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CONVERSION OF GLYCOGEN SYNTHETASE D OF RAT LIVER TO A FORM THAT RESEMBLES SYNTHETASE D OF SKELETAL MUSCLE

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SUMMARY

Glycogen synthetase D (UDPglucose:α-1,4-glucan-α-4-glucosyltransferase, EC 2.4.1.11) partially purified from rat liver and free of glycogen synthetase D phosphatase is converted by incubation with $MgCl_2$ to a form (the D′-form) that is distinct from the I-form. The D to D′ conversion is independent of the D to I conversion catalyzed by D phosphatase, but is stimulated by glucose 6-phosphate and inhibited by exogenous glycogen. Unlike the D- and I-forms, the activity of the D′-form is absolutely dependent on glucose-6-*P* as is the case with the skeletal muscle D-form. The D to D′ conversion may thus serve as a regulatory mechanism for glycogen synthesis in rat liver in response to fluctuation in intracellular glucose 6-phosphate levels.

INTRODUCTION

Glycogen synthetase (UDPglucose:α-1,4-glucan-α-4-glucosyltransferase, EC 2.4.1.11) of rat liver differs from that of skeletal muscle in that in Tris–maleate buffer at pH 7.4, the D-form (or b) exhibits little activity even at high concentrations of glucose 6-phosphate[1–3]. If assayed under these conditions, the conversion of the D-form to the I-form (or a) catalyzed by glycogen synthetase D phosphatase results in a concurrent increase in the activities with and without glucose-6-*P*[1–3]. Similar pattern of the activation of liver synthetase has been observed in the presence of phosphate or sulfate[4,5]. These anions, however, also inhibit the D-form of skeletal muscle synthetase[6].

The present report shows that partially purified liver synthetase D free of D phosphatase is converted by incubation with $MgCl_2$ to a form that is distinct from the I-form. When compared in Tris–maleate buffer at pH 7.4, this form is similar to the skeletal muscle synthetase D in kinetic properties and, therefore, is called the D′-form of liver synthetase.

MATERIALS AND METHODS

Animals

All rats used were Donryu adult male (120–180 g) and fed a commercial diet *ad libitum*. The animals were fasted for 24 h and then refed for 24 h before sacrifice.

Partially purified glycogen synthetase D

Glycogen synthetase D was partially purified from the liver in the same manner as described previously[1]. The final sample was suspended in one-fifth the original homogenate volume of 50 mM glycylglycine buffer (pH 7.4) and used for the experiments. The purification was approx. 300-fold from crude extracts and the final sample, still bound to patriculate glycogen, was free of synthetase I and synthetase D phosphatase.

Preparation of glycogen synthetase D phosphatase

The liver from a single rat was homogenized in a glass-Teflon homogenizer for 2 min using 4 vol. of 0.5 M sucrose–62.5 mM Tris–HCl (pH 7.4)–6.25 mM EDTA. The homogenate was centrifuged for 10 min at 5000 × *g*. The supernatant was then centrifuged at 105 000 × *g* for 60 min. As reported previously[1], the final supernatant was active in converting the partially purified D- to the I-form.

The pH of the supernatant was adjusted to 5.2 with 1 M acetic acid. After 10 min, the solution was centrifuged; the resulting supernatant was neutralized with 1 M KOH and brought to 30% saturation with saturated $(NH_4)_2SO_4$. After centrifugation, the supernatant was brought to 60% saturation. The precipitate was collected by centrifugation, dissolved in 1 ml of 20 mM Tris–HCl (pH 7.4) containing 10 mM mercaptoethanol and passed through a desalting column of Sephadex G-25. The entire procedure was conducted at 0–4 °C. Most of the D phosphatase activity present in the original high-speed supernatant was recovered in the final sample.

Assay of glycogen synthetase

Glycogen synthetase was assayed as described previously[1]. The standard assay mixture contained 50 mM Tris–maleate (pH 7.4), 1 mM UDP[U-^{14}C]glucose, 10 mM glucose-6-*P* (if present), 40 mM NaF, 0.6 mg of rabbit liver glycogen and enzyme in a final volume of 0.2 ml and incubated for 5 min at 30 °C. A unit of enzyme was defined as the amount which catalyzed the incorporation of 1 μmole of [U-^{14}C]glucose into glycogen per hour.

