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PURIFICATION AND PROPERTIES OF GLYCOGEN SYNTHASE *I* FROM SKELETAL MUSCLE: TWO KINETIC FORMS

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SUMMARY

Rabbit muscle glycogen synthase *I* (UDPglucose:glycogen α-4-glucosyltransferase, EC 2.4.1.11) containing less than 5% *D* form was purified over 500-fold. The enzyme, like the heart enzyme, exhibited unusual reaction kinetics best explained by two kinetic forms. The rate of conversion of Form 1 to Form 2 was analyzed graphically and a $T_{1/2}$ of 1.8–2.5 min was found. The kinetics of Form 1, the initial transient form, and of Form 2, the second stable form, were separately analyzed. Form 1 had a lower K_m than Form 2 whereas V was the same. Na_2SO_4 increased V 2-fold and decreased K_m 2- to 3-fold for both Forms 1 and 2.

INTRODUCTION

In the preceding paper[1] the purification and some properties of the totally converted *I* form of glycogen synthase (UDPglucose:glycogen α-4-glucosyltransferase, EC 2.4.1.11) from bovine heart was described. In this paper we describe a procedure for the purification of totally converted synthase *I* from rabbit skeletal muscle. This procedure yields a highly purified enzyme which is essentially free of synthase *D*. Some of the kinetic properties of this enzyme are presented. In the next paper[2], a study of anion activation of this enzyme and of cardiac muscle synthase *I* is reported. In a previous study from our laboratory, synthase *I* prepared by this procedure was used as the substrate in a detailed study of the purified synthase *I* kinase[3]. Recently, we have reported the preparation of the enzyme from which the glycogen has been removed by α-amylase (EC 3.2.1.1) digestion followed by gel filtration[4]. Although there was some loss of activity, this treatment yielded completely converted essentially glycogen-free synthase *I* which was virtually homogeneous as judged by sodium dodecyl sulfate-gel electrophoresis and by ultracentrifugation[5].

METHODS

Enzyme assays

Synthase activity was determined by measuring the transfer of [^{14}C]glucose from UDP[^{14}C]glucose into glycogen[6]. Total synthase ($I + D$) was determined in the presence of 6.7 mM glucose 6-phosphate while synthase I was assayed in the presence of 10 mM Na_2SO_4 (ref. 2). The I/total ratio is the activity in the presence of Na_2SO_4 divided by the activity in the presence of glucose 6-phosphate. Synthase I kinase and synthase D phosphatase and phosphorylase were assayed by published procedures[3,7,8].

Analytical procedures

Protein concentration was determined by the Folin–Lowry[9] procedure. Glycogen was determined by the phenol–H_2SO_4 method[10].

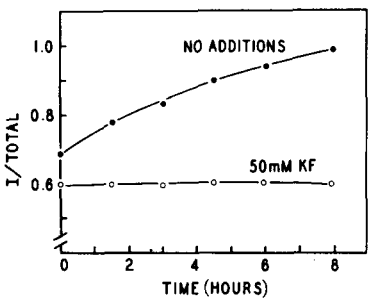
RESULTS

Purification of synthase I

Albino male New Zealand rabbits (approx. 3 kg) were anesthetized by intravenous injection of 140 mg secobarbital in 2 ml of 0.9% NaCl. The animals were bled through the jugular vein, skinned, and the hind leg and back muscles were excised and chilled on ice. The muscle was trimmed of fat and connective tissue, cut into small pieces, and weighed. The tissue (400–450 g) was placed in 3 vol. (w/v) of chilled 50 mM Tris–HCl and 5 mM EDTA buffer (pH 7.8*). All subsequent steps were done at 4 °C unless otherwise stated. The muscle was homogenized in a 1-gallon Waring Blender for 2 min. The homogenate was centrifuged at 15 000 × g for 30 min and the supernatant was filtered through glass wool. Column-treated oyster glycogen[6] was added to a concentration of 1 mg/ml and the solution was chilled to about 0 °C in an ice–salt bath. The solution was placed on a magnetic stirrer, and ethanol (95%, chilled to −60 °C was added to a final concentration of 30% (v/v). The ethanol was added slowly so that the temperature did not exceed 4 °C. The suspension was then stirred for 10 min in the ice–salt bath, and centrifuged at 15 000 × g for 15 min at −10 °C. The supernatant was discarded and the pellets were transferred to a 400-ml beaker and dissolved in a pH 6.8 buffer (0.5 ml of buffer per g of original tissue) containing 50 mM Tris–HCl, 5 mM EDTA, 50 mM mercaptoethanol, 2 mM Na_2SO_4. The mixture was stirred gently until solution was achieved (10–15 min). The solution was transferred to dialysis bags and dialyzed against 4 l of the same buffer for 6–8 h. During this dialysis there was an essentially complete conversion of synthase D into the I form with only about a 10% loss of total activity (Fig. 1). The conversion was completely inhibited by 50 mM KF. If the conversion was carried out by incubation at 4 °C without dialysis, there was a considerable loss of total activity. After conversion, the enzyme solution was removed from the dialysis bags and the pH was adjusted to 7.6 by the addition of Tris–HCl–EDTA–mercaptoethanol–sulfate buffer (pH 8.2). The solution was warmed in a 30 °C water bath for 15 min (final temperature 25 to 28 °C). This brief heat treatment prevented the enzyme from precipitating during the subsequent centrifugation at 50 000 × g. The enzyme was chilled to 4 °C and centrifuged

