# Studies on UDPG: α-1,4-Glucan α-4-Glucosyltransferase. VII. Conversion of the Enzyme from Glucose-6-phosphate-dependent to Independent Form in Liver*

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The partially purified liver UDPG: α-1,4-glucan α-4-glucosyltransferase associated with particulate glycogen was shown to contain a system for converting the transferase from the original glucose-6-phosphate-dependent (*D*) form to the glucose-6-phosphate-independent (*I*) form. In this partially purified preparation the conversion was carried out by incubating the particulate glycogen suspension with $Mg^{2+}$ and $SO_3^{2-}$. In the highly purified enzyme preparation, however, the conversion did not occur without an additional factor. This factor was separated from transferase by means of ultrasonic disintegration and centrifugation of the particulate glycogen. The factor was found in a fraction containing the smooth-surface vesicles of the endoplasmic reticulum. The activity of the factor was lost when heated in boiling water for 5 minutes at neutral *p*H. The 200-fold purified *D* and *I* forms of the enzyme were characterized and differentiated by several criteria. In addition, the presence of a third form that was inactive even in the presence of glucose-6-phosphate was demonstrated.

UDPG: α-1,4-glucan α-glucosyltransferase (transferse)[1] was first found in liver by Leloir and Cardini (1957). Studies carried out with the enzyme from skeletal muscle have demonstrated a new mechanism for the control of glycogen synthesis. In the first paper of this series (Rosell-Perez *et al.*, 1962), the preparation of two different forms of transferase from muscle was reported. One form was fully dependent upon glucose-6-phosphate (*D* form); the other was almost independent of this cofactor (*I* form). The interconversion of these two forms of the enzyme by phosphorylation and dephosphorylation was demonstrated by Friedman and Larner (1962, 1963). In the present work, the liver enzyme was studied in order to understand more fully the control mechanism of glycogen synthesis. We have now obtained the enzyme almost completely in the *I* form by the conversion of the *D* form. This conversion of the *D* form to the *I* form requires $Mg^{2+}$ and a heat-labile subcellular fraction (conversion factor). In addition, a form was found that was inactive even in the presence of glucose-6-phosphate. A preliminary note of this work has appeared (Hizukuri and Larner, 1963).

## EXPERIMENTAL

*Animal.*—Male Wistar rats weighing 130–220 g were used. These rats were fed *ad libitum*.

*Preparation of the Partially Purified D-Form Enzyme (Glycogen Pellet).*—The glycogen pellet that contained the *D* form of transferase was prepared by a modification of the method of Leloir and Goldemberg (1960). After decapitation and exsanguination of the rats, the livers were removed and immersed in a large volume of ice-cold 0.25 M sucrose–0.005 M Versene (*p*H 7.5). The procedures described were carried out at 2° unless otherwise stated. The chilled livers were drained and blotted well, then homogenized for 4 minutes with 3 volumes (v/w) of cold 0.25 M sucrose–0.005 M Versene (*p*H 7.5) in a motor-driven glass homogenizer with a Teflon pestle. The homogenate was centrifuged at 8500 × *g* for 10 minutes. The supernatant fluid (crude extract) contained practically all the activity. The crude extract (12 ml) was kept frozen (*ca.* −20°) in a test tube for at least 24 hours. Stored in this manner the transferase and conversion factor activities were well maintained for periods exceeding 1 month. Frozen crude extracts were thawed slowly and were centrifuged at 41,000 × *g* for 40 minutes in a Spinco Model L centrifuge (rotor 40). The particulate glycogen was present as a firmly packed pellet in the bottom of the tube and was covered with a loose microsomal layer. This upper layer was poured off and the surface of the glycogen pellet was washed carefully with 0.25 M sucrose-0.005 M Versene (*p*H 7.5) to remove the remainder of the microsomal fraction.

*Separation of the Conversion Factor from the Glycogen Pellet.*—The glycogen pellet from 12 ml of crude extract was suspended in 6 ml of ice-cold 0.25 M sucrose–0.005 M Versene (*p*H 7.5) by the use of a ground-glass tissue grinder. This suspension was then subjected to ultrasonic disintegration for 30 seconds at 15 amp (MSE unit).[2] The disintegrated-glycogen-pellet suspension was centrifuged at 41,000 × *g* for 40 minutes in a Spinco Model L centrifuge (rotor 40). After the supernatant fluid had been removed with a capillary pipet, a reddish-brown loose layer containing the conversion factor (immediately over the white firm glycogen pellet) was drawn off with a capillary pipette. The white, thick, washed glycogen pellet in the bottom of the tube contained most of the transferase activity. The specific activity of the transferase was enhanced about seven times with a 55% recovery. For further purification of the transferase from the conversion factor, the washed glycogen pellet was resuspended in 0.25 M

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[1] Abbreviations: UDPG, uridine diphosphoglucose; transferase, UDPG: α-1,4-glucan α-glucosyltransferase; SER, smooth-surface endoplasmic reticulum.