Chemicals

The sources of the chemicals used were described in the previous paper[1].

RESULTS

In the experiment shown in Fig. 1, partially purified glycogen synthetase D was incubated at 30 °C in the presence of 5 mM $MgCl_2$. When the concentration of the enzyme was low (0.2 ml enzyme/ml incubation mixture), no increase in enzyme activity could be observed unless synthetase D phosphatase was deliberately added (Fig. 1A).

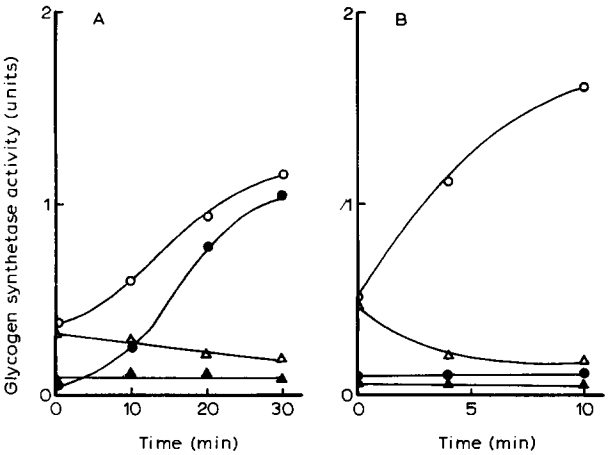

Fig. 1. Activation of partially purified glycogen synthetase D with or without glycogen synthetase D phosphatase. (A) 0.2 ml of synthetase D was incubated at 30 °C with 50 mM glycylglycine (pH 7.4), 5 mM $MgCl_2$, 2 mM Na_2SO_4, 1 mM caffeine in the presence (○, ●) or absence (△, ▲) of D phosphatase (0.47 mg in protein). The final volume was 1 ml. Aliquots were removed at the times indicated for assay of glycogen synthetase activity with (○, △) or without (●, ▲) glucose-6-*P*. 40 mM NaF included in the assay mixture was sufficient to terminate the phosphatase reaction. (B) 0.6 ml of synthetase D was incubated as described above except that D phosphatase (○, ●) or D phosphatase *plus* $MgCl_2$ were omitted (△, ▲). Activity was then determined with (○, △) or without (●, ▲) glucose-6-*P*.

Upon addition of the latter enzyme, activities measured with and without glucose-6-*P* were increased in parallel as has been reported previously[1]. These results confirmed the previous finding[1] that the synthetase D purified as above is free of D phosphatase.

When the concentration of synthetase D was raised to 0.6 ml/ml, however, incubation resulted in an activation of the enzyme which differed from the phosphatase-catalyzed D to I conversion in that only the activity measured with glucose-6-*P* was increased with time (Fig. 1B). The activation was rapid and there was no indi-

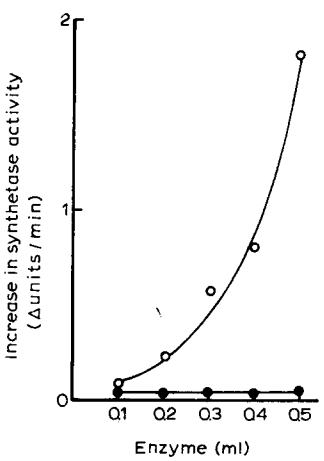

Fig. 2. Activation of synthetase D as a function of its concentration. Varying amounts of synthetase D were incubated at 30 °C with 50 mM glycylglycine (pH 7.4) and 5 mM $MgCl_2$ in a final volume of 1 ml. After 5 min, activity was determined with (○) or without (●) glucose-6-*P*.

cation of the occurrence of lag period which characterized the activation by D phosphatase (Figs 1A and 9).