* Buffers were prepared as a 10-fold concentrated stock solution. The pH of the concentrated buffer was measured at room temperature.

at 50 000 × *g* for 30 min. The supernatant was applied to a DEAE-cellulose column (4 cm × 24 cm) equilibrated with Tris–HCl–EDTA–mercaptoethanol–sulfate buffer (pH 7.8). The column was washed with one bed volume of the same buffer and then washed with 6–8 bed volumes of the same buffer containing 100 mM NaCl. The effluent contained most of the phosphorylase and was discarded. Synthase *I* was eluted from the column with Tris–HCl–EDTA–mercaptoethanol–sulfate buffer containing 300 mM NaCl. Column treated rabbit liver glycogen[6] was added to the eluted enzyme at a concentration of 0.25 mg/ml. The solution was chilled to 1 °C with an ice–salt bath and ethanol (95%, chilled to −60 °C) added to a final concentration of 15% (v/v). The solution was then stirred in the ice–salt bath for 10 min and centrifuged at



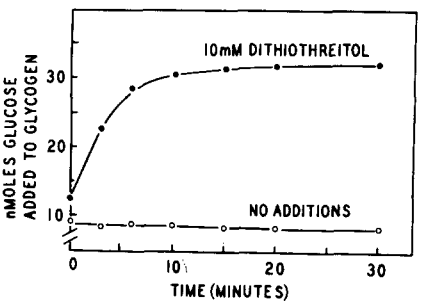


Fig. 1. Conversion of synthase *D* to synthase *I*. The resuspended first ethanol precipitate was dialyzed at 4 °C as described in the text. At the indicated times, aliquots were removed and assayed for synthase *I* and total synthase.

Fig. 2. Activation of synthase *I* by preincubation with dithiothreitol. Frozen synthase *I* was thawed and diluted with a pH 7.8 preincubation mixture containing 50 mM Tris–HCl, 5 mM EDTA, 10 mg/ml rabbit liver glycogen with or without 10 mM dithiothreitol and incubated at 30 °C. At the indicated times aliquots were removed and assayed for synthase *I*. Synthase *I* assays were run for 2 min.

−10 °C for 20 min at 20 000 × *g*. The precipitate was dissolved in 25 ml of 50 mM Tris–HCl–EDTA buffer (pH 7.8) and dialyzed for 3 h against 2 l of the same buffer. The enzyme was frozen in small aliquots and stored at −60 °C. After thawing, the enzyme required a preincubation in the presence of a reducing agent such as mercaptoethanol or dithiothreitol to activate synthase *I*. Routinely, the enzyme was preincubated with Tris–HCl–EDTA buffer containing 10 mg/ml glycogen and 10 mM dithiothreitol (pH 7.8) for 20 min at 30 °C Fig. 2). The enzyme could be stored at −60 °C for at least two months and regained complete activity with the above preincubation. The activated enzyme was stable at 4 °C for several days. Table I summarizes the purification of synthase *I* as described. Synthase *I* (*I*/total >0.95) was purified over 500-fold with a yield of 36%.

