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PURIFICATION AND PROPERTIES OF GLYCOGEN SYNTHASE *I* FROM BOVINE HEART: TWO KINETIC FORMS*

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SUMMARY

Bovine heart glycogen synthase (*I* form) (UDPglucose: glycogen α-4-glucosyltransferase, EC 2.4.1.11) containing less than 10% contamination by the *D*-form of the enzyme was purified approximately 3000-fold. The enzyme exhibited unusual reaction kinetics that are best explained by two kinetic forms. Form 1 was produced only by preincubation of the enzyme with glycogen ($\geqq$ 1 mg/ml) and this form was unstable under assay conditions. Form 2 was found after preincubation at low glycogen concentrations and was stable to the assay conditions. Forms 1 and 2 were shown to have slightly different pH optima and significantly different Michaelis constants for UDPglucose. Form 1 had a lower K_m than form 2, while the V was similar. Activators decreased the K_m and increased the V of both enzyme forms. Form 2 was also found to be inhibited by UDPglucose and calculations showed that this inhibition decreased the activity by approximately half and/or increased the K_m for UDPglucose by 2–3-fold.

INTRODUCTION

Since the original discovery of the *I* and *D* forms of glycogen synthase[1] (UDPglucose: glycogen α-4-glucosyltransferase, EC 2.4.1.11), several research groups have reported procedures for purifying this enzyme from different sources. Recently, essentially homogeneous preparations containing partially converted[2] or totally converted *I* or *D* forms of this enzyme were obtained from rabbit skeletal muscle[3,4], from rat and rabbit hearts[5] and from rat liver[6].

The above enzyme preparations were used to examine the stoichiometry of the reaction of ATP with glycogen synthase *I* to give glycogen synthase *D*[4]. Soderling, *et al.*[2], measured the amount of phosphate incorporated into synthase *I* during partial conversion to the *D* form and reported that 1 mole of phosphate was incorporated

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for every 90 000 g of protein converted to the *D* form. However, there was a substantial amount of the *I* form that was not phosphorylated in this experiment. Smith, *et al.*[4], compared the phosphate content of both the totally converted essentially homogeneous *I* and *D* forms of the enzyme and found a total of 7 moles of alkali-labile phosphate for every 100 000 g of protein or 6 per 90 000-dalton subunit. The conversion of heart synthase *I* to the *D* form has also been reported to require approximately 5 moles of phosphate per 100 000 g of synthase protein[5].

In kinetic studies, the skeletal muscle glycogen synthase *D* form was shown to have glycogen and UDPglucose kinetics compatible with a ping-pong mechanism[3]. In addition, the K_m for UDPglucose and glycogen for the *D* form of skeletal muscle synthase[3] and K_m for UDPglucose for the *D* form of heart synthase[5] were reported. The constants for the skeletal muscle enzyme were similar to those determined earlier with less pure enzyme preparations[7].

This paper reports a method for purification of bovine heart glycogen synthase *I*. The effects of both UDPglucose and several activators on the enzyme activity are described. Further, it is shown that the kinetics of the enzyme with respect to UDP-glucose may be significantly affected by interaction of glycogen synthase with glycogen. The second paper presents the purification and some kinetic properties of the completely converted *I* form from skeletal muscle. The third paper reports the specificity of anion activation of both completely converted glycogen synthase *I* forms from heart and skeletal muscle.