The activation of synthetase D is markedly concentration dependent: as shown in Fig. 2, the rate of activation (as determined by increase in the activity measured with glucose-6-*P*) was accelerated with increase in synthetase D concentration. The activity measured without glucose-6-*P* was not increased even at extremely high enzyme concentrations, excluding again the possibility of D phosphatase participating in the activation reaction. The activation is also temperature dependent: it occurred at 0 °C but in much slower rate than at 30 °C.

As is the case with D phosphatase reaction, the above activation reaction is absolutely dependent on Mg^{2+} (Fig. 1B) and is stimulated by glucose-6-*P*. With 2 mM glucose-6-*P*, the degree of activation was usually 400–500%; half maximal activation being attained at 0.17 mM (Fig. 3). The activation reaction, however, differed from

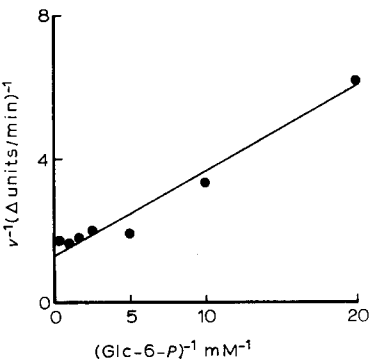

Fig. 3. Stimulation of synthetase D activation by glucose-6-*P*. 0.6 ml of synthetase D was incubated for 8 min as described in the legend to Fig. 2, in the presence of varying concentrations of glucose-6-*P*. Activity was then determined with glucose-6-*P*. Illustrated is a double reciprocal plot of the increase in the rate of activation due to glucose-6-*P* *vs* glucose-6-*P* concentration.

the D to I conversion in that caffein and glucose were without effect and that dithiothreitol was stimulative rather than inhibitory. Furthermore as shown in Fig. 4, 10 mM NaF, which inhibited the phosphatase reaction by 50%, did not inhibit the activation reaction. Added glycogen was inhibitory to both reactions. The activation reaction, however, was inhibited much more profoundly than the phosphatase reaction (Fig. 5).

The kinetic properties of the glycogen synthetase that had been activated fully in the above manner were studied in comparison with the original D-form and the I-form prepared from the D-form by D phosphatase. Fig. 6 compares the effect of increasing concentrations of glucose-6-*P* on activity with 1 mM UDPglucose as substrate. While the D- and I-forms failed to respond to glucose-6-*P* significantly as previously reported[1], the activity of the synthetase activated in the above manner was totally dependent on glucose-6-*P*, as is the case with the D-form of skeletal muscle enzyme[2]. The third form of rat liver synthetase is thus called the D′-form. Double reciprocal plot of the D′-form extrapolated to an apparent K_m of 0.33 mM, which is comparable to the value of 0.23 mM obtained for the D-form of skeletal muscle enzyme under similar conditions[2].

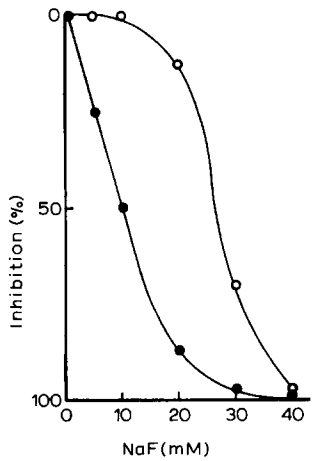

Fig. 4. Effect of NaF on the activation of synthetase D. Incubation with D phosphatase was carried out for 30 min as described in the legend to Fig. 1A, in the presence of varying concentrations of NaF, after which activity was determined without glucose-6-*P* (●). The activation of synthetase D without phosphatase was determined as described in the legend to Fig. 3 except that the activation reaction was conducted in the presence of varying concentrations of NaF (○).