Sepharose 6B gel filtration

The enzyme prepared by this procedure contained approximately 250 μg of glycogen per unit of synthase *I*. To determine if all of the synthase *I* was bound to glycogen (glycogen–enzyme complex), purified synthase *I* was chromatographed on Sepharose 6B. Enzyme activated in the presence of glycogen was applied to a Sepharose 6B column. As shown in Fig. 3, most of the glycogen was excluded from the Sepharose

TABLE I

PURIFICATION OF GLYCOGEN SYNTHASE *I*

Step	*Total units***	*Spec. act. (units/mg protein)*	*Synthase I* — *Total*	*Recovery (%)*
Homogenate*	639	0.01	0.49	—
15 000 × *g* supernatant	588	0.03	0.59	92
Ethanol ppt. I	565	0.11	0.63	88
After conversion	506	0.09	0.97	79
50 000 × *g* supernatant	347	0.08	0.96	54
0.3 M NaCl effluent	183	1.1	0.95	29
Ethanol ppt. II	228	5.2	0.99	36

* 405 g rabbit muscle was treated as described in text.
** μmoles of glucose added to glycogen per min at 30 °C.

6B gel. The exclusion limit of Sepharose 6B for polysaccharides is approximately $1 \cdot 10^6$. However, a significant amount of the glycogen was included demonstrating the heterogeneity of the KOH-treated rabbit liver glycogen. All of the synthase *I* was eluted with the high molecular weight excluded glycogen. When the enzyme was preincubated without additional glycogen in the preincubation mixture, the same elution pattern was obtained but there was a considerable loss of enzyme activity. Synthase *I* essentially free of glycogen following amylase digestion[5] was included and eluted after the thyroglobulin marker.

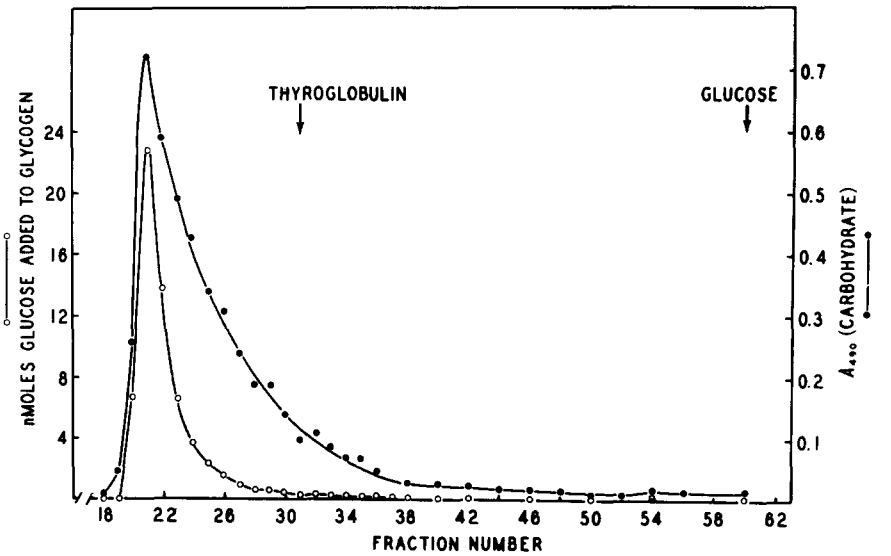


Fig. 3. Chromatography of synthase *I* on Sepharose 6B. Synthase I was preincubated as described in Fig. 2 except 50 mM mercaptoethanol replaced dithiothreitol. The enzyme was placed on a Sepharose 6B column (0.9 cm × 55 cm) equilibrated at 27 °C with 50 mM Tris–HCl, 5 mM EDTA, 50 mM mercaptoethanol (pH 7.8). Buffer was pumped through the column at 6.3 ml/h and 0.5-ml fractions were collected. Aliquots of the fractions were assayed for synthase *I* and carbohydrate. The column was also monitored at 260 nm. The absorbance at 260 nm (not shown) paralleled the elution of glycogen. The location of thyroglobulin (mol. wt = 630 000) and glucose (mol. wt = 180) markers are indicated.

Sulfate stabilization of synthase I

Skeletal muscle and cardiac muscle synthase *I* was stimulated about 2-fold by divalent anions[2]. Liver synthase predominately in the *I* form was also stimulated and protected from thermoinactivation at 37 °C by divalent anions[11]. Sulfate protection of muscle synthase *I* at increased temperature was accordingly studied. Synthase *I* was incubated at 42 °C in the absence or presence of 10 mM Na_2SO_4 and at the indicated times, aliquots were removed and assayed at 30 °C. As shown in Fig. 4, synthase *I* was almost completely inactivated within 30 min when incubated without sulfate at 42 °C. Na_2SO_4 greatly stabilized the enzyme protecting it from heat inactivation. When the incubation was carried out at 30 °C the enzyme was stable for up to 1 h even in the absence of sulfate. Yet, the inclusion of Na_2SO_4 (2 mM) throughout the purification increased the overall yield.

