The Partial Purification and Properties of Pig Brain Glycogen Synthase

JANET V. PASSONNEAU AND JOAN P. SCHWARTZ

From the Section on Cellular Neurochemistry, Laboratory of Neuropathology and Neuroanatomical Sciences, National Institute of Neurological Diseases and Stroke, National Institutes of Health, Bethesda, Maryland 20014

DAVID A. ROTTENBERG

From the Department of Neurology, The New York Hospital-Cornell University Medical College, New York, New York 10021

SUMMARY

Both the I (independent of glucose 6-phosphate) and D (dependent on glucose 6-phosphate) forms of glycogen synthase (UDP-glucose:glycogen α-4-glucosyltransferase EC 2.4.1.11) have been partially purified from pig brain and the kinetic constants of the enzymes have been examined. The $K_m$ for UDP-glucose for the I form increased from 0.11 to 0.5 mM when the temperature was raised from 25 to 37°C. When glucose 6-phosphate was present, the $K_m$ for UDP-glucose was decreased to 0.03 and 0.08 mM at 25 and 37°C, respectively. The amount of glucose 6-phosphate required to produce half-maximal stimulation decreased with increasing UDP-glucose concentration at both temperatures but increased with increasing temperature. The $K_m$ for glucose 6-phosphate at 0.03 and 0.20 mM UDP-glucose was 0.13 and 0.10 mM, respectively, at 25°C. At 37°C and 0.125 and 4.0 mM UDP-glucose the $K_m$ for glucose 6-phosphate was 0.32 and 0.04 mM, respectively. The $K_m$ for UDP-glucose for the D form at 0.75, 2.0, and 10 mM glucose 6-phosphate was 0.71, 0.50, and 0.42 mM at 25°C. At higher temperatures the apparent affinity for the substrate was decreased; at 37°C, the $K_m$ for UDP-glucose at 0.75 and 2.0 mM glucose 6-phosphate was 5.75 and 1.42 mM, respectively. The requirement for glucose 6-phosphate was decreased when UDP-glucose concentrations were increased; at 0.5 and 5.0 mM UDP-glucose concentrations, the $K_m$ for glucose 6-phosphate was 22.7 and 1.82 mM at 25°C. As was the case with the I form, the apparent $K_m$ for glucose 6-phosphate increased at higher temperatures. At 37°C, the $K_m$ for glucose 6-phosphate at 0.5 and 5.0 mM UDP-glucose was 43.5 and 6.15 mM. The temperature coefficient for the maximum velocity was 10.1% per degree for synthase I and 8.5% per degree for synthase D between 25 and 37°C. The D form of synthase was calculated to be virtually inactive under normal physiological conditions with the substrate concentrations found in the brain. The enzymatic activity calculated for synthase I correlates well with the observed rate of incorporation of UDP-[U-14C]glucose into brain glycogen.

Glycogen synthesis from uridine diphosphoglucose was first observed by Leloir and Cardini (1) in a crude homogenate of rat liver. Following this initial observation, the enzyme glycogen synthase (UDP-glucose:glycogen α-4-glucosyltransferase EC 2.4.1.11) has been described in a wide variety of tissues (2). The stimulation of glycogen synthase activity of glucose-6-P was noted originally by Leloir (2); two interconvertible forms of glycogen synthase subsequently were described in muscle, one of which was completely dependent on the presence of glucose-6-P (D or b) (3), and the other which was independent of glucose-6-P for catalytic activity (I or a). The two forms have since been shown to exist in a number of tissues including liver (4), heart (5), white blood cells (6), brain (7), spleen (8), adrenal gland (9), adipose tissue (10), and kidney (11, 12).

Kinetic studies have been made with brain glycogen synthase using either crude homogenates (7, 13) or partially purified cell-free preparations (14, 15). However, the I and D forms of brain synthase have not been isolated separately nor have any kinetic studies of the separated species been undertaken. In the present study we describe the partial purification of glycogen synthase I and D from pig brain and attempt to define the kinetic properties of the partially purified enzymes.

MATERIALS AND METHODS

Enzymes used for the analysis of glycogen synthase and phosphorylase activity were purchased from Boehringer Mannheim Corp. UDP-[U-14C]glucose (210 mCi per mmol) was purchased from the International Chemical and Nuclear Corp. All other chemicals were purchased from Sigma Chemical Co., St. Louis. Pig brains were purchased from Esskay, Baltimore, Md.