[2] Measuring and Scientific Equipment Ltd., 25-28 Buckingham Gate, London SW 1.

TABLE I
ENZYMIC ACTIVITIES OF FRACTIONS DURING PURIFICATION

| Fraction | Protein Content (mg/ml) | Glycogen Content (mg/ml) | UDPG:α-Glucan Transferase (μmole/mg/hr) −Glucose-6-P | UDPG:α-Glucan Transferase (μmole/mg/hr) +Glucose-6-P | Phosphorylase (μmole/mg/hr) | Glucose-6-phosphatase (μmole/mg/hr) |
|---|---|---|---|---|---|---|
| Homogenate | 48.1 | | 0.09 | 0.53 | | |
| Crude extract | 30.1 | | 0.20 | 0.91 | | |
| Crude extract[a] | 32.0 | 13.3 | 0.45 | 0.79 | 0.09 | 0.07 |
| Glycogen pellet | 1.63 | 13.2 | 3.19 | 14.33 | 0.97 | 0.13 |
| Washed-glycogen pellet | 0.11 | 10.0 | 50.00 | 139.09 | 6.45 | 0.02 |
| Conversion factor | 2.22 | 2.5 | 0.13 | 1.35 | 0.25 | 0.21 |

[a] Stored at −20° for 13 days.

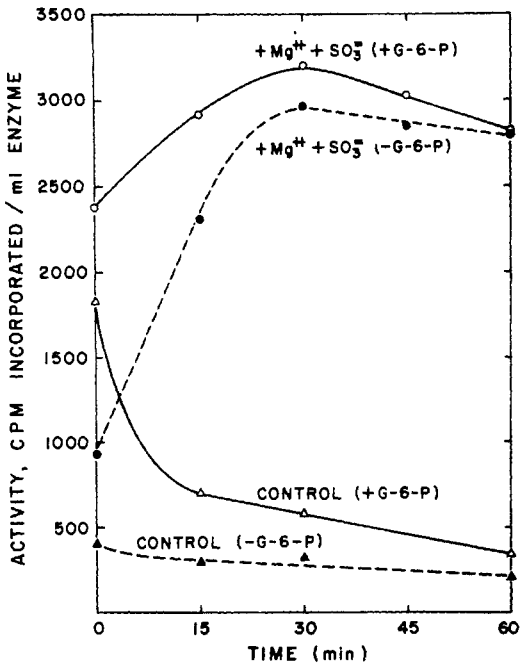


FIG. 1.—Time course of conversion reaction in glycogen pellet. Unbroken line measured with glucose-6-P and broken line measured without glucose-6-P. Activity was assayed after incubation by using enzyme diluted 3-fold with cold 0.05 M Tris–0.005 M Versene (*p*H 7.8). ○—○, ●—● contained 0.01 M $MgCl_2$ and 0.01 M $Na_2SO_3$; ▽—▽, ▼—▼ indicates control ($H_2O$) (see Experimental).

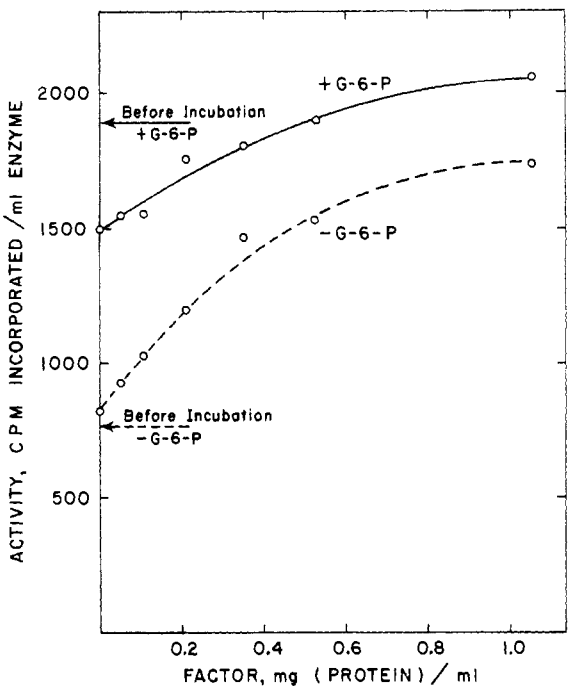


FIG. 2.—Effect of the conversion factor in the conversion of the *D* form to the *I* form of the enzyme. The twice-washed glycogen pellet (0.184 mg of protein/ml) was suspended in 0.04 M Tris–0.004 M Versene (*p*H 8.2) containing 0.01 M $MgCl_2$ and 0.01 M $Na_2SO_3$ and incubated with the conversion factor as indicated at 30° for 30 minutes.

sucrose–0.005 M Versene (*p*H 7.5) and rewashed by centrifugation. This second washing of the glycogen pellet resulted in an additional 1.5- to 2-fold increase in specific activity of the transferase and a preparation free of conversion factor.