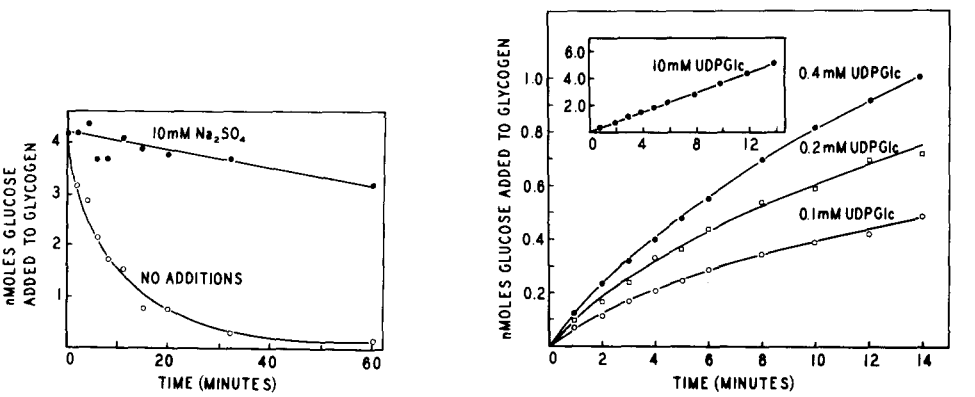


Fig. 4. Sulfate stabilization of synthase *I*. Synthase *I* was preincubated as described in Fig. 2. The enzyme was then incubated at 42 °C with or without 10 mM Na_2SO_4 and aliquots removed at the indicated times for synthase *I* assay at 30 °C. In both cases, the concentration of Na_2SO_4 in the synthase *I* assay was 10 mM.

Fig. 5. The effect of UDPglucose concentration on the time course of the reaction. The enzyme, 3 units/ml, was preincubated as described in Fig. 2. After activation it was diluted 100-fold with preincubation mixture and stored on ice. Synthase *I*, about 6 munits/ml in the final assay, was assayed at 30 °C in a reaction mixture containing 50 mM Tris–HCl, 5 mM EDTA, 10 mg/ml rabbit liver glycogen, (pH 7.8), and the concentration of UDPglucose indicated (spec. act. between 910 and 1850 dpm/nmole). 75-μl aliquots were removed at the times indicated and the glycogen isolated.

Factors affecting the time course of the reaction

When synthase *I* was assayed with saturating UDPglucose concentration (10 mM) the reaction was linear with time (Fig. 5, insert). However, when low concentrations of UDPglucose (0.1 to 0.4 mM) were employed, there was a relatively rapid initial rate which decreased with time to a slower constant rate (Fig. 5) (see Fig. 1, ref. 1). The curvilinear progress curves were not eliminated when the enzyme was diluted to concentrations employed in the assay and incubated with a reaction mixture lacking only UDPglucose. The same type of progress curves were also obtained if the enzyme concentration was double or 1/2 of that shown in Fig. 5. In all the remaining experiments the enzyme concentration was adjusted so that less than 10% of the substrate was consumed during the experiment.

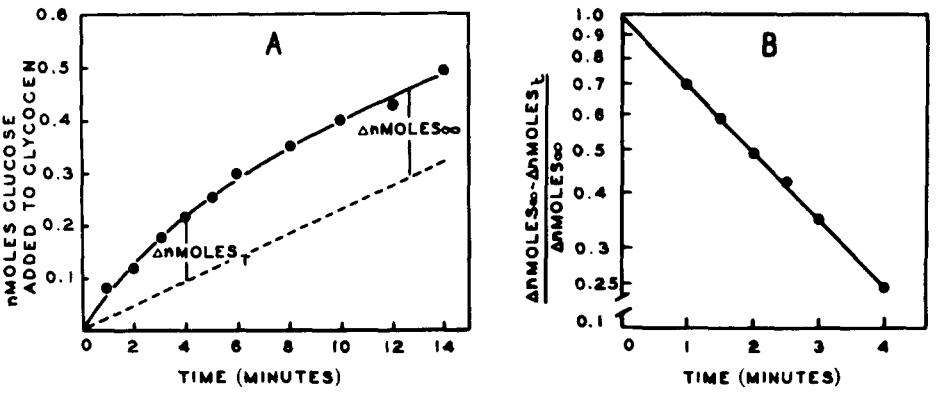


Fig. 6. Graphical analysis of the curvilinear time courses. A reference line was drawn through the origin parallel to the linear portion of the progress curve. The vertical distance, $\triangle$ nmoles$_t$, between the reference line and the curved portion of the progress curve was determined at a number of times, t. The vertical distance between the reference line and the linear portion of the time course was designated Δnmoles$_\infty$. Values of the log of (Δnmoles$_\infty$ — Δnmoles$_t$)/nmoles$_\infty$ were plotted against time. The data were obtained from the 0.1 mM UDPglucose time course in Fig. 5.