METHODS

Purification of glycogen synthase I

Glycogen synthase *I* was purified 3000-fold from bovine heart by a procedure similar to that described previously[3,5]. The heart was obtained from a local slaughter house and used within 1½ h. It was trimmed of fat and connective tissue, sliced, cooled to 4 °C and homogenized in a blender for 2–3 min. (In a typical preparation, 800 g of tissue *plus* 1.6 l 50 mM Tris–5 mM EDTA buffer, pH 7.8, were used). The homogenate was centrifuged (12 000 × *g*, 1 h) and the supernatant was filtered through glass wool. After cooling the extract to nearly 0 °C in an ice–salt bath, 95% ethanol (chilled to −60 °C) was added to a final concentration of 30% (v/v), while maintaining the temperature below 5 °C. The suspension was allowed to stand for 5–10 mins and then centrifuged (12 000 × *g*, 1 h) in a centrifuge pre-chilled to −10°C. The supernatant was discarded, the pellets were drained and frozen in liquid nitrogen (2 h or less) (The freezing step was used as a convenient stopping point in the purification; it also facilitated the removal of the sediment from the centrifuge tubes.) The pellets were dispersed in Tris–EDTA buffer, pH 6.8, containing 2 mM Na_2SO_4 and 50 mM mercaptoethanol with a Teflon glass homogenizer to give a total volume of approximately 1/10 the original homogenate. The pH was adjusted to 7.0 with 1 M acetic acid and the preparation was allowed to stand several days in the refrigerator. During this incubation, the enzyme could be almost completely converted to glycogen synthase *I* (<90%) by a reaction that was inhibited by 50 mM KF (presumably a phosphatase reaction). The enzyme preparation was dialyzed every other day against fresh buffer to replenish the mercaptoethanol lost through evaporation and oxida-

tion. With some preparations, warming to 30 °C for an hour or two speeded up the conversion reaction.

When the conversion reaction stopped, approximately 20 units of human salivary α-amylase (EC 3.2.1.1) (prepared by the method of Bernfeld[8]) or porcine pancreatic α-amylase (Sigma Chemical Co.) were added to the preparation and it was dialyzed at room temperature in a continuous flow dialyzer against Tris–EDTA buffer, pH 6.8, containing 2 mM Na_2SO_4 and 50 mM mercaptoethanol for 2 h. The pH of the enzyme preparation was adjusted to 7.7, the extract was centrifuged (78 000 × *g*, 3 h), and the pellets were discarded. (This treatment with α-amylase was necessary to prevent sedimentation of the enzyme during centrifugation.). Rabbit liver glycogen (0.5 mg/ml) isolated by KOH digestion was added to the supernatant just before the preparation was applied to a DEAE-cellulose column previously equilibrated with the same pH 7.8 buffer. The total bed volume of DEAE-cellulose was not critical for this purification, but the column was found to work well with a 200-ml bed volume for each kg of original tissue processed. The column was washed with 5–7 bed volumes of buffer containing 0.1 M NaCl and the effluent was discarded. The α-amylase was found in this eluate. The NaCl concentration of the buffer was increased to 0.25 M and the enzyme was eluted from the column. Glycogen synthase was precipitated by adding ethanol to 15% (v/v) according to the procedure described above and the precipitate was collected by centrifugation. These pellets were suspended in Tris–EDTA buffer, pH 7.8, and dialyzed against the same buffer for 2 h. The above enzyme preparation could be stored frozen at −70 °C for up to six months.

TABLE I

PURIFICATION OF GLYCOGEN SYNTHASE *I*

Step	*Vol. (l)*	*Units**	*Spec. act. (units/mg protein)*	*Synthase I (total)*	*Recovery (%)*
Homogenate	2.5	362	0.0016	—	—
22 000 × *g* supernatant	1.2	183	0.0071	0.242	50.5
Ethanol ppt. I	0.215	154	0.014	0.253	42.5
78 000 × *g* supernatant**	0.117	163	0.20	0.958	45.0
0.25 M NaCl effluent	0.102	159	0.61	0.982	44.0
Ethanol ppt. II	0.005	138	4.7	0.946	38.2

* μmoles glucose added to glycogen per min.
** Corrected for α-amylase action during assays for glycogen synthase.

Table I shows data from an enzyme preparation purified by the above procedure. The final enzyme product contained 11 mg/ml carbohydrate or approximately 3 units of enzyme per mg of carbohydrate (1 unit = 1 μmole/min at 30 °C).