Preparation of Glycogen Synthase, I Form—Pig brains (1 kg) were homogenized at 0°C in a Waring Blender with 4 volumes of 25 mM Tris, pH 7.5, containing 5 mM dithiothreitol (DTT) and 1 mM EDTA (Tris-DTT-EDTA). The homogenate was centrifuged at 8000 × g (Table I) and the supernatant fractions were combined and brought to 0.75 M ammonium sulfate by the addition of the solid reagent and 1 M ammonium hydroxide to maintain the pH at 7.5. The precipitate was collected and dialyzed for 24 hours against Tris-DTT-EDTA, after which the dialyzed sample was centrifuged to remove denatured proteins and treated with calcium phosphate gel. The calcium phosphate gel (Sigma, type I) was suspended in water at a concentration of 25 mg of solids per ml and the final protein to solids ratio was 1:10.25. The gel then was sedimented by centrifugation and the supernatant fluid was discarded. The gel was washed with the Tris-DTT-EDTA reagent

1The abbreviations used are: DTT, dithiothreitol; TES, N-tris-(hydroxymethyl)methyl-2-aminoethanesulfonic acid.
Preparation of Glycogen Synthase, D Form—The preparation of the D form of the synthase was essentially the same as described for the I form with the following exceptions. Pig brain (500 g) was homogenized in 4 volumes of Tris-DTET-EDTA containing 20 mm KF and the supernatant fluid was collected after centrifugation at 500 x g. To the supernatant fluid (2 liters) was added 2 liters of 50 mm TES buffer at pH 7.0 containing 50 mm KF, 20 mm MgCl₂, 0.6 mm EGTA, 20 mm theophylline, 20 mm ATP, and 20 μM cyclic adenosine 3',5'-monophosphate. The purpose of this step was to effect conversion to the D form by protein kinase present in the homogenate (10). The combined mixture was brought to 30° and incubated for 20 min. Solid ammonium sulfate was added to 2 M concentration along with ammonium hydroxide to maintain the pH at 7.5. After the precipitate was collected, the calcium phosphate gel step and the second ammonium sulfate precipitate were performed as described for synthase I, but in the presence of 20 mM KF. After dialysis, when some synthase activity was found in Fraction 7, suitable correction was made for the negative temperature coefficient of the DPNH fluorescence (1.6% per degree) (18). In simultaneous assays, synthase activity was also measured by the incorporation of UDP-[U-14C]glucose into glycogen as described by Thomas et al. (19) with some minor modifications (17). Analyses of crude extracts by the incorporation of radiosotope into glycogen resulted in apparently lower activities than the measurement of UDP. The discrepancy was found to be due to phosphorylation of the newly formed glycogen (17). Agreement between the results of the two assays improved as enzyme purification proceeded.

Commercial loss of UDP-glucose was contaminated with UDP (0.3 to 1% on a molar basis) which created a troublesome blank in assays which depend on the measurement of UDP formation. This contamination was removed by column chromatography as described by Goldberg. UDP-glucose (1 g = 1.6 mmol) was dissolved in 5 ml of water and applied to a DE52 column (2 x 30 cm) which had been prewashed with 250 ml of 50 mm NaHCO₃, 500 ml of 100 mm NaHCO₃, and 250 ml of 25 mm redistilled triethylammonium bicarbonate, pH 7.5. UDP-glucose was eluted from the column with a linear gradient of 25 to 250 mM triethylammonium HCO₃ (1 liter), monitored at 260 nm, and collected in 10-ml fractions. The fractions containing UDP-glucose (Fractions 25 to 45) were pooled and evaporated to dryness in a rotary flash evaporator, water having been added several times until the amine odor disappeared. In some trials the UDP-glucose broke down to UDP and glucose during the prolonged evaporation step, apparently because of excessive heating. This was avoided by lyophilizing subsequent samples; although the amine odor was not completely removed, there was no apparent interference with the synthase reaction.