Transient reaction progress curves of this type may be indicative of a relatively slow conversion of one kinetic form of an enzyme into a second stable form with reduced activity[1,12]. The rate of approach to the second less active form was analyzed graphically by the procedure outlined by Hatfield *et al.*[13]. As shown in Fig. 6A, a reference line was drawn through the origin parallel to the linear portion of the time course. Differences between the reference line and the curved portion of the time course were determined at several times. These differences in nmoles of glucose from UDPglucose added to glycogen were designated Δnmoles$_t$. The difference between the reference line and the linear portion of the time course was designated Δnmoles$_\infty$. The log of (Δnmoles$_\infty$ — Δnmoles$_t$)/Δnmoles$_\infty$ was plotted *versus* time (Fig. 6B). The plots obtained by this process were linear as predicted for a first order process and the half time ($T_{1/2}$) for the formation of the stable form of synthase *I* could be estimated from the graph[13]. The data in Fig. 6 are from the time course at 0.1 mM UDPglucose shown in Fig. 5. The $T_{1/2}$ was approximately 1.9 min. The $T_{1/2}$ increased slightly as UDPglucose concentration was increased to 0.4 mM. There was some variation in the $T_{1/2}$ obtained from different preparations of the enzyme. Using standard preincubation conditions (Fig. 5) the $T_{1/2}$ for several preparations varied from 1.8 to 2.4 min when assayed at 0.13 mM UDPglucose.

UDP, a product of the reaction, can markedly inhibit muscle synthase *I*[2,14]. It did not seem likely that UDP production was causing the non-linear early time course since the curvature was not greatly altered by the amount of reaction and therefore by UDP concentration. In our standard assay when 0.375 nmole of glucose was transferred from UDPglucose onto glycogen, the UDP formed would constitute a concentration of 5 μM. When the time course at 0.13 mM UDPglucose was determined in the presence and absence of 5 μM UDP, there was an inhibition observed (Fig. 7). However, UDP did not change the general shape of the reaction progress curve, and the $T_{1/2}$ was increased 40%. Furthermore, when the enzyme was preincubated with 5 μM UDP, the curvature was not eliminated (data not shown).

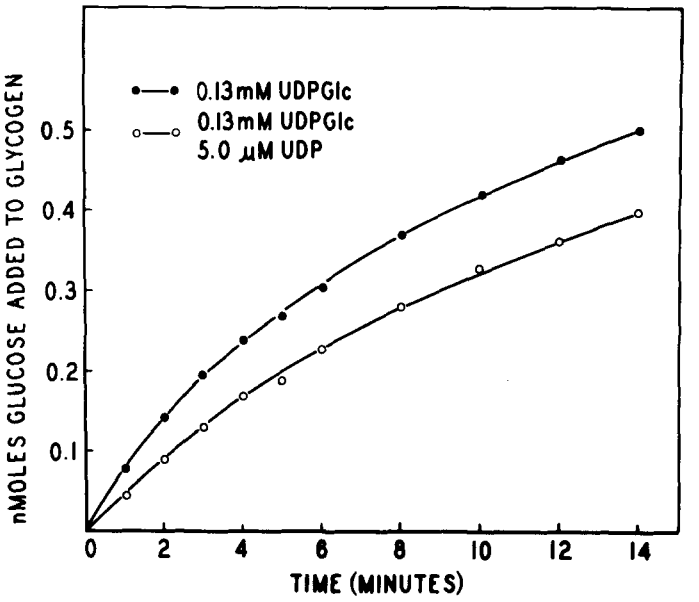


Fig. 7. The effect of UDP on the time course of the reaction. The enzyme was preincubated and diluted as described in Fig. 5 and then assayed with 0.13 mM UDPglucose with or without 5 μM UDP.