The D-form of rat liver does not respond to glucose-6-*P* under the above conditions chiefly because of its extremely low affinity towards UDPglucose[1]. The results with varying concentrations of UDPglucose in the presence and absence of 10 mM glucose-6-*P* are shown in Fig. 7. It can be seen that the D to D′ conversion is accompanied by a decrease in K_m for UDPglucose in the presence of glucose-6-*P* from 2.0 to 0.57 mM. Although the latter value is almost identical to the corresponding value for the I-form, K_m obtained in the absence of glucose-6-*P* was still much greater for the D′-form than for the I-form. The ratio of the v obtained in the absence of glucose-6-*P* to that in the presence of glucose-6-*P* was almost unity for the I-form, but only 0.25 for the D′-form.

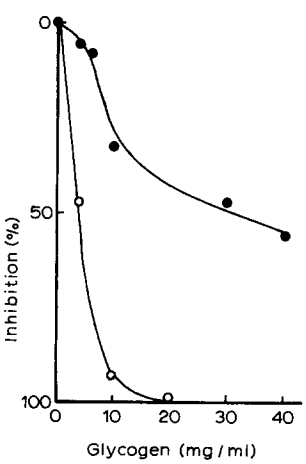

Fig. 5. Effect of glycogen on the activation of synthetase D. Experimental conditions and symbols were the same as those for Fig. 4 except that glycogen was tested instead of NaF.

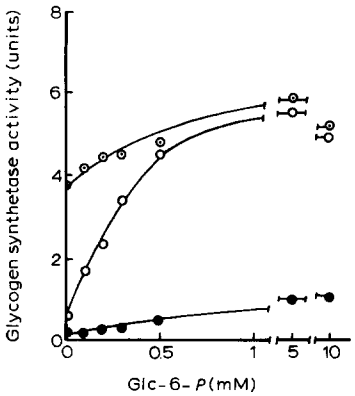

Fig. 6. Effect of glucose-6-*P* on the activity of the D- (●), D'- (○) and I-forms (○) of glycogen synthetase. The D'- and I-forms were prepared by incubating the D-form as described in the legend to Fig. 4 except that NaF was omitted.

It is evident from these observations that the D'-form is distinct from the I-form that is derived from the same D-form. Kinetically, the D'-form resembles closely the D-form of muscle enzyme, for which the values of 0.5 (with glucose-6-*P*) and 1 mM (without glucose-6-*P*) were reported for K_m for UDPglucose and the value of 0.2 was found for the v ratio[2].

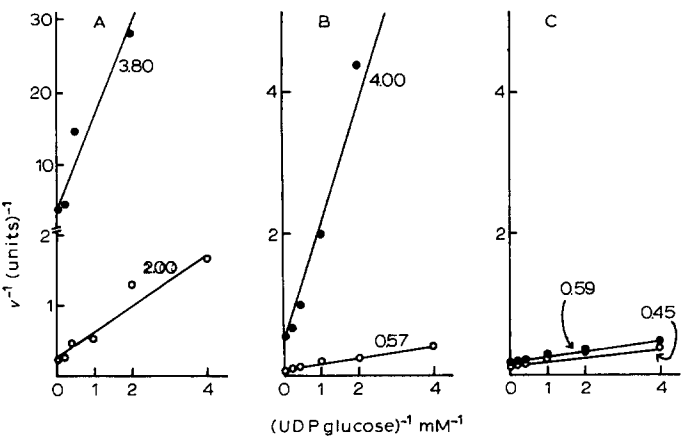

Fig. 7. Double reciprocal plots of velocity *vs* UDPglucose concentration of the D- (A), D'- (B) and I-forms (C). Assays were made with (○) or without (●) glucose-6-*P*. K_m values for UDPglucose are given as mM.

The pH–activity curves of the three forms of liver glycogen synthetase are shown in Fig. 8. The D'-form differs from the D-form in that the activity in physiological pH range is reasonably high when glucose-6-*P* is present. It nevertheless differs from the I-form since the activity measured in the absence of glucose-6-*P* still remains extremely low at all the pH values examined. The pH–activity relationship of the D'-form again resembles closely that of the D-form of muscle enzyme[2].