Phosphorylation activity was assayed as described by Lowry et al. (20). Protein was determined according to Lowry et al. (21). [14C]-Labeled glycogen was prepared by incubating either a crude muscle or one of the partially purified synthase fractions with UDP-[U-14C]glucose as precursor. The specific activity of UDP-[U-14C]glucose in the reaction mixture was 25 cpm per amol. The incubation mixture was otherwise identical with that used to assay synthase activity except that 10 mm glycogen was primed. After incubation at 37° for 1 or 2 hours, a solution of 95% KOH was added and the mixture heated 15 min at 100°. After cooling, 0.4 volume of 1% NaCl and 3.6 volumes of 95% ethanol were added, the precipitate was sedimented by centrifugation at 10,000 rpm for 10 min, the supernatant fluid was decanted, and the precipitate was resuspended in 1 ml of water and precipitated with 3 ml of 95% ethanol. The last step was repeated for a total of three times, and the residual glycogen was dissolved in distilled water. The specific activity of the resultant glycogen ranged from 155 to 1640 cpm per nmol (anhydroglucosyl units).

**RESULTS**

The purification of a representative preparation of the I form of synthase is outlined in Table I. A 300-fold over-all purification of the I form was achieved during which the proportion of synthase I increased from 77 to 94%. Although the degree of purification is comparable to that achieved by Soderling et al. (22) in rabbit muscle, synthase activity in brain is so low relative to its activity in muscle that the specific activity attained is only 1/20 that of the muscle preparation. Nevertheless, the enzyme was sufficiently free of phosphorylase (Table I) or other contaminating enzymes such as NADH oxidase, which would react in the analytical system. Recovery was 3%. Since the enzyme was dia-

### Table I

| Fraction | Volume | Protein | Total units | % Recovery | % I | Ratio of units of synthase to units phosphorylase |
|----------|--------|---------|-------------|------------|-----|-----------------------------------------------|
| Homogenate | 4300 | 29.3 | 103 | 1.53 | 100 | 77 | 0.23 |
| 2 M (NH₄)₂SO₄ precipitate | 700 | 31.4 | 129 | 5.86 | 67 | 0.64 |
| Calcium phosphate gel eluate | 738 | 6.66 | 87 | 17.7 | 45 | 1.69 |
| 1 M (NH₄)₂SO₄ precipitate | 28.34 | 52 | 51 | 27 | 96 | 7.15 |
| DE52 column concentrate | 10 | 12.8 | 21 | 210 | 14 | 1275 |
| Sepharose 4-B200 concentrate | 4 | 3.2 | 6 | 470 | 3 | 94 |

in a volume equal to the volume of the dialyzed sample. The enzyme was eluted from the gel with 0.2 M phosphate buffer, pH 7.5 (0.033 M KH₂PO₄-0.167 M K₂HPO₄); the gel was eluted twice, each time with a volume of buffer equivalent to the sample volume before gel treatment. Next, the solubilized enzyme was concentrated by precipitation with 1 M ammonium sulfate (neutralized as before) and the precipitate was collected and dialyzed against 100 volumes of Tris-DTET-EDTA reagent overnight at 4°. The dialyzed sample (18 ml, 600 mg of protein) was loaded on a Whatman DE52 cellulose column (20 x 2.5 cm). The enzyme was eluted in the cold with a linear Tris-DTET-EDTA gradient consisting of 250 ml of 25 mm buffer and 250 ml of 500 mm buffer. The effluent was collected in 5 ml fractions, monitored at 280 nm, and analyzed for glycogen synthase activity. The peak of enzyme activity was found in Fractions 40 to 42. These fractions were combined and concentrated by vacuum dialysis. The concentrated enzyme was loaded on a Sepharose 4B-200 column (13.5 x 2.5 cm) and eluted with 25 mm Tris-DTET-EDTA. Fractions, 3.5 ml, were collected and monitored at 280 nm, and the peak of enzyme activity was found in Fraction 7.

**Preparation of Glycogen Synthase, D Form**—The preparation of the D form of the synthase was essentially the same as that described above for the I form with the following exceptions. Pig brain (500 g) was homogenized in 4 volumes of Tris-DTET-EDTA containing 20 mm KF and the supernatant fluid was collected after centrifugation at 500 x g. To the supernatant fluid (2 liters) was added 2 liters of 50 mm TES buffer at pH 7.0 containing 50 mm KF, 20 mm MgCl₂, 0.6 mm EGTA, 20 mm theophylline, 20 mm ATP, and 20 μM cyclic adenosine 3',5'-monophosphate. The purpose of this step was to effect conversion to the D form by protein kinase present in the homogenate (10). The combined mixture was brought to 30° and incubated for 20 min. Solid ammonium sulfate was added to 2 M concentration along with ammonium hydroxide to maintain the pH at 7.5. After the precipitate was collected, the calcium phosphate gel step and the second ammonium sulfate precipitation were performed as described for synthase I, but in the presence of 20 mm KF. After dialysis, when some synthase had been converted again to the I form (Table III), the dialyzed sulfate fraction again was incubated with the TES reagent described above to effect I to D conversion of the synthase. The DE52 column chromatography was carried out as above. No Sepharose treatment was attempted since there was considerable loss of I form activity after chromatography without substantial purification (Table I).