*Conversion of the D Form to the I Form of Enzyme.*—The glycogen pellet from the crude extract was suspended in about 6 ml of 0.05 M Tris-HCl–0.005 M Versene (*p*H 7.8; half the volume of the original crude extract) by using a ground-glass tissue grinder. One volume each of 0.1 M $Na_2SO_3$ and 0.1 M $MgCl_2$ were added to 8 volumes of the glycogen-pellet suspension. This mixture was incubated at 30° for 60 minutes. The protein that denatured during the incubation was removed by centrifugation at 3000 rpm for 20 minutes. The supernatant fluid that contained all the activity was then centrifuged at 41,000 × *g* for 40 minutes in a Spinco Model L centrifuge (rotor 40). The loose layer (microsomal fraction) on the glycogen pellet was poured off, and the surface of the glycogen pellet was washed with 0.25 M sucrose–0.005 M Versene (*p*H 7.5). The glycogen pellet thus obtained contained the *I* form of the enzyme; the specific activity of the transferase was increased 200- to 300-fold when compared with the homogenate (measured with added glucose-6-phosphate) with a 30% recovery.

*Analytical.*—Transferase activity was assayed by measuring the incorporation of the radioactivity from [$^{14}$C]glucose UDPG into acceptor glycogen, as described by Villar-Palasi and Larner (1961). The reaction mixture used was modified slightly. In the present study 0.1 ml of the test mixture, which contained 0.667 μmole of UDPG (6500 cpm/μmole), 1 mg of rabbit liver glycogen, 5 μmoles of Tris-HCl (*p*H 7.8), 0.5 μmole of Versene, and 1.1 μmoles of glucose-6-phosphate (when needed), was mixed with 0.05 ml of the enzyme suspension. This reaction mixture was then incubated at 30° for 5 minutes. Total activity was assayed with added glucose-6-phosphate; *I*-form activity was assayed without glucose-6-phosphate.

Phosphorylase activity was measured according to the method of Hers (1959) with AMP. Glucose-6-phosphatase was assayed according to the method described by Swanson (1955). Protein was determined by the method described by Lowry *et al.* (1951) with the use of bovine serum albumin as a standard. Glycogen content was determined as follows: Two volumes of ethanol

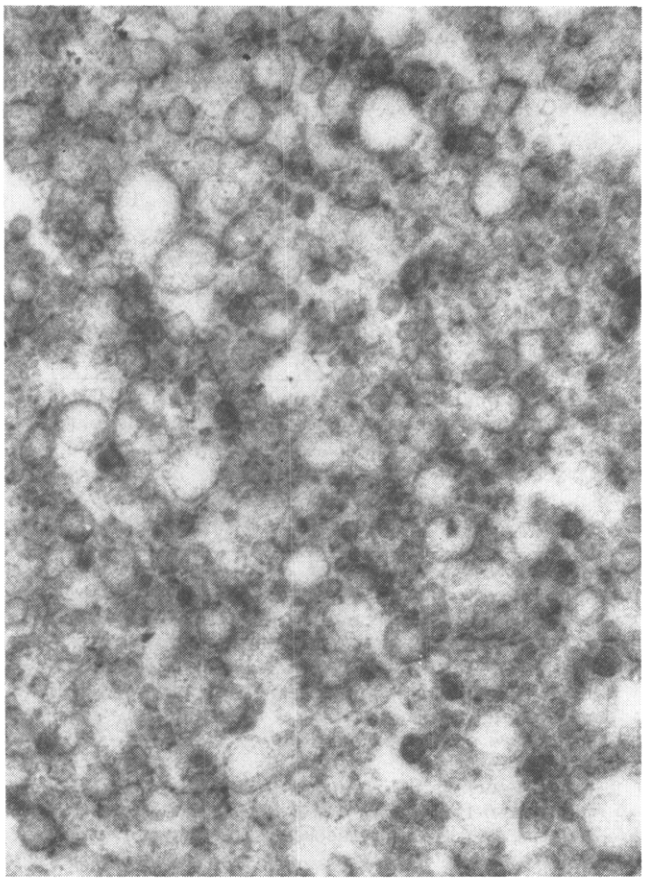

FIG. 3.—Electron micrograph of the conversion factor. The sample was fixed with osmium tetraoxide embedded in methacrylate and sectioned (× 32,200).