The pH optimum for synthase *I* in the absence of an anion activator is pH 7.0 (ref. 2). The time course at pH 7.0 and 7.8 is shown in Fig. 8. Both the initial rate and the steady state rate were greater at pH 7.0. However, at both pH values the time

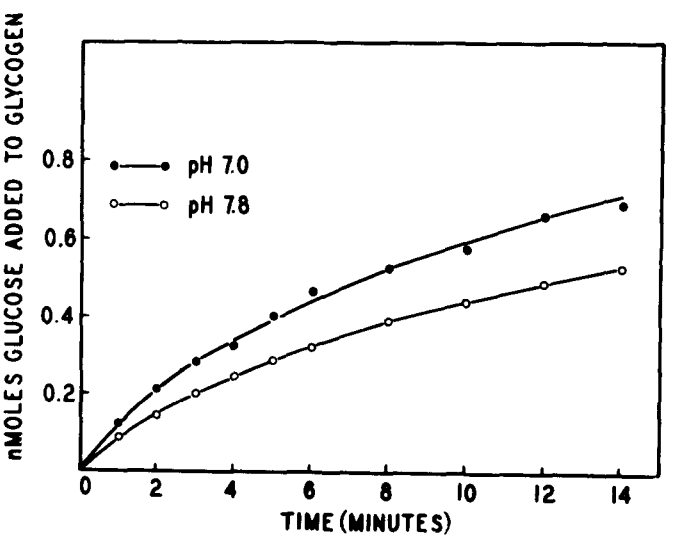
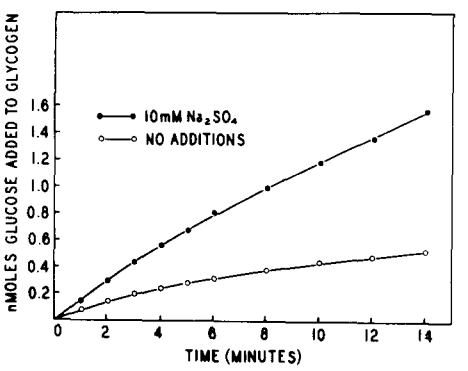


Fig. 8. The effect of pH on the time course of the reaction. The enzyme was preincubated for 30 min at 30 °C with 25 mM Tris–HCl, 5 mM EDTA, 10 mM dithiothreitol, and 10 mg/ml rabbit liver glycogen (pH 7.8). The activated enzyme was diluted 100-fold with 5 mM EDTA, 10 mM dithiothreitol, and 10 mg/ml rabbit liver glycogen (pH 7.0). Synthase *I* was assayed in a reaction mixture containing 50 mM Tris, 50 mM maleate, 5 mM EDTA, 10 mg/ml glycogen, and 0.13 mM UDPglucose at pH 7.8 or 7.0 (the pH of the final enzyme assay mixture was determined).

course had a similar curvature. The $T_{1/2}$ was somewhat higher at pH 7.8 (1.8 min *vs* 2.1 min).

A number of divalent anions activate synthase *I* and shift the pH curve to a broad optimum around pH 7.8 (ref. 2). The time course in the presence and absence of Na_2SO_4 (10 mM) is presented in Fig. 9. Both the initial rate and the steady state rate were increased by the divalent activator. The curvature of the time course and the $T_{1/2}$ were the same in both cases.



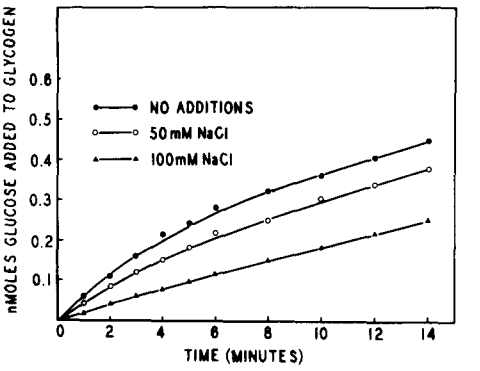


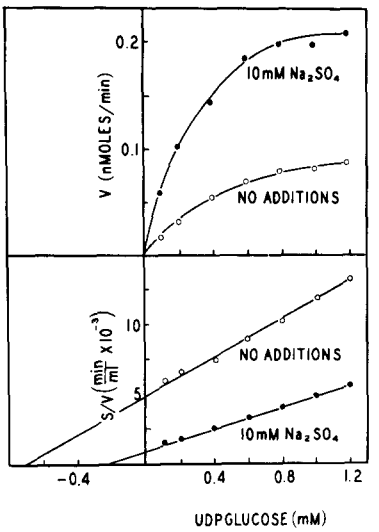
Fig. 9. The effect of Na_2SO_4 on the time course of the reaction. The enzyme was preincubated and diluted as described in Fig. 5 and then assayed with 0.13 mM UDPglucose in the presence or absence of 10 mM Na_2SO_4.

Fig. 10. The effect of NaCl on the time course of the reaction. The enzyme was preincubated and diluted as described in Fig. 5 and then assayed at the indicated concentration of NaCl and 0.13 mM UDPglucose.