During the early stages of purification synthase activity was assayed using a two-step procedure in which the enzyme was incubated at 37° and the resultant UDP was measured fluorometrically (14, 16). The reaction mixture routinely contained 50 mm Tris (pH 7.5) with 20 mm Na₂SO₄, 5 mM EDTA, and 25 mm KF. The concentrations of glycogen, UDP-glucose and glucose-6-P were varied as described in the separate procedures. The enzymatic reaction was stopped by heating for 2 min at 100°. During the purification procedure the reagent contained 10 mm glyco-

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1. N. D. Goldberg, personal communication.
The ratio of pig brain synthase I to glycogen has been calculated at early stages of purification. Units of enzyme activity are total micromoles per min in each fraction.

| Fraction                      | Glycogen | Synthase I | Ratio of units synthase I to glycogen |
|-------------------------------|----------|------------|---------------------------------------|
| Homogenate                    | 1.17     | 193        | 165                                   |
| 2.0 m (NH₄)₂SO₄ precipitate.. | 0.600    | 129        | 215                                   |
| Calcium phosphate gel eluate..| 0.072    | 87         | 1208                                  |
| 1.0 m (NH₄)₂SO₄ precipitate. | 0.002    | 52         | 2600                                  |

### Table III

**Purification of glycogen synthase D from pig brain**

The D form of brain glycogen synthase was prepared and assayed as described in the text. Units are micromoles of UDP-glucose converted to glycogen and UDP per min.

| Fraction        | Volume | Protein | Total Units | 1st Units/mg | % Recovery | % D  |
|-----------------|--------|---------|-------------|--------------|------------|------|
| Homogenate      | 2500   | 19.8    | 70          | 1.53         | 100        | 4    |
| 1st I → D conversion | 4000  | 1.49    | 43          | 7.2          | 57         | 99   |
| 2 m (NH₄)₂SO₄ precipitation | 427   | 11.5    | 37          | 7.6          | 48         | 84   |
| Calcium phosphate gel eluate | 607   | 2.32    | 14          | 9.8          | 18         | 84   |
| 1.0 m (NH₄)₂SO₄ precipitation | 47    | 8.68    | 14          | 34           | 18         | 75   |
| Second I → D conversion | 47    | 8.09    | 14          | 34           | 18         | 96   |
| DE52 column concentrate | 1     | 5.8     | 1.7         | 292          | 2          | 97   |

### Table IV

**Effect of incubation with glycogen on activity of brain glycogen synthase I and D**

The incubation mixture contained 25 mM Tris (pH 7.5), 1 mM EDTA, 0.02% bovine plasma albumin, 5 mM dithiorthietol, 25 mM KF, 25 mM glycogen (as anhydroglucosyl units), and, in the case of the form, D, 25 mM glucose-6-P. Enzyme was added to a concentration of 2.5 to 5 mg per ml. After incubation at room temperature (25°) for the stated times, a 2- to 10-μl aliquot was assayed in 1 ml in the one-step assay described under "Materials and Methods," according to Passonneau and Rottenberg (16). The reagent for I contained no glucose-6-P; that for D contained 5 mM glucose-6-P.

| Time of preincubation (min) | I Synthase | D Synthase |
|-----------------------------|------------|------------|
| 0                           | 98         | 134        |
| 15                          | 422        | 235        |
| 30                          | 461        | 335        |
| 60                          | 541        | 434        |
| 90                          | 637        | 447        |
| 90 (without glycogen)       | 113        | 154        |

The purification of synthase D from pig brain is summarized in Table III. The procedure of purification was similar to that of the I except that an I to D conversion was carried out in a large volume at Step 2 and repeated a second time in a smaller volume at Step 6. Synthase D was purified almost 200-fold and was 80% as pure of the I form from brain. The 4% present at Step 1 increased to 99% D after treatment to effect the I to D conversion (as determined by measurements in the presence and absence of 5 mM glucose-6-P). In subsequent steps there was a decrease in the percentage of the D form, in spite of the presence of KF to inhibit phosphatase activity. Since protein kinase had co-purified with D through Step 5 (Table III) (16), a second incubation with ATP was carried out. The resulting preparation was 98% in the D form which persisted through column chromatography. The over-all recovery was 2%.