To prepare enzyme with low glycogen content, two procedures were used. The first method depended on separation of glycogen synthase from glycogen during purification of the enzyme. That is, after the enzyme preparation had been treated with α-amylase, no glycogen was added before applying to the DEAE-cellulose column. This method gave somewhat poorer yields of enzyme than reported in Table I (approx. 15%) and the final enzyme product had a lower specific activity (approx. 1). The

second method was to purify the enzyme first (as described above) and then remove the glycogen. The purified enzyme was treated with 5–10 units of α-amylase. The α-amylase was then removed by gel filtration on Biogel A 0.5 m (BioRad Laboratories). On these columns, the glycogen synthase eluted with the front while the α-amylase was retarded. By adjusting the size of the column to the minimum necessary for separation of glycogen synthase and α-amylase, the preparation of glycogen synthase was not significantly diluted in this step. Enzyme preparations used in these studies were prepared by both of the methods described and contained less than 2 μg carbohydrate per munit of enzyme.

Analytical methods

Total carbohydrate was determined by the phenol–H_2SO_4 method described by Dubois *et al.*[9], and protein was determined by the Folin–Lowry[10] method.

Assays

The synthase assay is based on the incorporation of [^{14}C]glucose into glycogen from UDP[^{14}C]glucose[11]. Assays in the absence and presence of glucose-6-*P* give, respectively, the activity due to glycogen synthase *I* and total synthase.

α-Amylase activity was determined by measuring the decrease in radioactivity of ^{14}C-labeled glycogen when incubated with the amylase at pH 6.8 in the presence of 10 mM $CaCl_2$ (The radioactive glycogen substrate was prepared by incubating UDP-[^{14}C]glucose with glycogen and glycogen synthase. The product was isolated by ethanol precipitation, redissolved in water and then precipitated again.) The amylase incubation mixture was spotted on filter paper and washed with 66% ethanol to remove alcohol soluble radioactive compounds, *i.e.* low molecular weight oligosaccharides. The washing procedure was identical to that used for the assay of glycogen synthase. The radioactivity on the paper was determined with a liquid scintillation spectrometer.

RESULTS

Factors affecting the time course of the reaction

In the course of determining the kinetic constants of glycogen synthase *I* for UDPglucose, it was found that low substrate concentrations gave progress curves that were curvilinear. The following experiments were designed to examine the cause of this phenomenon.

The effect of UDPglucose concentration on the reaction time course is shown in Fig. 1. The data give curvilinear plots with UDPglucose concentrations between 0.05 and 0.32 mM. However, 5 mM UDPglucose gave a linear time course over the same period of incubation. At all UDPglucose concentrations, the progress curves were linear after 4 or 5 min had elapsed. Thus, there appeared to be a time-dependent change in enzyme activity when assayed with low substrate.

Curves of this type may be produced by enzyme forms that are unstable under the assay conditions. In order to test this possibility, the conditions for activation of the frozen glycogen synthase preparations used for the experiment in Fig. 1 were studied. Frozen enzyme is activated routinely by incubation with a reducing agent such as mercaptoethanol, Na_2SO_3, or dithiothreitol and with glycogen. Since our en-

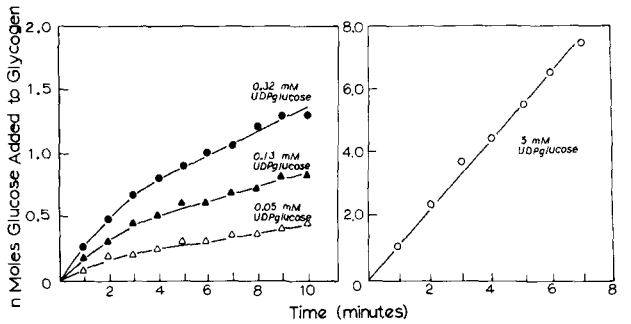

Fig. 1. The effect of UDPglucose concentration on the time course of the reaction. The enzyme was preincubated 20 min at 30 °C with 50 mM Tris, 5 mM EDTA, 20 mM dithiothreitol, and 10 mg/ml rabbit liver glycogen (pH 7.8) to obtain maximum activity. It was then assayed in a reaction mixture containing 50 mM Tris, 5 mM EDTA, 10 mg/ml rabbit liver glycogen, and the concentrations of UDPglucose indicated (spec. act. between $4 \cdot 10^5$ and $2 \cdot 10^6$ cpm/μmole). Aliquots of each reaction mixture were removed at the times indicated, spotted on filter paper squares, and stopped in an ethanol bath as described in ref. 11.