were added to 1 volume of the sample in order to precipitate glycogen and protein. The precipitate was collected by centrifugation and was digested with 30% KOH at 100° for 2 hours. After the solution was cooled glycogen was precipitated by the addition of 2 volumes of ethanol. The collected precipitate was then dissolved in water, precipitated again with 2 volumes of ethanol, and collected by centrifugation. The last step was repeated three times. The final white precipitate was dissolved in water, and glycogen was determined by the anthrone method (Roe, 1955) using glucose as a standard.

*Chemicals.*—The source of chemicals is cited in paper I of this series (Rosell-Perez *et al.*, 1962).

## RESULTS

*Preparation and Purification of Enzymes.*—The transferase activities in the fresh homogenate and crude extract are shown in Table I. The enzyme had a 4- to 10-fold increase in activity in the presence of glucose-6-phosphate. The fact that the activation by glucose-6-phosphate was somewhat lower than that observed in other studies (Leloir and Goldemberg, 1960; Steiner *et al.*, 1961) might be explained on the basis of the differences in assay conditions. Purification of the enzyme was carried out routinely starting with frozen crude extract. However, it was found that light ultrasonic disintegration was as effective as freezing and that it could be used with fresh crude extracts. The purification steps are also shown in Table I. After freezing and thawing, the *I*-form activity increased 1.5- to 2-fold over the fresh crude extract, whereas total activity did not increase. The transferase in the washed glycogen pellet, which was mainly present as the *D* form, was purified 200- to 350-fold when compared with the homogenate with a 25–30% recovery of total activity. The specific activity of the washed glycogen pellet was enhanced 1.5- to 2-fold after further washing with a 50% recovery of activity.

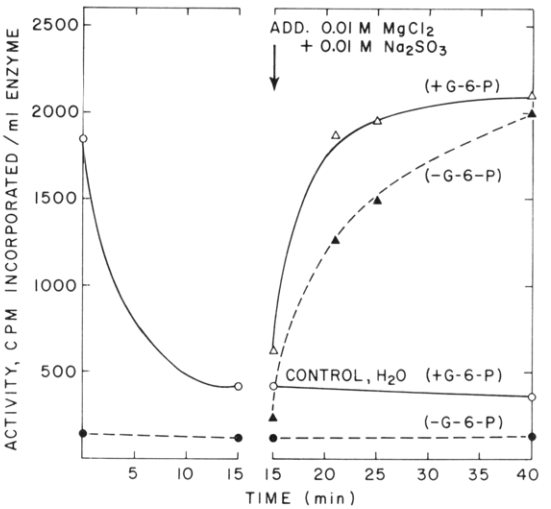


FIG. 4.—Conversion of the *D* form to the *I* form of the enzyme through an inactive form (unfractionated). The glycogen pellet was suspended in 0.05 M Tris–0.005 M Versene, 1.6 ml, *p*H 8.2, and incubated at 30°. After 15 minutes of incubation, the enzyme was chilled immediately in an ice bath for 5 minutes, then reincubated at 30° with the addition of 0.2 ml each of 0.1 M $MgCl_2$ and 0.1 M $Na_2SO_3$. Water (0.4 ml) was added to the control experiment. In the second incubation, activity was corrected by increasing the volume to give the comparative value in terms of protein content. −G-6-P and +G-6-P denote absence and presence of glucose-6-phosphate, respectively, in the assay.

*Conversion of the D Form to the I Form of the Enzyme.*—(Fig. 1). When the glycogen pellet was incubated with $MgCl_2$ (0.01 M) and $Na_2SO_3$ (0.01 M), a marked increase in the activity of the *I* form occurred. The total activity also increased, but it was not comparable to the increase of the *I*-form activity. The ratio of activities measured with and without glucose-6-phosphate was 2.6 at zero time, 1.3 at 15 minutes, and 1.0 at 60 minutes. This conversion of activities was not due to the formation of glucose-6-phosphate or some other soluble cofactor during the incubation period. Glucose-6-phosphate was not detectable by means of glucose-6-phosphate dehydrogenase and NADP. Also, the enzyme that was recovered by centrifugation after complete conversion to the *I* form was not activated with glucose-6-phosphate. In the control experiment without $MgCl_2$ and $Na_2SO_3$, the total activity decreased rapidly during the first 10 minutes and the *I*-form activity also decreased to a small extent. The optimum concentration of $MgCl_2$ required was found to be $8–10 \times 10^{-3}$ M in the presence of $4 \times 10^{-3}$ M Versene. $Na_2SO_3$ (0.01 M) was found to be a good stabilizing agent of the I form of transferase and was added to the incubation mixture.