The effect of increasing the ionic concentration by addition of NaCl is shown in Fig. 10. When 50 mM NaCl was included during the assay, the time course of the initial rapid rate appeared to be shortened, but the same second steady state rate was obtained. When 100 mM NaCl was present during the assay the initial rapid rate almost completely disappeared and the second linear rate was equal to the linear rate obtained in the absence of added NaCl. Increasing the concentration of NaCl further, gave a linear time course but inhibited the reaction, *i.e.* the slope was reduced (see Fig. 3, ref. 1). The effect of NaCl did not appear to be a simple acceleration of the conversion of one form into another. When the data obtained with 50 mM NaCl present were analyzed as described in Fig. 6, the resulting plot was not linear and a direct comparison of the $T_{1/2}$ values could not be made.

Kinetic analysis of two forms of synthase I

As with the heart enzyme, the curvilinear time courses found at low substrate concentrations and the linear time courses at high concentrations were compatible with two kinetic forms of the enzyme. An initial transient form was designated "Form 1", and a second stable form was accordingly designated "Form 2". At low substrate concentrations Form 1 would have a greater rate than Form 2, while at high substrate concentrations (presumably saturating levels of UDPglucose) their rates would be similar. Kinetic studies were carried out on both forms of the enzyme. For these studies, time courses were carried out for 16 min at different UDPglucose concentra-



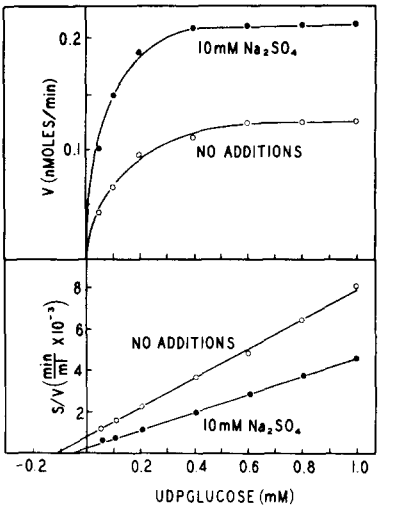


Fig. 11. Kinetic plots for Form 1 of synthase *I*. The enzyme was preincubated and diluted as described in Fig. 5. The time course of the reaction was determined at each UDPglucose concentration and the initial rates were estimated.

Fig. 12. Kinetic plots for Form 2 of synthase *I*. The enzyme was preincubated and diluted as described in Fig. 5. The time course of the reaction was determined at each UDPglucose concentration and the rate was determined from the linear portion of the curve, *i.e.* 10–16 min.

tions. The rates for Form 1 were estimated from the initial slopes. The rates for Form 2 were obtained from the linear portion of the plots, *i.e.* 10–16 min portion. The data were analyzed by plotting S/v *versus* S and the maximum velocity and K_m for each form was estimated from the resulting plots. The kinetic plots obtained from Form 1 are presented in Fig. 11 (see Fig. 6, ref. 1). The plots both in the presence and absence of Na_2SO_4 were linear. The kinetic plots for Form 2 are shown in Fig. 12. Again, under the conditions of these assays, the S/v *versus* S plots were linear. We did not observe the downward curvature at low concentrations (lowest concentration employed was 0.1 mM) which was obtained with Form 2 of synthase *I* (see Fig. 7, ref. 1) purified from cardiac muscle[1]. The kinetic constants obtained for Form 1 and Form 2 are sum-

TABLE II

KINETIC CONSTANTS FOR UDPGLUCOSE AND THE CALCULATED RELATIVE *in vivo* VELOCITY

	K_m	V	*Relative in vivo velocity**
Form 1			
(−) Na_2SO_4	0.11	0.15	37
10 mM Na_2SO_4	0.05	0.23	100
Form 2			
(−) Na_2SO_4	0.72	0.12	6
10 mM Na_2SO_4	0.27	0.25	29

* Calculated from the Michaelis–Menten equation, $v = V[S]/(K_m + [S])$. A UDPglucose concentration of 0.03 mM (ref. 18) and the appropriate kinetic constants were used for the calculations.

marized in Table II. The K_m for UDPglucose was 6- to 7-fold lower for Form 1 than Form 2. The V was essentially the same for both forms. Na_2SO_4 increased the V 2-fold and decreased the K_m 2- to 3-fold for both Form 1 and Form 2.

