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GLYCOGEN SYNTHETASE [UDP GLUCOSE: α-1,4-GLUCAN α-4-GLUCOSYL TRANSFERASE (EC 2.4.1.11)] OF HUMAN EPIDERMIS*

AKIRA OHKAWARA, M.D., KENNETH M. HALPRIN, M.D. AND VICTOR LEVINE

ABSTRACT

Normal human epidermis contains glycogen synthetase in activities which are approximately 10% of that found in brain and muscle. The enzyme is activated by glucose-6-phosphate (Glc-6-P) and seems to have characteristics which are similar to those reported for the same enzyme in other tissues. About 50% of the enzyme is normally in the I form which presumably correlates with the very low levels of glycogen (0.35 μg/mg) normally present in human epidermis.

As a result of the wide spread use of the PAS (Periodic acid-Shiff) stain combined with diastase (to remove glycogen) a great deal has been learned about the histochemically demonstrable accumulation of glycogen in the human epidermis. For example, it is known that glycogen is present in large amount in fetal epidermis (1) but is practically absent from normal adult epidermis (2). Also stainable glycogen accumulates during many disease processes (psoriasis vulgaris, Bowen's disease, clear cell acanthoma and squamous cell carcinoma) and can be provoked by a variety of experimental processes (ultraviolet light irradiation, X-ray irradiation, "Scotch-tape" stripping, topical vitamin A acid application and intradermal bradykinin).

The mechanism responsible for this accumulation and the purpose it might serve remain unknown. Unlike the situation in the sweat duct where it does seem to serve as a source of energy (3), epidermal glycogen seems to accumulate at exactly the wrong times if it is to serve such a function (i.e. it accumulates when it should be being used, such as for the great energy needs in psoriasis).

As part of a more detailed study of this phenomenon of glycogen accumulation, we would like to report the result of biochemical studies of the epidermal enzyme which manufactures glycogen (glycogen synthetase) and to compare its properties with the properties reported for this enzyme in other tissues.

MATERIALS AND METHODS

Materials. C^{14}UDP Glucose (227 mC/mmole, 0.025 mg/0.5 ml 70% ethanol) was obtained from New England Nuclear, Boston, Mass. It was diluted 1:5 with distilled water before use. Glycogen-shell fish; Sodium glucose-6-phosphate and Sodium Uridine diphosphoglucose were purchased from Sigma Co., St. Louis, Mo. The glycogen was purified with repeated precipitation in alcohol, dried, and dissolved in distilled water to make an 8% solution. The glucose-6-phosphate (Glc-6-P) was dissolved in distilled water to make a 100 mM solution and the pH was adjusted to 8.0 with NaOH. Whatman #41 ET filter paper was cut into 2 cm squares and 2 adjoining corners were cut off according to Thomas, *et al.* (4).

Preparation of epidermal homogenate. Human epidermal strips were obtained from the backs of normal male volunteers with a motor driven "Castroviejo" keratome without anesthesia. The keratome was set at 0.2 mm thickness which usually results in a skin slice which is 75 to 85% epidermis. 5% water homogenates were prepared with a pre-chilled glass homogenizer in the cold. Homogenates were centrifuged at 700 g for 10 minutes in the cold and the supernatants obtained were used as the enzyme source.

Assay procedure. The assay procedure was that of Thomas, *et al.* (4). The reaction mixture consisted of 0.8 ml of diluted C^{14}UDP Glucose; 0.15 ml of an 8% solution of purified glygogen; 0.12 ml of 0.5 M Tris HCl buffer, pH 7.8 containing 50 mM EDTA; 0.13 ml of 100 mM Glc-6-P, pH 8.0; 0.12 ml of 52 mM UDPG in water. Final volume was 1,320 μl and this amount of mixture was enough for 20 determinations.