zyme had been treated with α-amylase to remove glycogen, it was possible to vary the amounts of glycogen and enzyme in the preincubation. The results are shown in Fig. 2. At 50 munits/ml enzyme the addition of glycogen (0.1%) to the activation mixture produced a curvilinear time course. Without glycogen the time course was linear. The insert shows the time course when the enzyme was activated at 5.0 munits/ml with glycogen. The progress curve was linear and the activity was one-tenth that of the enzyme preincubated at 40 munits/ml without glycogen. Apparently, both glycogen and an adequate enzyme concentration are required to observe a curvilinear time course.

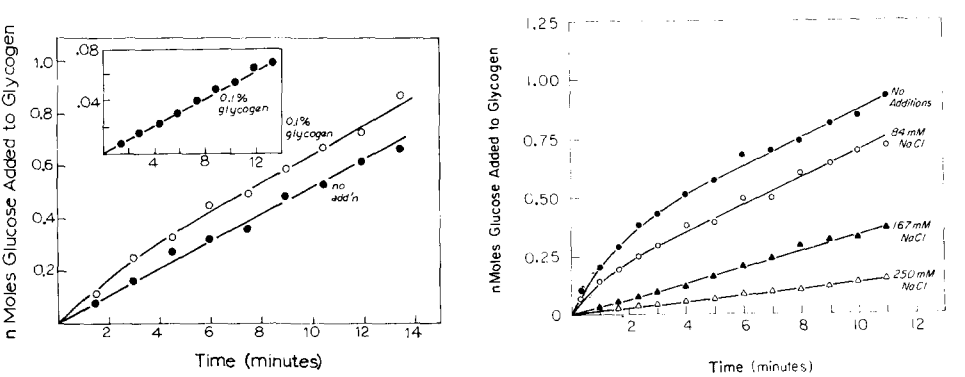

Fig. 2. The effect of preincubation conditions on the time course of the reaction. The glycogen-free enzyme was preincubated 15 min at a concentration of 50 munits/ml (main figure), or 5.0 munits/ml (insert figure). The enzyme at the higher concentration was preincubated with (○—○), or without (●—●) 1 mg/ml rabbit liver glycogen. The enzyme at low concentration was incubated with 1 mg/ml glycogen. Time courses were run as in Fig. 1 in reaction mixture containing 0.17 mM UDPglucose. The enzyme concentrations during assay were 7 munits/ml and 0.7 munits/ml. The enzyme preparation contained approx. 1–2 μg of glycogen per munit of enzyme.

Fig. 3. The effect of NaCl on the time course of the reaction. The enzyme (approximately 50 munits/ml) was preincubated in the presence of 1 mg/ml rabbit liver glycogen and then assayed in reaction mixtures containing the concentrations of NaCl shown, and 0.11 mM UDPglucose. The time course was run as described in Fig. 1.

The effect of NaCl on the time course is shown in Fig. 3. The enzyme was preincubated under standard conditions, *i.e.* both glycogen and reducing agent present and NaCl was added to the assay tubes. In the presence of 84 mM NaCl the progress curve became linear after 2.5–3 min with a linear rate from 4–11 min identical to the rate in the absence of NaCl during that same incubation period. Higher concentrations of NaCl completely obliterated the curvature in the progress curve and also decreased the linear rate.