The possible influence of these two forms as well as divalent activators on the *in vivo* rate of synthase *I* was determined by solving the Michaelis–Menten equation, $v = V[S]/(K_m + [S])$ using the appropriate substrate concentration and the kinetic constants in Table II. The *in vivo* concentration of UDPglucose in rabbit muscle is about 0.03 mM and remains constant under a variety of physiological conditions[15]. As shown in Table II, the *in vivo* velocity could be greatly altered by the kinetic form of the enzyme present as well as anion activators.

DISCUSSION

The procedure for the purification of skeletal muscle synthase *I* described in this paper yields a 500-fold purified glycogen-bound enzyme essentially free of synthase *D* (*I*/total >0.95). While this preparation is not homogeneous, it is stable when frozen and it is useful for many studies. When a homogeneous glycogen free enzyme is required, the glycogen can be removed by amylase digestion and a virtually homogeneous preparation obtained[4]. The glycogen-bound synthase *I* is free of phosphorylase and usually does not have detectable levels of glycogen synthase *I* kinase activity. However, when synthase *I* was to be used as the substrate for studies of synthase *I* kinase[3], it was essential that the preparation be carefully assayed for synthase *I* kinase since an occasional preparation was contaminated with the interconverting enzyme. The synthase *I* preparation appeared to be free of synthase *D* phosphatase activity, when it was assayed according to Villar-Palasi[7]. However, when synthase *I* prepared by this procedure was assayed for synthase *D* phosphatase in the presence of Mn^{2+}, there was considerable phosphatase activity (personal communication from J. S. Bishop, also see ref. 16).

At saturating substrate levels (10 mM UDPglucose) the time course was linear while at lower concentrations the time courses were not linear (Fig. 5). There was an initial fast rate which continuously decreased to a slower constant rate. The formation of the product UDP, an inhibitor of synthase *I*[2,14] did not appear to cause the non-linear course. When UDP was included in the assay, there was some inhibition but the time course had the same general shape (Fig. 7). In addition, preincubation with UDP did not eliminate the curvature of the progress curve. This data is consistent with a change in synthase from an unstable enzyme with a relatively fast rate at low substrate concentration (designated as "Form 1") to a second stable form with a reduced rate (designated as "Form 2"). A similar interpretation of transient reaction progress curves was presented by Shill and Neet[12] for yeast hexokinase and Thomas and Larner[1] for cardiac synthase *I*.

The rate of conversion of Form 1 to Form 2 was analyzed graphically (Fig. 6). The resulting semilogarithmic plots were linear as expected for a first order process[13] and the half-time ($T_{1/2}$) could be estimated from the graph. Under a number of conditions including different enzyme preparations, varied enzyme concentrations, different pH values, several UDPglucose concentrations, and anion activators, the $T_{1/2}$ ranged from 1.8 to 2.5 min (Figs 5, 8 and 9).

The change(s) which occur when Form 1 is converted to Form 2 are unknown.

The effect of increasing the ionic strength by the addition of NaCl to the assay suggests that aggregation–disaggregation may be involved (Fig. 10). Varying the glycogen concentration from 1 to 10 mg/ml did not have any apparent effect on the progress curve. However, in all of these studies the synthase *I*–glycogen complex was used and it is not known what effect the complete absence of glycogen would have on the two forms. It appears that UDPglucose was necessary for the transition of Form 1 to Form 2. Both Form 1 and Form 2 showed standard Michaelis–Menten kinetics when UDPglucose concentration was varied (Figs 11 and 12). While the V was similar for both forms, the K_m for Form 1 was 6- to 7-fold less than for Form 2 (Table II). The anion activator, Na_2SO_4, stimulated both forms of the enzyme by increasing the V and decreasing the K_m.

The importance of these two kinetic forms *in vivo* remains to be determined. The difference in K_m for UDPglucose of the two forms of synthase *I* could have a profound effect on the *in vivo* rate of glycogen synthesis. Skeletal muscle UDPglucose concentration is quite low and constant under a variety of physiological conditions. Piras and Staneloni[15] reported a UDPglucose concentration in rabbit skeletal muscle of 0.03 mM. Assuming the enzyme forms followed standard Michaelis–Menten kinetics, the relative *in vivo* velocities of the two forms in the presence and absence of an activator were calculated (Table II). As can be seen under these extremes, the velocity of the enzyme could vary as much as 17-fold. In addition to this direct effect on catalytic activity of synthase *I*, it will be important to establish if these forms of synthase *I* and anion activators have any modulating effects on the activity of the interconverting enzymes. Thus the fraction of total synthase in phosphorylated and dephosphorylated form (*D* and *I* form) could be regulated in part by changes in synthase *I*, the substrate for synthase *I* kinase.

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