**Effect of Glycogen Primer on Enzyme Activity**—It has been shown that incubation of purified glycogen synthase with glycogen results in an activation of the enzyme (16, 23). The effect of incubation with glycogen on both I and D synthase is shown in Table IV. Further experiments were carried out to determine whether the incubated enzyme was affected by additional glycogen in the assay system. After incubation with 25 mM glycogen (anhydroglucosyl units) the enzyme was diluted in the assay system so that the final glycogen concentration was 0.07 to 0.14 mM. Addition of 10 mM glycogen to the assay system increased the reaction velocities of both synthase I and D only slightly, with similar results over a 35-fold range of UDP-glucose concentrations. Neither the maximum velocities without glucose-6-P nor the affinity for UDP-glucose were substantially affected by the presence of glycogen added to the assay system.

Incubation of the D form of synthase with 25 mM glycogen, and subsequent analyses with and without added glycogen showed results similar to those for the I form of the enzyme. The reaction velocities were slightly greater in the presence of 10 mM than in 0.07 to 0.14 mM glycogen. The maximum velocities were 730 and 768 nmol per ml per min and for the absence of added glycogen, respectively.

**Kinetic Studies of Synthase I Form**—In another series of experiments, the Kₘ for UDP-glucose of the I form of synthase was measured at 25° in the presence and absence of glucose-6-P (Fig. 1). The enzyme was preincubated routinely for 60 min at 25° in the presence of 25 mM glycogen, and 10 mM glycogen was present in the assay medium. The apparent Kₘ for UDP-glucose in the absence of glucose-6-P (according to least squares regression analysis) was 0.113 mM and the maximum velocity was 1.2 μmol per ml per min, or 0.088 unit per mg of the protein. In the presence of 5 mM glucose-6-P the apparent Kₘ for UDP-glucose was reduced by two-thirds to 0.0333 mM; the Vₘₐₓ was essentially unchanged at 1.0 μmol per ml per min. At 37° the Kₘ for UDP-glucose in the absence of glucose-6-P was increased to 0.508 mM and the Vₘₐₓ to 4.0 μmol per ml per min. In the presence of 5 mM glucose-6-P, the Kₘ was reduced to one-sixth,
The activity of glycogen synthase I from pig brain as a function of UDP-glucose (UDPG) concentration. The velocities were measured at 25°C in the one-step assay described under "Materials and Methods." Velocities are expressed at micromoles per ml per min. Glucose-6-P (G-6-P) when present was 5 mM. Least squares regression lines are drawn in.

![Graph 1](https://via.placeholder.com/150)

**FIG. 1.** Activity of glycogen synthase I from pig brain as a function of UDP-glucose (UDPG) concentration. The velocities were measured at 25°C in the one-step assay described under "Materials and Methods." Velocities are expressed at micromoles per ml per min. Glucose-6-P (G-6-P) when present was 5 mM. Least squares regression lines are drawn in.

0.082 mM, and the \( V_{\text{max}} \) was somewhat less, 3.0 \( \mu \text{mol} \) per ml per min.

The effect of glucose-6-P concentration on glycogen synthase I activity at 25°C with two UDP-glucose concentrations is shown in Fig. 2. There was a marked effect of glucose-6-P in both cases (UDP-glucose was below saturation in each instance); however, as would be expected from the affinity of the enzyme for UDP-glucose, much larger effects were seen at lower concentrations of UDP-glucose. A 4.5-fold increase in synthase I activity was observed at 0.03 mM UDP-glucose. When the UDP-glucose concentration was increased to 0.21 mM, the maximum effect of glucose-6-P was a 3.8-fold increase in velocity.

Because glycogen synthase I is active in the absence of glucose-6-P, the data were analyzed for \( AV \), the difference between the velocity in the presence and absence of 5 mM glucose-6-P. If one plots \( 1/AV \) against \( 1/\text{glucose-6-P} \) the amount of glucose-6-P required for half-maximal stimulation at 0.03 and 0.21 mM UDP-glucose is 0.13 and 0.11 mM, respectively.

![Graph 2](https://via.placeholder.com/150)

**FIG. 2 (left).** The activity of glycogen synthase I at 25°C at two concentrations of UDP-glucose (UDPG) and varying concentrations of glucose-6-P. The assays were conducted as described under "Materials and Methods" and Fig. 1.