As will be shown below (Fig .5), glycogen synthase *I* has a pH optimum of 7.0 in the absence of an activator (Na_2SO_4, ref. 12) and 7.9 with an activator. Since the previous studies in this paper were done at pH 7.8 without an activator, it was necessary to determine the effect of pH and activation on the reaction time course. Fig. 4 shows that the time course was still curvilinear at pH 6.8 as well as in the presence of 3.8 mM Na_2SO_4. In fact, Na_2SO_4 increased the curvature of the progress curves. The initial rate of reaction was enhanced twice as much as the rate between 4 and 11 min.

Definition of two kinetic forms of glycogen synthase I

The studies discussed in the previous section showed that initial rates for the glycogen synthase *I* catalyzed reaction as well as the shape of the progress curve for the reaction depended both on preincubation and on reaction conditions. The curvilinear time courses found at low substrate concentrations and the linear time courses found at high concentrations was compatible with two different kinetic forms of the enzyme. The first of these, designated Form 1, was examined by measuring the initial rate obtained when the enzyme was preincubated at concentrations greater than 25 munits/ml in the presence of added glycogen (1 mg/ml) (see Fig. 2). This

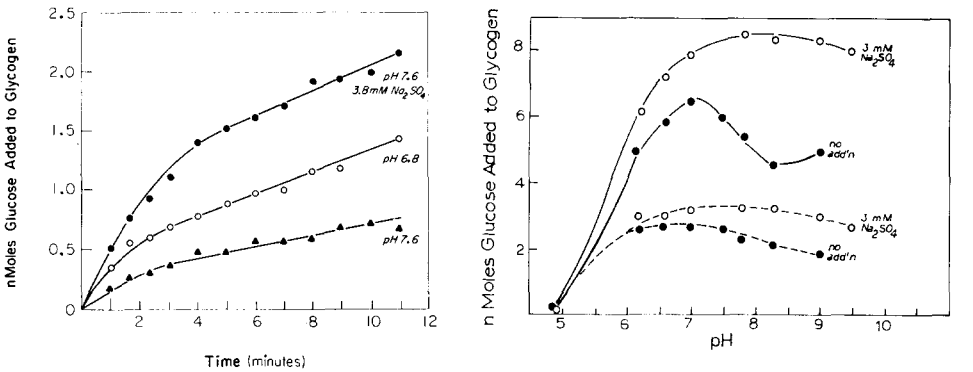

Fig. 4. The effect of Na_2SO_4 and pH on the time course of the reaction. The enzyme (approximately 25 munits/ml) was preincubated in the presence of 10 mg/ml rabbit liver glycogen and assayed with either 3.8 mM Na_2SO_4 or in reaction mixture buffered with 50 mM 2-(*N*-morpholino)-ethanesulfonic acid, pH 6.8. The time course was run as described in Fig. 1 at 0.11 mM UDP-glucose.

Fig. 5. pH activity curves for two forms of glycogen synthase *I*. The enzyme treated with α-amylase to decrease its carbohydrate content (see Methods), was preincubated at 15 munits/ml with or without glycogen. Form 1 (dashed curves; preincubated with glycogen) was then assayed for 1.5 min in reaction mixtures containing 4.4 mM UDPglucose ($1 \cdot 10^6$ cpm/μmole), and buffered with 50 mM Tris–maleate at the pH values indicated. Form 2 (solid curves; preincubated without glycogen) was assayed in identical test mixtures for 10 min.

form corresponds to the form of the enzyme that was unstable in our assay and was responsible for the initial fast reaction rate at low UDPglucose concentrations. The second kinetic component, Form 2, was examined by measuring the rate produced by an enzyme preincubated at the same concentration without added glycogen. This enzyme form gave linear progress curves during assay and therefore was stable to the assay conditions. Since the rate of reaction of an enzyme preincubated without glycogen and assayed at low UDPglucose was the same as the second linear phase of reaction produced by an enzyme preincubated with glycogen (Fig. 2), we assumed that these two enzyme forms must be the same.

pH optima

The pH optima for both Form 1 and 2 are shown in Fig. 5. Form 1 was assayed by using short incubation times (1.5 min) after preincubating the enzyme with glycogen. As shown, the pH optima for this form are approximately 6.7 without, and 7.7 with Na_2SO_4. The pH optima for Form 2 were obtained by preincubating without added glycogen and determining the rate after 10 min. Form 2 had a pH optima at 7.1 without and 8.0 with Na_2SO_4. The curves obtained without activator for the two forms have distinctly different shapes, but with activator the curves are similar.