*Requirement of Heat-labile Factor for Conversion.*—The $Mg^{2+}$-dependent conversion occurred to a lesser extent in the more highly purified enzyme. The twice-washed glycogen pellet fraction, when incubated with $MgCl_2$ and $Na_2SO_3$, showed practically no conversion. The factor responsible for the conversion was recovered from the washings of the glycogen pellet (see Experimental). When the twice-washed glycogen pellet was incubated with this conversion factor, an in-

TABLE II

REQUIREMENT OF THE HEAT-LABILE FACTOR FOR THE CONVERSION OF THE *D* FORM TO THE *I* FORM OF THE ENZYME

| Incubation Conditions[a] | Activity (cpm/ml of enzyme) 0-Time Incubation (−) Glucose-6-P | 0-Time Incubation (+) Glucose-6-P | 30-Minute Incubation (−) Glucose-6-P | 30-Minute Incubation (+) Glucose-6-P |
|---|---|---|---|---|
| Enzyme + factor (150 μg protein/ml) | 865 | 1480 | 1400 | 1590 |
| Enzyme + heated factor (protein)[b] | 710 | 1330 | 696 | 1260 |
| Enzyme + $H_2O$ (control) | 680 | 1280 | 552 | 1020 |
| Factor only | 28 | 71 | 14 | 128 |

[a] Conditions. The twice-washed glycogen pellet (72 μg protein in the final mixture) was suspended in 0.04 M Tris-0.004 M EDTA, *p*H 8.2, containing 0.01 M $MgCl_2$ and 0.01 M $Na_2SO_3$ with and without indicated factor, and incubated at 30°. [b] Heated for 5 minutes at 98°.

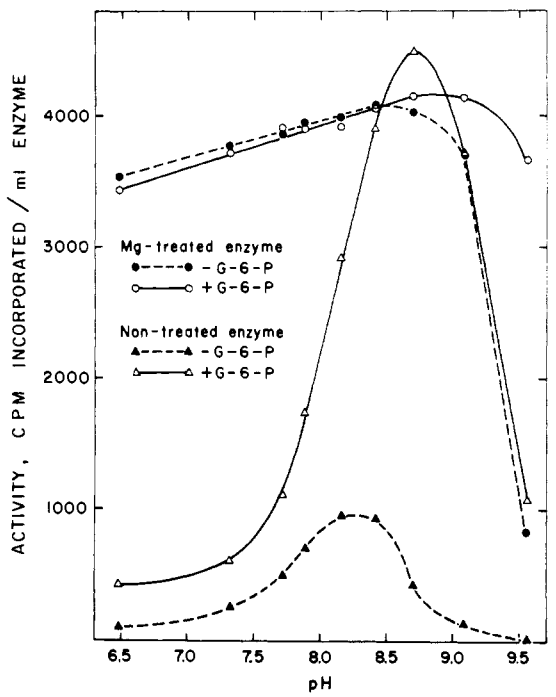


FIG. 5.—*p*H-Activity curves of the *D* form and the *I* form of the enzyme in 0.15 M Tris-HCl buffer containing $4 \times 10^{-3}$ M Versene, $3.8 \times 10^{-2}$ M sucrose, $1.7 \times 10^{-3}$ M $Na_2SO_3$, 0.43% glycogen, $4.45 \times 10^{-3}$ M UDPG, and $6.2 \times 10^{-3}$ M glucose-6-P (when added). Unbroken lines represent measurements with glucose-6-P and broken lines represent measurements without glucose-6-P. The enzyme was purified approximately 200-fold on the basis of protein content. ○—○, ●—●, *I* form of the enzyme; △—△, ▲—▲, *D* form of the enzyme.

crease in the *I*-form activity was noted; without the factor, the *I*-form activity did not increase. The factor lost its activity when heated for 5 minutes in boiling water at neutral *p*H, as shown in Table II. The amount of the conversion reaction was dependent upon the amount of the factor, as shown in Figure 2.

*Nature of the Conversion Factor.*—The fraction that contained the conversion factor was reddish-brown and rich in glucose-6-phosphatase (Table I). It was rich in smooth-surfaced vesicles with variable diameters and densities (Fig. 3). It had a weak transferase activity which was lost following digestion of the glycogen with α-amylase.