20 μl of the enzyme preparation (equivalent to 1.0 mg of fresh skin) was added to 50 μl of the reaction mixture and incubation was carried out for 30 minutes at 30° C. 0 time incubation and boiled tissue controls were also run. At the end of incubation, the reaction was stopped by placing the tubes in the ice and 50 μl from each reaction tube was spotted on filter papers. The filter papers were placed in 66% ethanol and washed for 10 minutes with constant stirring. Washing was repeated 5 times with changes of ethanol each time. By this washing procedure the glycogen was precipitated onto the filter papers at the same time that non-reacted UDPG was washed free. At the end of the wash period, the ethanol was decanted and the filter papers were washed for 10 minutes in acetone.† After the acetone wash filter papers were dried under an infra-red or UV lmap for 10 minutes. Each paper was placed in a scintillation vial containing 10 ml of scintillation "cocktail"‡ and the

* From The Dermatology Service of the Miami V. A. Hospital and the Department of Dermatology of the University of Miami. (Reprint requests to Doctor Halprin, 1201 N. W. 16th Street, Miami, Florida.)

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† Spotting known amounts of glycogen on the filter paper, followed by processing through the precipitation and wash procedures, followed by chemical analysis of the recoverable glycogen on the filter paper have shown complete recovery (95–98%).

‡ "cocktail" Beckman TLA Scintillation Formula: Butyl-PBD 8 gram/L, PBBO 0.5 gram/L and toluene g.s.a.d. 1000 ml + 500 ml of Triton X-100.

radioactivity was counted with a Packard Liquid Scintillation Counter.

RESULTS AND DISCUSSION

1. *Linearity of the reaction with time*

Figure 1 shows the time course of the reaction with saturating amount glucose-6-phosphate (Glc-6-P) and UDPG present at pH 7.8 and 30° C. The reaction is linear up to 60 minutes by which time only 1% (approx. 1000 cpm) of the available UDPG has been converted to glycogen. 30 minutes was chosen as our standard incubation time in the subsequent experiments.

2. *Linearity of the reaction with differing amounts of tissue enzyme preparation*

Figure 2 demonstrates that the reaction velocity under the above conditions is directly proportional to the amount of tissue extract (enzyme) added.

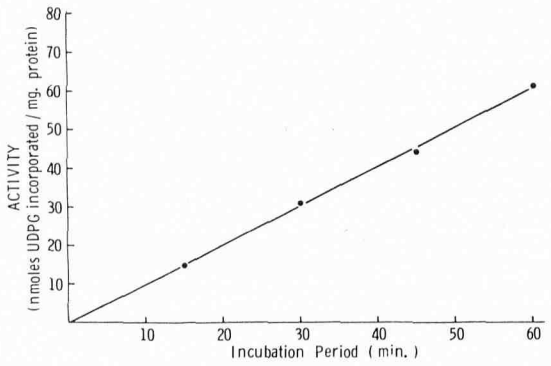

FIG. 1. Time course of the reaction. Enzymes were assayed as described in the text in the presence of 10 mM Glc-6-P, 4.7 mM UDPG, 4.6 mM EDTA, 0.9% glycogen and epidermal homogenate corresponding to 1.0 mg of wet tissue (20 μl). pH 7.8 at 30° C.

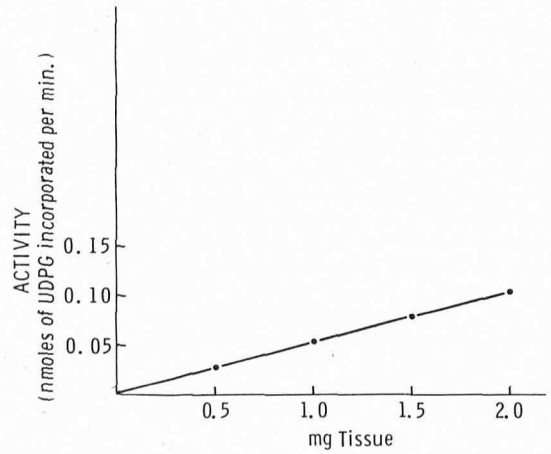

FIG. 2. Relation between UDPG incorporation into glycogen and enzyme concentration. Enzymes were assayed as described in the text in the presence of 10 mM Glc-6-P, 4.7 mM UDPG, 4.6 mM EDTA, 0.9% glycogen and various concentrations of enzyme preparation. pH 7.8 at 30° C.