Kinetic analysis of glycogen synthase I

Fig. 6, 7 and 8 show kinetic plots of the two forms of glycogen synthase. At all UDPglucose concentrations, time courses were run to permit accurate determinations of the initial velocity, and the data was analyzed by plotting $[S]/v$ *versus* $[S]$. By this method, the slope represents the reciprocal of the maximum velocity and the intercept at the abcissa is $-K_m$.

The plots for Form 1 (Fig. 6) are linear and both slopes and intercepts were

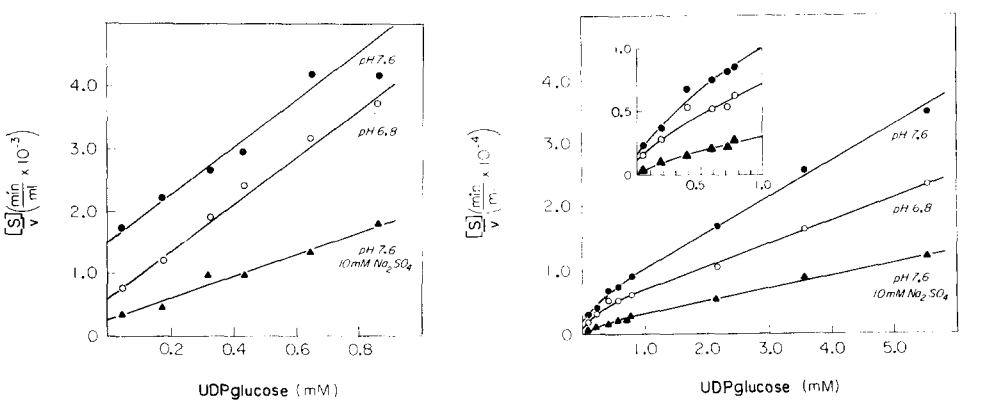

Fig. 6. Kinetic plots for Form 1 of glycogen synthase *I*. The enzyme, treated with α-amylase, was preincubated with 1 mg/ml rabbit liver glycogen. The time couse of the reaction was determined at each UDPglucose concentration and the initial rate was determined from a tangent drawn at time zero. The conditions of each assay were as shown in the figure.

Fig. 7. Kinetic plots for Form 2 of glycogen synthase *I*; effect of pH and Na_2SO_4. The enzyme, treated with α-amylase, was preincubated without added glycogen. The time course of the reaction was determined at each UDPglucose concentration under the conditions indicated in the figure and the initial rate was determined. These time courses were linear over the time interval studied from time zero.

TABLE II

KINETIC CONSTANTS FOR UDPGLUCOSE

Enzyme form	*Conditions*	K_m *(mM)*	V *(nmoles/min)*
Expt 1			
Form 1	pH 7.6	0.39	0.26
	pH 7.6 + 10 mM Na_2SO_4	0.12	0.56
	pH 6.8	0.18	0.28
Form 2	pH 7.6	0.82	0.18
	pH 7.6 + 10 mM Na_2SO_4	0.48	0.47
	pH 6.8	0.86	0.27
Expt 2			
Form 2	pH 7.6	0.90	0.46
	pH 7.6 + 20 mM Na_2SO_4	0.30	0.93
	pH 7.6 + 20 mM NaH_2PO_4	0.25	0.81
	pH 7.6 + 1 mM Glc-6-*P*	0.07	0.79