*Conversion to an Inactive Intermediate.*—As mentioned previously, if the glycogen pellets that contained transferase were incubated at 30° for 10–15 minutes without $Mg^{2+}$ and $SO_3^{2-}$, the transferase became inactivated (even when tested in the presence of glucose-6-phosphate; Fig. 1). If the inactivated enzyme was chilled immediately in ice and then was reincubated in the presence of 0.01 M $MgCl_2$ and 0.01 M $Na_2SO_3$, the *I*-form activity increased rapidly during this second incubation and rose to a level greater than the original total activity (Fig. 4). Thus in the two incubations the *D* form was converted first to an inactive intermediate and then to the *I* form. A similar inactivation and reactivation has been reported by Steiner (1961). He reactivated transferase in the presence of glucose-6-phosphate and NaF. These compounds and their combinations were tested in the present reactivation reaction (Table III). The combination of $MgCl_2$ and $Na_2SO_3$ was as effective as glucose-6-phosphate alone, whereas $MgCl_2$, $Na_2SO_3$, and glucose-6-phosphate together comprised the most effective combination; NaF had no effect. After the activation with glucose-6-phosphate, the enzyme was recovered by centrifugation in order to remove glycose-6-phosphate. The precipitated enzyme was not activated by this cofactor.

TABLE III

ACTIVATION OF THE INACTIVE FORM BY VARIOUS CHEMICALS

| | Activity (%) −Glucose-6-P | +Glucose-6-P |
|---|---|---|
| Expt 1 | | |
| Original (crude glycogen pellet) | 8.4 | 100 |
| Inactivated[a] | 7.2 | 23 |
| Inactivated enzyme[b] incubated with 0.01 M of | | |
| $MgCl_2$ and $Na_2SO_3$ | 72 | 101 |
| Glucose-6-P | | 93 |
| $MgCl_2$, $Na_2SO_3$ and glucose-6-P | | 121 |
| $H_2O$ (control) | 8 | 22 |
| Expt 2 | | |
| Original (crude glycogen pellet) | 11 | 100 |
| Inactivated[a] | 11 | 41 |
| Inactivated enzyme[b] incubated with 0.01 M of | | |
| $MgCl_2$ | 44 | 52 |
| $Na_2SO_3$ | 37 | 53 |
| $MgCl_2$ and $Na_2SO_3$ | 94 | 105 |
| $CaCl_2$ and $Na_2SO_3$ | 70 | 90 |
| Glucose-6-P | | 93 |
| NaF | 9 | 27 |
| Glucose-6-P and NaF | | 94 |
| Glutathione | 14 | 38 |
| $H_2O$ (control) | 13 | 43 |

[a] Incubated for 15 minutes at 30° in 0.05 M Tris–0.05 M EDTA, *p*H 8.2. [b] Incubated for 10 minutes at 30° in 0.04 M Tris–0.04 M EDTA, *p*H 8.2.

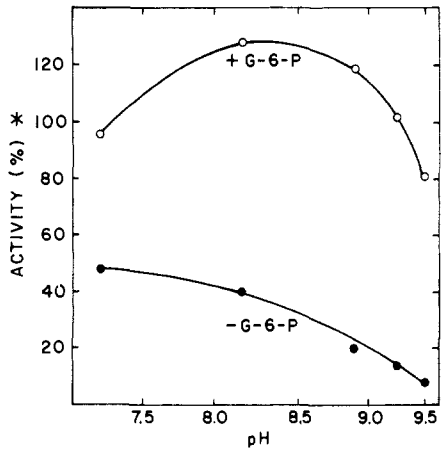


FIG. 6.—*p*H-Stability of the *I* form of the enzyme. The enzyme was incubated in the same buffer solution de-described in Figure 5 without UDPG for 5 minutes at 30°. Activity was assayed after incubation, using the enzyme diluted 3-fold and at *p*H adjusted to 6.8 with Tris-HCl buffer.

Therefore, in all probability, Steiner (1961) observed not a *reversible* inactivation and reactivation of transferase, but an activation to a type of enzyme which was different from the original.

*Characterization and Differentiation of the Two Forms of the Enzyme.*—Figure 5 shows the *p*H dependence of both the *I* form ($Mg^{2+}$, $SO_3^{2-}$ treated) and the *D* form (untreated) enzyme. The *D* form had a sharp maximum activity at *p*H 8.9 in the presence of glucose-6-phosphate and at *p*H 8.4 without this cofactor. The degree of activation by glucose-6-phosphate was dependent upon *p*H in a manner similar to the yeast enzyme (Algranati and Cabib, 1962) and to the muscle enzyme (Traut, 1962). The *I* form of the enzyme, on the other hand, had a broad *p*H-activity curve but showed the same maximum activity at *p*H 8.9 and 8.4 when measured with and without glucose-6-phosphate, respectively. The *I* form of the enzyme was not activated with glucose-6-phosphate when the *p*H was below 8.5. However, it was activated by glucose-6-phosphate at a higher *p*H. This apparent activation by glucose-6-phosphate was caused partly by the unstable nature of the enzyme at the higher *p*H in the absence of glucose-6-phosphate.

