzymatic reaction (CoA) contains sulfhy dryl groups, and the presence of a sulfhydryl containing compound-like L-cysteine in the incubation mixture may result in inhibition of the enzymatic reaction by a mechanism similar to product inhibition.

![Figure 8](image8.png)

**Fig. 8.** A: Rate of acetyltransferase activity as a function of Gm-6-P concentration; B: double reciprocal plot of A. The assays were done according to the standard condition 2, except that Gm-6-P concentrations were varied as indicated. The enzyme source was 61.5 μg of the second CM-Sephadex fraction per 0.25 ml of the incubation mixture. Velocities (v) are expressed as nmoles of AcGm-6-P produced per hour per ml of the incubation mixture.

![Figure 9](image9.png)

**Fig. 9.** A: Rate of acetyltransferase activity as a function of acetyl CoA concentration; B: double reciprocal plot of A. The assays were done according to the standard condition 2, except that acetyl-CoA concentrations were varied as indicated. The enzyme source was 61.5 μg of the second CM-Sephadex fraction per 0.25 ml of the incubation mixture. Velocities (v) are expressed as in Figure 8.
When ml enzyme (7.8 M) was added to the reaction under standard assay conditions, a maximum velocity of 1060 nmoles of Gm-6-P/ hr·ml of the incubation mixture was obtained, as calculated from the y-intercept of Figure 8B.

Similar plots for the relationship between the velocity of the enzymatic reaction and the concentration of acetyl-CoA are shown in Figure 9, A and B. From the x- and the y-intercepts of Figure 9B, a Km value of 7.1 × 10⁻⁴ M acetyl-CoA and a maximum velocity (Vₘ) of 384.6 nmoles of AcGm-6-P/hr·ml of the incubation mixture were obtained, respectively.

The Km values of 15.1 × 10⁻⁴ M obtained for Gm-6-P and 7.1 × 10⁻⁴ M obtained for acetyl-CoA are approximately three times of those reported for the sheep brain enzyme (10). The Km value of 7.1 × 10⁻⁴ M observed for acetyl-CoA is comparable to that obtained by Davidson et al. (3) for the N. crassa enzyme (7.8 × 10⁻⁴ M), while the value obtained for Gm-6-P (15.1 × 10⁻⁴ M) is almost double that of N. crassa enzyme (7.8 × 10⁻⁴ M).

**Enzymatic Specificity.** The enzyme was completely inactive when either Gm or Galm was substituted for Gm-6-P under similar assay conditions.

**LITERATURE CITED**