changed by our experimental conditions. The plots for Form 2 (Fig. 7) are curvilinear at low substrate concentration. This fact is shown more clearly in expanded curves in the inset. The kinetic constants derived from the preceding plots and additional kinetic plots using P_i and glucose-6-*P* (Fig. 8) are compiled in Table II. Both the K_m and v of Form 1 were affected by Na_2SO_4 (3-fold and 2-fold, respectively), whereas the effect of decreased pH was only on the K_m (2-fold). For Form 2, activation with several activators affected both K_m and V (data from both experiments 1 and 2). Also, a lower pH changed only the V and not the K_m (V increased 1.5-fold). It is quite clear that the two kinetic forms of glycogen synthase *I* respond differently to a decreased pH and that Form 1 shows the largest effects of activation. The Michaelis

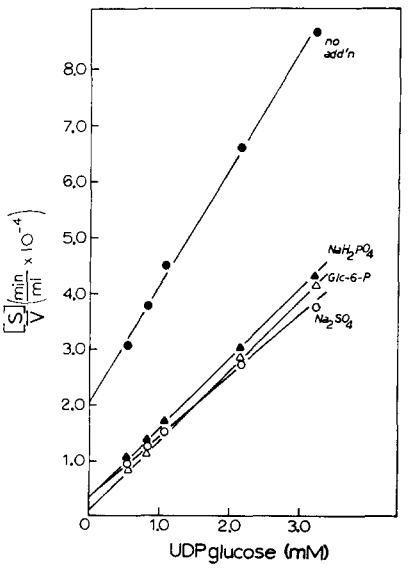

Fig. 8. Kinetic plots for Form 2 of glycogen synthase *I*; effect of phosphate, sulfate, and glucose-6-*P*. Experimental conditions are as described in Fig. 7. Reactions were terminated after 5 min incubation.

constants for Form 1 were all lower than those of Form 2, but the values of V were similar under equivalent conditions.

The kinetic plots for Form 2 (Fig. 7) indicate that UDPglucose is an inhibitor of the reaction as well as a substrate, since the kinetic plots curved downward as substrate concentration increased. To estimate the effect of this inhibition the following analysis was made.

Since the plots were linear above 0.8 mM UDPglucose, substrate was considered to have no modifying effect in that range. Therefore, kinetic constants determined from UDPglucose concentrations over 0.8 mM represent values at maximum inhibition by the substrate. This inhibition could result from an effect on V, K_m, or on both.

Assuming an effect only on V (V system) and using the initial velocity found at 0.038 mM UDPglucose for the velocity of relatively uninhibited Form 2, it was possible to solve for the unknown V of the uninhibited enzyme as follows:

(1) Data obtained for Form 2 when assayed with no activators at pH 7.6. K_m = 0.86 mM UDPglucose (assumed constant for this calculation) velocity at 0.038 mM UDPglucose = $1.7 \cdot 10^{-5}$ μmole/min.

(2) Calculation:

$$v = \frac{V}{1 + K_m/[S]} \quad \text{or} \quad V = v\left(1 + \frac{K_m}{[S]}\right)$$

$$V = 1.7 \cdot 10^{-5}\,(1 + 0.86/0.038) = 0.41 \text{ nmole/min.}$$

This calculation yields a maximum velocity for Form 2 that would be observed if the velocity dependence on UDPglucose followed Michaelian kinetics. However, the V observed in our studies was 0.19 nmole/min. Therefore, Form 2 of glycogen synthase I was reduced in activity by at least 56% by interaction of UDPglucose with an inhibitory site.

TABLE III

UDPGLUCOSE INHIBITION OF FORM 2

Conditions	*V system* (-fold decrease in V)*	*K system** (-fold increase in K_m)*
pH 7.6	1.8	2.4
pH 7.6 + 10 mM Na_2SO_4	1.4	3.0
pH 6.8	2.1	2.0

* Changes in V calculated by assuming the K_m to be unchanged by UDPglucose.
** Changes in K_m calculated by assuming the V to be unchanged by UDPglucose.