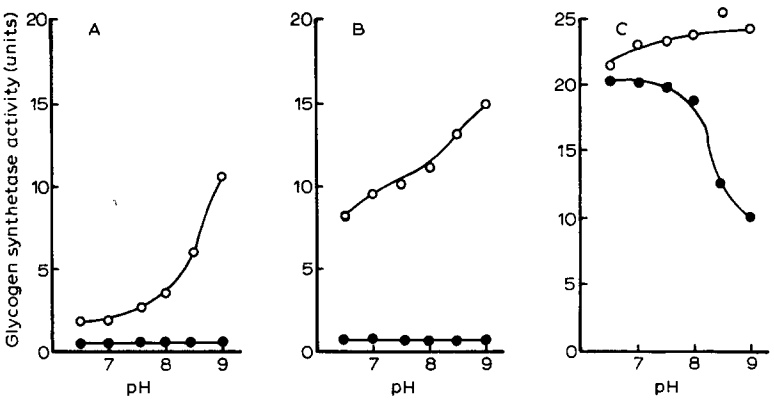

Fig. 8. pH dependence of the activity of the D- (A), D'- (B) and I-forms (C). Assays were made in 50 mM Tris–maleate buffer with (○) or without (●) glucose-6-*P*.

After the D to D' conversion had been completed, the incubation mixture was diluted with 5 vol. of 50 mM glycylglycine buffer (pH 7.4) and centrifuged at 16 000 × *g* for 30 min. All the glycogen synthetase activity was recovered in the pellet. Washing several times followed by resuspension of the pellet yielded a glycogen synthetase activity that was identical in kinetics to that of the above D'-form. It is therefore unlikely that the unique properties of the D'-form are due to low molecular metabolite(s) that was formed during the prior incubation.

The finding that the D'-form closely resembles the D-form of muscle enzyme raised the possibility that the D'-form might be an actual substrate for D phosphatase.

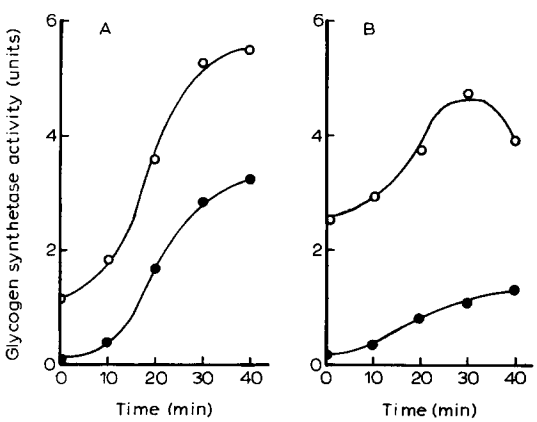

Fig. 9. Formation of the I-form from the D- (A) or D'-form (B) by D phosphatase. Phosphatase reaction was carried out as described in the legend to Fig. 1A with the D- or D'-form as substrate and synthetase activity was determined with (○) or without (●) glucose-6-*P*. For these experiments, D phosphatase was further purified as follows: 6 ml of the enzyme prepared as described in the text (8 mg protein/ml) were applied to a column (1.5 cm × 15 cm) of DEAE-cellulose previously equilibrated with 20 mM Tris–HCl buffer (pH 7.4)–50 mM mercaptoethanol. The column was first washed with 60 ml of the equilibrating buffer and this was followed by elution with 90 ml of the same buffer containing 80 mM NaCl and finally with buffer containing 200 mM NaCl. D phosphatase was precipitated from 200 mM NaCl eluates by $(NH_4)_2SO_4$ at 60% saturation, dissolved in 1 ml of 20 mM Tris–HCl buffer (pH 7.4)–50 mM mercaptoethanol and passed through a desalting column of Sephadex G-25. The final sample (0.18 mg in protein) was used.

If this was the case, the D to D′ conversion should be a pre-requisite to the D phosphatase reaction. In order to test this possibility, the D- and D′-forms were separately incubated with D phosphatase. As shown in Fig. 9, the rate of the formation of the I-form observed as an increase in the activity measured without glucose-6-*P* was much greater with the D-form than with the D′-form as substrate. It was therefore concluded that the D- but not D′-form is an actual substrate for D phosphatase. The D to D′ conversion appears to be an activation process entirely independent of the phosphatase-catalyzed D to I conversion.