![Graph 3](https://via.placeholder.com/150)

**FIG. 3 (right).** The effect of glucose-6-P concentration on the activity of brain glycogen synthase I at two UDP-glucose (UDPG) concentrations at 37°C. The assays were conducted as described under "Materials and Methods" and Fig. 1.

Similar analyses were made for the effect of glucose-6-P at 37°C (Fig. 3). At 4 mM UDP-glucose, a near-saturating concentration of substrate, only 1.2-fold increase in velocity is seen at high levels of glucose-6-P. When the UDP-glucose concentration is reduced to 0.125 mM, glucose-6-P increases the reaction velocity 2.8-fold. If one plots the \( 1/AV \) versus \( 1/\text{glucose-6-P} \), the concentration of glucose-6-P required to produce half-maximal stimulation at 4 and 0.125 mM UDP-glucose are 0.037 and 0.317 mM, respectively.

The effect of ATP on synthase I activity was minimal. When 5 mM ATP was tested with 1 mM UDP-glucose at 25°C, the enzyme velocity was 80% of control in the absence of glucose-6-P, and 75% of control in the presence of glucose-6-P. The lack of effect of ATP may be attributed in part to the relatively alkaline pH of the assay system as shown by Piras et al. (24).

**Kinetic Studies of Synthase D Form—**Unlike the I form, synthase D is completely inactive in the absence of glucose-6-P (in the present preparation the synthase was 97% in the D form, Table III). At 25°C, when glucose-6-P is 0.75 mM, the \( K_m \) for UDP-glucose is 0.71 mM and the \( V_{\text{max}} \) is 0.303 pmol per ml per min (0.05 unit per mg of protein) (Fig. 4). When glucose-6-P is increased to 2 mM the \( K_m \) for UDP-glucose is lowered to 0.50 mM and the \( V_{\text{max}} \) is increased to 0.440 pmol per ml per min. At 10 mM glucose-6-P the \( K_m \) is reduced to 0.42 mM and the \( V_{\text{max}} \) is increased to 0.768 pmol per ml per min.

The effect of glucose-6-P on the \( K_m \) for UDP-glucose is more marked at 37°C. When glucose-6-P concentration is 0.75 mM the apparent \( K_m \) for UDP-glucose is 5.76 mM and the \( V_{\text{max}} \) is 1.4 pmol per ml per min. When glucose-6-P concentration is increased to 2 mM, the \( K_m \) for UDP-glucose is decreased to one-fourth (1.42 mM), and the \( V_{\text{max}} \) is increased to 2.04 pmol per ml per min.

Because the D form of brain synthase was totally inactive in the absence of glucose-6-P, it was possible to use a Lineweaver-Burk plot with \( 1/V \) plotted against \( 1/\text{glucose-6-P} \) (Fig. 5). At high glucose-6-P concentrations there appeared to be some inhibition and these points, although shown in the figure, were not used in the regression analysis. At 25°C, the apparent \( K_m \) for glucose-6-P at 0.5 mM UDP-glucose was 22.7. When UDP-glucose was increased to a near saturating level, 5 mM, the \( K_m \) for glucose-6-P was reduced to 1.82 mM. The extrapolated maximum velocity
was lower with 5 mM than with 0.5 mM UDP-glucose. However, since all of the experimental points with 5 mM UDP-glucose show higher velocity, the extrapolated points may not be valid.

At 37°, the Kₘ for glucose-6-P at 0.5 mM UDP-glucose was 43.5 mM and the Vₘₐₓ was 1.04 µmol per ml per min. At near saturating levels of UDP-glucose (5 mM) the Kₘ for glucose-6-P was reduced to 6.15 mM and the Vₘₐₓ remained unchanged. As at 25°, the velocities at the highest glucose-6-P concentrations (4 and 7 mM) were decreased and therefore not considered in the regression analysis. Whether the decreased velocities were a result of an impurity in glucose-6-P which inhibits synthase activity, or inhibition due to excess substrate, was not investigated further.