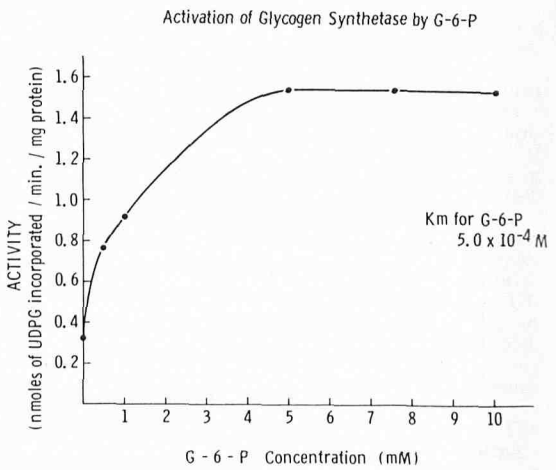

FIG. 3. The effect of various concentrations of Glc-6-P on the enzyme activities. Enzymes were assayed as described in the text in the presence of 4.7 mM UDPG, 4.6 mM EDTA, 0.9% glycogen and 20 μl of enzyme preparation. pH 7.8 at 30° C.

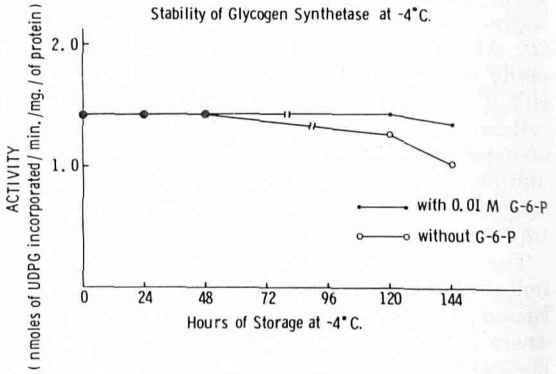

FIG. 4. The stability of glycogen synthetase at −4° C. The stability of glycogen synthetase in 5% water extract stored at −4° C in the presence and absence of 10 mM Glc-6-P. Enzymes were assayed as described in the text in the presence of 4.7 mM UDPG, 4.6 mM EDTA, 0.9% glycogen and 20 μl of the enzyme preparation. pH 7.8 at 30° C.

3. *Effect of Glc-6-P on enzyme activity*

Figure 3 demonstrates that in the presence of Glc-6-P synthetase activity is increased despite the fact that Glc-6-P is not a substrate of the reaction. Without any Glc-6-P present, the activity in the above tissue was approximately 20% of that obtainable in the presence of maximal stimulation by Glc-6-P (>5 mM). The concentration of Glc-6-P necessary for half maximal stimulation is 5×10^{-4} M (Km) which is in agreement with figures of 9×10^{-4} M for mouse muscle (5) and 6×10^{-4} M for rat liver (6). The activity found in the absence of Glc-6-P is presumably due to the I or Glc-6-P independent form of the enzyme while that found in the presence of Glc-6-P represents total activity of the I and D (Glc-6-P dependent) forms of the enzyme as described by Larner, *et al.* (7). In addition Glc-6-P had some protective ac-

tion upon the enzyme stored at −4° C (Fig. 4). The enzyme in 5% water homogenate stored at −4° C was stable at least for 48 hours and began to lose its activity after 5 days, however the enzyme preparation stored at −4° C with 10 mM Glc-6-P retained its full activity as long as 5 days. The protective action of Glc-6-P upon the enzyme has been described before (8).

4. *The effect of pH*

As illustrated in Figure 5, the pH optimum without Glc-6-P (I form) is at pH 6.5 while in the presence of Glc-6-P (I and D forms), it is at pH 7.8. These optima are fairly sharp in both cases. Similar pH changes depending on the presence or absence of Glc-6-P have been reported for lamb muscle (8).