DISCUSSION

The present results show that upon incubation with $MgCl_2$, the glycogen synthetase D of rat liver undergoes a conversion to a form that is distinct from the I-form. This new form has been called the D′-form. The conversion does not require D phosphatase. It is stimulated by glucose 6-*P* and is inhibited by glycogen.

We have previously reported that the D-form of rat liver glycogen synthetase differs from that of skeletal muscle[2]. In view of this observation which has recently by confirmed by Sato *et al.*[3], it is of special interest that at least kinetically, the D′-form closely resembles the D-form of muscle. This makes it likely that the D to D′ conversion is of physiological significance.

Several studies have been reported which indicate that liver glycogen synthetase bound to glycogen pellet may undergo interconversion between active and inactive forms. According to Steiner *et al.*[7], the enzyme is inactivated by incubation at 37 °C and reactivated by incubation with glucose-6-*P*. Like the D to D′ conversion, these processes are highly dependent on the concentration of the enzyme preparation: dilution resulted in more extensive inactivation and less complete reactivation. Hizukuri and Larner[8] reported that the D-form inactivated by incubation at 30 °C is noteworthy in view of the present finding that the D-form but not the D′-form is the substrate for D phosphatase (Fig. 9). In these previous studies, high concentrations of glucose-6-*P* were used for synthetase assay. The inactive and active forms of glycogen synthetase D as revealed by them may thus correspond to the D- and D′-forms, respectively, of the present study. If this assumption was correct, then one might expect that the D to D′ conversion should be reversible. Sanada and Segal[9] reported that soluble glycogen synthetase D of rat liver is activated by incubation with glucose-6-*P* without changing to the I-form. Similar activation of synthetase D from trout liver was stimulated by Mg^{2+} and glucose -6-*P* and inhibited by fluoride[10]

Neither D- nor I-form of liver glycogen synthetase is influenced by the concentration of glucose-6-*P* especially when physiological concentrations of P_i (refs 2 and 3) or maleate (Fig. 6) are present and it has been stated that in the liver, glucose-6-*P* level cannot be an important regulatory factor for glycogen synthetase[11].

It is therefore of interest that even in the presence of maleate (and possibly of P_i), the activity of the D′-form is totally dependent on glucose-6-*P* (Fig. 6) as is the case with the D-form of skeletal muscle enzyme[2]. Furthermore, the D to D′ conversion is stimulated markedly by levels of glucose-6-*P* (around 0.2 mM, Fig. 3) which are within the levels found in rat liver physiologically (0.08–0.41 μmole/g, ref. 12). The conversion may therefore serve as a sensitive regulatory mechanism for glycogen synthetase in rat liver in response to fluctuations in intracellular glucose-6-*P* levels.

The mechanism by which the D-form is converted to the D'-form is not yet clear. The conversion proceeds faster at 30 °C than at 0 °C and is dependent on the concentration of the synthetase D preparation. Although the possibility exists that the conversion might be an enzyme-mediated process, attempts to disclose such enzyme activity in high-speed supernatant of rat liver have been unsuccessful.

An alternative explanation is that the D'-form differs from the D-form only conformationally. In this connection, it is noteworthy that glucose-6-*P* has been shown to enhance the aggregation of glycogen synthetase from liver and muscle[7,9,13,14]. The aggregation, however, has been detected only with the enzyme preparations made free of glycogen and it is not known whether the enzyme undergoes similar changes while bound to glycogen as in the case with the present enzyme preparation. Furthermore, the D-form appears to aggregate much less extensively than the I-form[15,16].

The present study showed that even after conversion to the D'-form, glycogen synthetase remains tightly bound to particulate glycogen. Fischer and co-workers[17,18] reported that only phosphorylase associated with glycogen undergoes an activation–inactivation reaction similar to that observed *in vivo*. It is therefore conceivable that the conversion to the D'-form described above may be a functional property of the glycogen synthetase D associated with particulate glycogen. This possibility is currently under investigation.

ACKNOWLEDGEMENTS

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