Figure 6 demonstrates the stability of the *I* form of the enzyme as a function of *p*H. The enzyme retained activity in the presence of glucose-6-phosphate in the *p*H range tested. The slight increase of activity at about *p*H 8.4 during the short incubation was probably caused by the presence of small amounts of the inactive form and its activation in the presence of glucose-6-phosphate. In the absence of glucose-6-phosphate, the enzyme was quite unstable and especially at higher *p*H. At *p*H 9.5 more than 90% of the activity was lost. In the presence of glucose-6-phosphate, less than 20% of the activity was lost.

Figure 7 shows the UDPG concentration dependence of the *D* form of the enzyme. The $K_m$ at *p*H 8.9 was found to be $9 \times 10^{-4}$ M in the presence of glucose-6-phosphate. The degree of activation by glucose-6-phosphate varied with the UDPG concentration. The enzyme was activated to a lesser degree by glucose-6-phosphate at higher concentrations of UDPG. A similar observation has been made with the muscle enzymes (Rossell-Perez *et al.*, 1962; Kornfeld and Brown, 1962; Traut, 1962). Figure 8 shows the glucose-6-phosphate concentration dependence of the *D* form of the enzyme. The $K_m$ for activation by

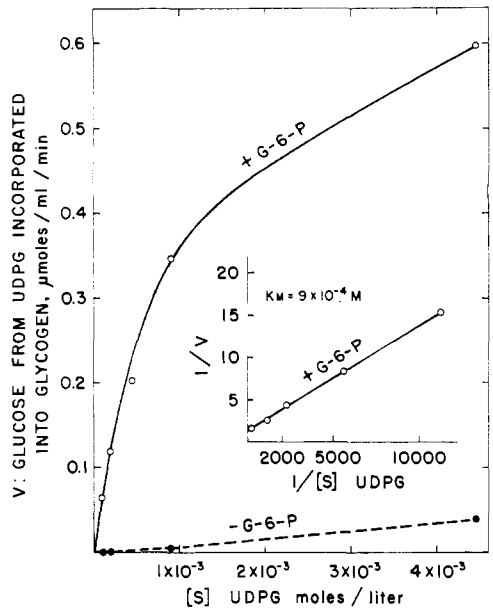


FIG. 7.—UDPG concentration dependence of the *D* form of the enzyme activity. Conditions are the same as in the activity assay (see Experimental), except for UDPG concentration indicated and a *p*H of 8.9.

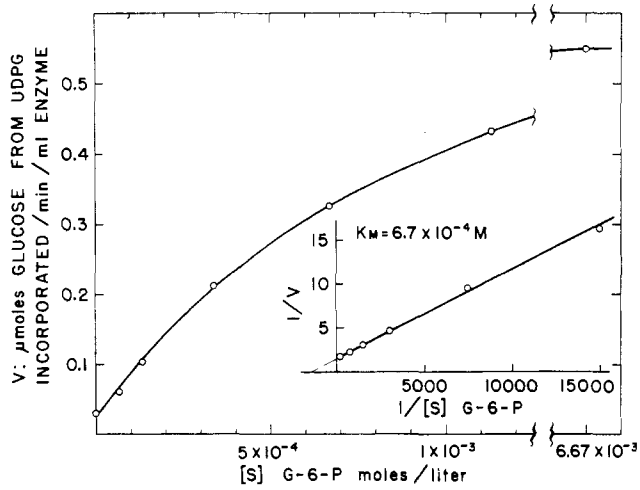


FIG. 8.—Glucose-6-P concentration dependence of the *D* form of the enzyme. Conditions are the same as in the activity assay (see Experimental), except for glucose-6-P concentration indicated and a *p*H of 8.9.

glucose-6-phosphate was found to be $6.7 \times 10^{-4}$ M at *p*H 8.9. Figure 9 shows the UDPG concentration dependence of the *I* form of the enzyme. A linear reciprocal plot was obtained with added glucose-6-phosphate and the $K_m$ was determined as $6.2 \times 10^{-4}$ M at *p*H 8.4. Nonlinear kinetics was obtained in the absence of glucose-6-phosphate. The slope was less at the higher concentrations of UDPG than at the lower concentrations of UDPG. This was found to be significant in repeated experiments. A $K_m$ of $1.1 \times 10^{-3}$ M was determined from the lower slope. Glucose-6-phosphate decreased the $K_m$ for UDPG but did not alter the *V*. This was similar to the mercaptoethanol-treated muscle enzyme (Rosell-Perez *et al.*, 1962) and the lamb muscle enzyme (Traut, 1962).