**Binding Experiments**—The [¹⁴C]glycogen prepared in our laboratory ("Materials and Methods") was polydisperse in the ultracentrifuge, and consequently not suitable for binding experiments, so we attempted to make a more uniform preparation in an effort to determine the molar ratio of synthase bound to the glycogen. The glycogen was centrifuged in a 10 to 30% sucrose gradient, using the SW 39 rotor in a Spinco model L centrifuge. The heaviest fractions (0.6 ml) were combined and concentrated by vacuum dialysis to a final volume of 0.2 ml. The molecular weight of the main component was found to be 4 x 10⁶ using keyhole limpet hemocyanin as a marker (25). A portion of this glycogen (250 nmol of anhydroglucosyl units, 155 cpm per nmol) was incubated for 60 min at room temperature with 15 pmol of either [¹⁴C]glycogen, glycogen synthase, or the [¹⁴C]glycogen-enzyme mixture. The tubes were centrifuged 16 hours at 25,000 rpm. Twenty-five 200-µl fractions were collected and portions of each were assayed for glycogen synthase activity, [¹⁴C]glycogen radioactivity, and glycogen content. The position of the glycogen synthase peak was not markedly affected by incubation with [¹⁴C]glycogen, being displaced only one fraction toward the bottom (Fig 6). It is clear that, in this preparation at least, the synthase binds preferentially to low molecular weight glycogen. Other binding experiments gave essentially the same results.

Using phosphorylase a as a marker (molecular weight, 400,000 (26)) the molecular weight of the synthase was calculated to be 311,000. Soderling et al. (22), using gel electrophoresis and density gradient centrifugation, found the molecular weight of the rabbit muscle glycogen synthase subunit to be 90,000 to 100,000 and suggested that the native enzyme is a tetramer. Our results agree reasonably well if one assumes the lower molecular weight of the monomer. The molecular weight is also in close agreement with
that found for rat liver synthase (27) and for the enzyme from swine kidney (12).

**DISCUSSION**

A summary of the kinetic characteristics of the I and D forms of brain glycogen synthase is shown in Table V. The affinity for UDP-glucose of the I form in the absence of glucose-6-P is of the same order of magnitude as that observed for the renal (11, 12), liver (27), rat muscle (28), and leukocyte enzymes (6), but is 10 times greater than that observed for rabbit skeletal muscle (28). When the temperature is increased from 25 to 37°C the apparent $K_m$ for UDP-glucose is increased 5-fold in the absence of glucose-6-P, and 2.5-fold in the presence of glucose-6-P.

The affinity of the D form of synthase for UDP-glucose is several times higher than that reported for the rabbit (29) or rat liver synthase (27) and for the enzyme from swine kidney (12). Of special interest is the observation of the tremendous decrease in affinity for the UDP-glucose of the I form in the absence of glucose-6-P is of the same order of magnitude as that observed for the renal (11, 12), liver (27), rat muscle (28), and leukocyte enzymes (6). Of special interest is the observation of the tremendous decrease in affinity for the substrate with increasing temperature. At low glucose-6-P concentration (0.75 mm) the apparent $K_m$ for UDP-glucose is 8 times greater at 37 than at 25°C. These data indicate that in the brain synthase $D$ must be virtually inactive under physiological conditions.

Since brain tissue is 10% protein the calculated amount of total synthase in pig brain from Tables I and III is 150 µmol per min per kg wet weight. Assuming Michaelis-Menten kinetics and given the concentrations of glucose-6-P and UDP-glucose in brain tissue (100 and 80 µmol per kg, respectively), synthase $D$ would operate at less than 0.2% of capacity. In rapidly frozen brain tissue (100 and 80 µmol per kg, respectively), synthase $D$ would operate at less than 0.2% of capacity. In rapidly frozen brain tissue, about 80% of the synthase is in the D form (7, 13); thus, the maximum rate of the D synthase activity would be 0.24 µmol per min per kg. Synthase I, on the other hand, could function at about 20% of its $V_{max}$. Thus, the calculated velocity of the I form would be in the neighborhood of 6 µmol per min per kg.

The total activity of glycogen synthase in the brain is very low compared to that in liver and muscle, and also low compared to that of brain glycogen phosphorylase, which is 5300 µmol kg⁻¹ min⁻¹ (30). Brain glycogen stores are only 10% as large as those of resting muscle and 1% of those of liver from a fed animal. Although potential phosphorylase activity in brain far exceeds that of synthase (3300 versus 150 µmol kg⁻¹ min⁻¹), the kinetic properties of brain phosphorylase are such that the glycogen is virtually inaccessible for phosphorylase (20). Under ordinary physiological conditions glycogen stores in the brain are maintained by a balance between low glycogen synthase activity and an effective “kinetic inhibition” of phosphorylase activity.

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