5. *The effect of UDPG concentration upon enzyme activity*

a. In the absence of Glc-6-P when the I form is presumably being measured the Km for UDPG is 1.25×10^{-3} M at the pH optimum of 6.5 as shown in Figure 6. In the presence of 12.5 mM magnesium, the Vmax is increased and the Km for UDPG is 6.2×10^{-4} M (Table I and Figure 6). High concentrations of magnesium, however, are inhibitory (Table I).

b. In the presence of Glc-6-P when both the I and D forms are being measured the Km for UDPG at the otpimum pH of 7.8 is 1.33×10^{-4} M (Fig. 7). This compares with 4.8×10^{-4} M for rat liver (6) and 2.2×10^{-4} M for mouse muscle (5) and 3×10^{-5} M for lamb muscle (8). With the magnesium concentration of 10 mM the Km for UDPG is 2.5×10^{-4} M and there is no appreciable effect on the maximal activity.

6. *The effect of glycogen primer*

The amount of glycogen usually present in the epidermis is approximately 0.35 μg/mg fresh tissue and in psoriasis a disease where glycogen accumulates within the epidermis it reaches approximately 1.8 μg/mg (9). Concentrations of glycogen covering this normal range when added to the assay mixture did not appreciably increase the basal rate without any added glycogen. This basal rate is approximately 25% of the maximal measured in the presence of the usual saturating primer concentration of 500 μg of glycogen (Fig. 8). This baseline production rate in the absence of added glycogen is probably due to glycogen associated with the enzyme in the preparation.

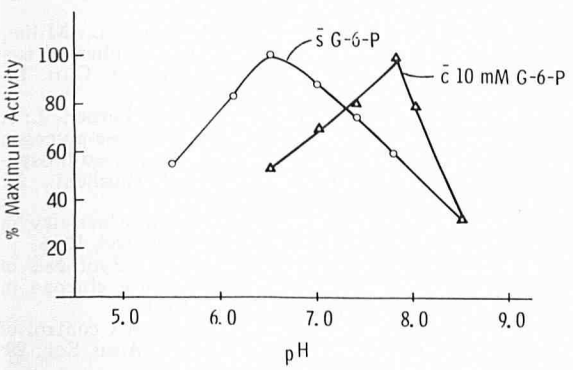

FIG. 5. The effect of pH on the enzyme activities in the presence and absence of 10 mM Glc-6-P. Enzymes were assayed as described in the text at 30° C. Each assay mixture contained 4.7 mM UDPG, 4.6 mM EDTA, 0.9% glycogen and 20 μl of enzyme preparation.

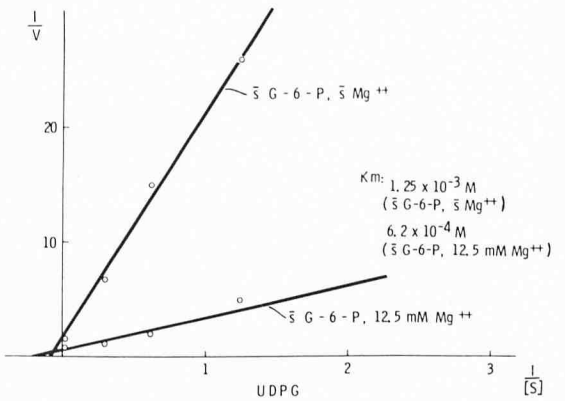

FIG. 6. The effect of various concentrations of UDPG on the enzyme activities in the absence of G-6-P. Enzymes were assayed as described in the text at 30° C, pH 6.5 in the presence of 4.6 mM EDTA, 0.9% glycogen and 20 μl of enzyme preparation. To study the effect of magnesium 10 mM $MgCl_2$ was added to the above mixture. Figures are plotted according to Lineweaver-Burk. UDPG concentrations were expressed as $\times 10^{-4}$ M and activities as nmoles of UDPG incorporated per minute per mg. of protein.