The sensitivity of the enzyme to inhibition by certain nucleotides was different for the two forms. The *D* form was more sensitive and was inhibited 50% by $6.7 \times 10^{-3}$ M ATP, ADP, and AMP. The *I* form was relatively insensitive and was inhibited only 0–5%

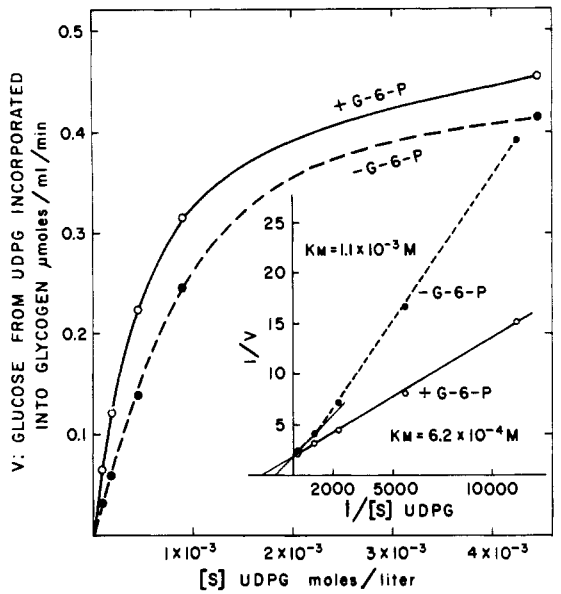


FIG. 9.—UDPG concentration dependence of the *I* form of the enzyme. Conditions were the same as in the activity assay (see Experimental) except for UDPG concentration indicated and at a *p*H of 8.4.

by the same nucleotides. Both forms of the enzyme were inhibited 90%–100% by $10^{-5}$ M *p*-mercuribenzoate.

## DISCUSSION

The conversion of the enzyme from the *D* form to the *I* form appears to be an enzymic reaction because it is time dependent and requires a heat-labile subcellular fraction. No clear evidence has yet been obtained concerning the structural difference between these two forms. In the muscle enzyme the conversion reaction of the *D* form to the *I* form has been proposed to be a dephosphorylation reaction (Friedman and Larner, 1963). The following chemicals had no effect on the conversion reaction in liver: 0.01 M NaF, 0.01 M L-serine, $10^{-3}$ M diisopropylfluorophosphate, and insulin (1 unit/ml).

Evidence has been brought forward that smooth-surface endoplasmic reticulum (SER) is closely related to glycogen (Yamada, 1960; Porter and Bruni, 1960). In liver-cell preparations, many SER particles have been included with amorphous glycogen (Luck, 1961). SER fractions cannot be readily separated from glycogen itself and the glycogen-pellet fraction (centrifuged from tissue homogenates) contains SER particles. In the present study the SER particles normally found in the glycogen pellet were separated from the glycogen by ultrasonic disintegration of the resuspended pellet followed by centrifugation. The transferase was recovered in the glycogen pellet but not in the SER fraction, which indicates its binding with glycogen (Leloir and Goldemberg, 1960; Luck, 1961). The SER fraction contained the factor for converting the *D* form to the *I* form of the enzyme. This conversion factor seemed to be specific since it did not act on the *D* form of the rat muscle enzyme (microsomal fraction).

Two methods of conversion of the *D* form to the *I* form of the enzyme have been observed; one is a direct conversion, and the other is an indirect conversion through an inactive intermediate. The velocity of conversion from the inactive form to the *I* form was faster than that of direct conversion of the *D* form to the *I* form in the same enzyme preparation. This suggests that the inactive form may be a normal intermediate in the conversion process. It is interesting that glucose-6-phosphate was just as effective in the conversion of the inactive form to the *I* form as was $Mg^{2+}$ plus $SO_3^{2-}$.

Thus, in agreement with the findings of Traut (1962) for the muscle enzyme, glucose-6-phosphate may control the transferase in two ways, i.e., by activation of the *D* form of the enzyme (as a cofactor) and by the conversion of the enzyme forms. Conceivably, then, through this dual effect an increase in the intracellular concentration of glucose-6-phosphate would result in an increase in glycogen synthesis.

From the results presented in this paper, together with those obtained from the studies on muscle enzyme, glycogen synthesis appears to be controlled by the interconversion of the different enzyme forms, just as glycogen degradation is controlled by the interconversion of phosphorylase *a* and *b*.

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