Similar calculations, using the velocity of the enzyme at 0.038 mM UDPglucose as the basis of calculation as shown above, were made for two other assay conditions. In addition, calculations assuming constant V were made. The results are shown in Table III. The inhibition was similar under all assay conditions; 2 to 3-fold change in either the K_m or V was found.

DISCUSSION

This paper shows that the time course of the reaction catalyzed by bovine heart glycogen synthase *I* is not linear at low substrate concentrations (0.05–0.32 mM) during the entire incubation period. However, it was linear with higher substrate (5 mM UDPglucose). At low substrate concentrations the reaction began rapidly and then slowed to a constant rate that was somewhat less than the initial rate after approximately 4 min. The initial fast rate was attributed to an unstable enzyme form designated "Form 1" and the slower linear reaction rate was termed "Form 2".

Several factors affected the linearity of progress curves. First, preincubation conditions were critical, *i.e.* glycogen was required during activation of the enzyme and the enzyme concentration had to be somewhat above 5 munits/ml to observe an initial fast rate of reaction (Fig. 2). Second, increasing the ionic strength of the reaction mixture with NaCl (84 mM) decreased the fast rate and the slower linear rate was unaffected (Fig. 3). The differences in the reaction kinetics produced by these factors are suggestive of an aggregation–disaggregation phenomenon. That is, if Form 1 were an aggregated form of the enzyme that was broken down to a more disaggregated form under reaction conditions, one might expect to obtain the above data.

It is not likely that these curvilinear time courses were the result of accumulation of an enzyme-bound intermediate. By assuming a minimum molecular weight of 13 000 for each active site[3,4] and a specific activity of 10 for pure enzyme, it is possible to estimate the molar concentration of enzyme active sites in the reaction mixture. One can also calculate the concentration of enzyme-bound intermediate that must be formed to produce the curvature obtained in any given experiment, *e.g.* in Fig. 2 the amount of enzyme-bound intermediate would correspond to the difference between the curves with and without glycogen. Thus, it can be shown that the concentration of intermediate necessary to produce the curvature of Fig. 2 is approximately 120 times the concentration of enzyme active sites.

From the data reported in this paper, it is also possible to speculate on the probable cause for the instability of Form 1 under our assay conditions. First, the amount of reaction was not a primary cause of the curved progress curve because it was shown (Figs 1–4) that the time after the reaction was initiated that gave nonlinear reaction rates was relatively constant despite significant differences in the extent of reaction during this period. This also argues against intermediate formation as the cause of the curvature. Second, because of this same data it is evident that formation of products does not cause the breakdown to Form 2. It was also found in experiments not reported here that UDP, a product of the reaction, added to the preincubation did not affect the shape of the time course. Third, addition of an anion activator or lowering the pH of assay did not result in a faster breakdown of Form 1, but instead stimulated Form 1 more than Form 2 (Fig. 4). Therefore, the most likely cause of the instability of Form 1 in the assay is the presence of UDPglucose. It was impossible to test this possibility conclusively because our enzyme preparations always contained enough glycogen to permit reaction in the presence of UDPglucose with no added glycogen. However, it should be noted that Form 2 was shown to be allosteric with respect to UDPglucose and kinetic plots were curvilinear. Form 1 did not show this property and, therefore, may not be capable of binding UDPglucose

at an allosteric site. It would seem possible for both kinetic forms of glycogen synthase to be present *in vivo* and the interconversion between these forms may serve as an additional regulatory mechanism in glycogen synthesis. This is possible since at normal tissue concentrations of UDPglucose, Form 1 may have more than twice the activity of Form 2 due to the difference in Michaelis constants for UDPglucose. However, at the high enzyme concentrations probable *in vivo*, especially when binding to glycogen is considered, and in the presence of other proteins that bind to glycogen, the significance of the kinetic forms of glycogen synthase *I* in the cell may be even more complex.

ACKNOWLEDGEMENTS

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