TABLE I

The effect of various concentrations of magnesium on the enzyme activities. Enzymes were assayed as described in the text in the presence of 4.7 mM UDPG, 4.6 mM EDTA, 0.9% glycogen and 20 μl of enzyme preparation at pH 7.8 in the presence of 10 mM Glc-6-P and 6.5 in the absence of Glc-6-P respectively at 30° C. Activities are expressed as nmoles of UDPG incorporated per minute per mg. of wet tissue.

Mg^{++} Concentration (mM)	0	0.8	1.6	3.0	6.2	12.5	25.0
c̄ 10mM G-6-P	0.086	0.086	0.086	0.088	0.090	0.096	1.00
s̄ G-6-P	0.010	0.011	0.010	0.011	0.011	0.016	0.003

7. *Glycogen synthetase activity in the normal human epidermis*

The average glycogen synthetase activity as measured in nine normal human skins is 0.048 nm/min/mg fresh tissue or 1.17 nm/min/mg of soluble protein (Table II). This is in good agreement with activities measured by Adachi in dog skin but much lower than that measured in mouse skin (10) and it is close to a previous report by ourselves using a relatively crude assay procedure (11). This activity in skin is about 1/10 of

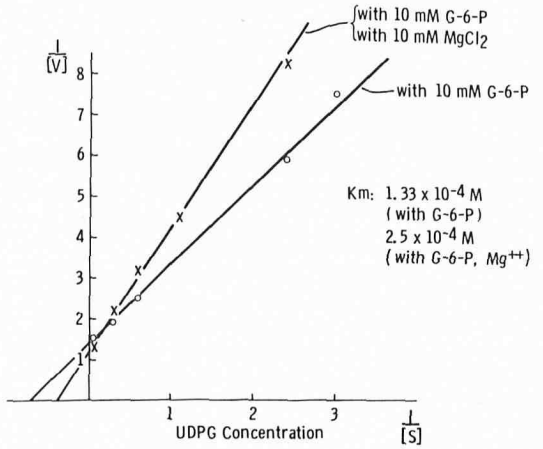

FIG. 7. The effect of various concentrations of UDPG on the enzyme activities in the presence of 10 mM G-6-P. Enzymes were assayed as described in the text at 30° C, pH 7.8 in the presence of 4.6 mM EDTA and 20 μl of enzyme preparation. To study the effect of magnesium, 10 mM $MgCl_2$ was added to the above mixture. Figures are plotted according to Lineweaver-Burk. UDPG concentrations are expressed as $\times 10^{-4}$ M and activities as nmoles of UDPG incorporated per minute per mg. of protein.

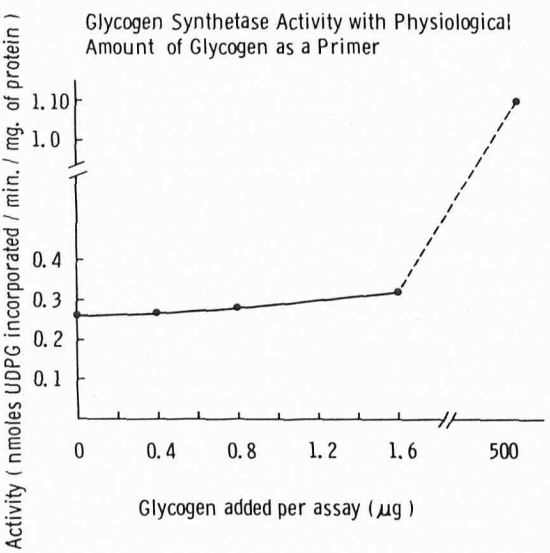

FIG. 8. The primer effect of glycogen on the enzyme activity. Enzymes were assayed as described in the text in the presence of 10 mM G-6-P, 4.7 mM UDPG, 4.6 mM EDTA, 20 μl of enzyme preparation. Glycogen concentration is expressed as microgram per assay and activities as nmoles of UDPG incorporated per minute per mg. of protein, pH 7.8 at 30° C.

that found in rabbit brain (12) and 1/15 of that of mouse muscle (5).

8. *The relation between glycogen concentration and the per cent I form*

Also shown in Table II is the percent of the activity which is independent of Glc-6-P which averages approximately 50% in human epidermis. This is a relatively high proportion of I form and is presumably due to the low concentration of glycogen in the tissue. In the graph shown by Larner, *et al.* using perfused rat heart the inverse relation between the glycogen concentration of the tissue and the percent I form is well illustrated (13). At a concentration of 0.35 μg/mg which would be comparable to normal epidermal glycogen concentration there is approximately 50% I form.

TABLE II

Glycogen synthetase activity in the normal human epidermis. Total activity was measured as described in the text in the presence of 10 mM Glc-6-P, 4.7 mM UDPG, 4.6 mM EDTA, 0.9% glycogen and 20 μl of enzyme preparation, pH 7.8 at 30° C. I form activity was measured in the absence of Glc-6-P from above mixture, pH at 6.5 at 30° C. The activities are reported with their standard deviations. The per cent I form from the above figures is 58%. It should be pointed out that total activity as measured at pH 7.8 only includes 60% of the maximal I form activity. Correction for this underestimate would give a figure of 1.44 nm/min/Mg protein and the actual per cent I form would be 47%. ( ) denotes the number of cases studied.

Glycogen Synthetase Activity in the Normal Human Epidermis

	nm/min/mg prot.	nm/min/mg Fresh Tissue
Total Activity	1.17 ± 0.34 (9)	0.048 ± 0.015 (9)
I - Form	0.675 ± 0.53 (4)	0.024 ± 0.018 (4)
Glycogen	(0.35 µg/mg) (4)	

( ) denotes the number of cases studied

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REFERENCES

1. Sundberg, C.: Das Glykogen in menschlichen Embryonen von 15, 27, und 40 mm. Z'tschr. f. Anat., *73:* 168, 1924.
2. Rothman, S.: *Physiology and Biochemistry of the Skin.* Ed., Rothman, S. The University of Chicago Press, Chicago, 1954.
3. Wolfe, S., Cage, G., Epstein, M., Tice, L., Miller, H. and Gordon, R. S.: Metabolic studies of isolated human eccrine sweat glands. J. Clin. Invest., *49:* 1880, 1970.
4. Thomas, J. A., Schlender, K. K. and Larner, J.: A rapid filter paper assay for UDP glucose-glycogen glucosytransferase, including an improved biosynthesis of UDP-C^{14}-glucose. Anal. Biochem., *25:* 486, 1968.
5. Danforth, W. G.: Glycogen synthetase activity in skeletal muscle. J. Biol. Chem., *240:* 588, 1965.
6. Leloir, L. F. and Goldenberg, S. H.: Synthesis of glycogen from uridine diphosphate glucose in liver. J. Biol. Chem., *235:* 919, 1960.
7. Larner, J.: Hormonal and nonhormonal control of glycogen metabolism. Trans. N. Y. Acad. Sci., *29:* 192, 1966.
8. Traut, R. R. and Lipmann, F.: Activation of glycogen synthetase by glucose-6-phosphate. J. Biol. Chem, *238:* 1213, 1963.

9. Halprin, K. M. and Ohkawara, A.: Carbohydrate metabolism in psoriasis: An enzymatic study. J. Invest. Derm., *46:* 51, 1966.
10. Adachi, K.: Metabolism of glycogen in skin and the effect of X-rays. J. Invest. Derm., *37:* 381, 1961.
11. Halprin, K. M. and Ohkawara, A.: Glucose and glycogen metabolism in the human epidermis. J. Invest. Derm., *46:* 43, 1966.
12. Brekenridge, B. M. and Crawford, E. J.: Glycogen synthesis from uridine diphosphate glucose in brain. J. Biol. Chem., *235:* 3054, 1960.
13. Larner, J., Villar-Palasi, C., Goldberg, N. D., Bishop, J. S., Huijing, F., Wenger, J. I., Sasko, H. and Brown, N. B.: *Control of Glycogen Metabolism*. Ed., Whelan, W. J., Academic Press, London and New York, 1968.