GLYCOGEN SYNTHESIS AND I TO D CONVERSION OF GLYCOGEN SYNTHETASE IN ASCITES HEPATOMA CELLS

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Yoshida ascites hepatoma (AH-130) cells obtained from the peritoneal cavity of rats lack glycogen and contain glycogen synthetase mostly in a G6P-independent (I) form. On incubation of these cells in a glucose-containing medium, a series of events takes place, which includes: i) glycogen formation declining with time; ii) almost total conversion of the synthetase to a G6P-dependent (D) form; iii) its redistribution from soluble to particulate fraction. A picture thus emerges which substantiates the operation of a feed back inhibition by glycogen of glycogen synthetase suggested by Danforth (1965) for rat muscle enzyme. The preferential binding of the synthetase D to glycogen appears to play an important role in this mechanism.

Materials and Methods: The operations described were conducted at 0-4° unless otherwise specified. AH-130 cells were harvested from the peritoneal cavity of rats (Donryu-strain) 7 days after inoculation and washed twice in physiological saline. One half of the washed cells was packed in 0.4 M sucrose-5 mM EDTA (pH 7.7) by centrifuging at 600 X g for 2 min, homogenized in 4 vol. of the sucrose-EDTA medium and centrifuged at 5000 X g for 10 min. The extract was then centrifuged at 105,000 X g for 60 min, yielding a clear supernatant fraction. The precipitate, referred to as particulate fraction, was suspended in the sucrose-EDTA medium by gentle homogenization.

Abbreviation used: G6P, glucose 6-phosphate.

Table I. Subcellular distribution and G6P dependence of glycogen synthetase before and after incubation with glucose

Incubation	Fractions	Glycogen synthetase activity*		-G6P/+G6P
		-G6P	+G6P	
Before	Extract	17.83	23.00	0.78
	Supernatant	14.21	17.92	0.79
	Particulate	1.04	1.66	0.62
After	Extract	5.08	20.68	0.25
	Supernatant	0.21	0.71	0.29
	Particulate	1.56	19.18	0.08

*Activity was expressed as μmoles of glucose incorporated into glycogen/hr/ml of packed cells.

The other half of the saline-washed cells was packed in Ca^{++}-free Krebs-Ringer phosphate buffer (pH 7.4). Six ml of the packed cells were mixed with 9 vol. of the phosphate buffer and incubated at 37° in the presence of 20 mM glucose plus 10 mM pyruvate while on a shaker oscillating 60 times/min. After 60 min, the cells were freed of residual glucose and pyruvate and washed twice in the sucrose-EDTA medium. The homogenization and fractionation of these cells were carried out as described for non-incubated cells.

Glycogen synthetase activity was estimated by measuring the amount of ^{14}C-glucose into glycogen from UDP-^{14}C-glucose. The standard assay mixture contained 50 mM Tris-maleate buffer (pH 7.7), 0.3% glycogen, 1.5 mM UDP-^{14}C-glucose, 10 mM G6P (when added) and 0.05 ml of enzyme in a final volume of 0.2 ml. After incubation for 8 min at 30°, the reaction was stopped by adding 15% KOH. The isolation and radioactivity measurement of glycogen were then conducted by a procedure essentially similar to that of Steiner et al. (1965).

Results: Table I compares the subcellular distribution and G6P dependence of glycogen synthetase in hepatoma cells before and after incubation with excess glucose and pyruvate. Pyruvate was used because of its effect on stimulating glycogen synthesis (Tsuiki and Saheki, 1966). The glycogen content

of freshly harvested hepatoma cells was 0.04 μmole glucose equivalent/ml of packed cells. After incubation, it rose to a value of 7.1 μmoles.

The most noticeable change that occurred during incubation is the redistribution of glycogen synthetase activity: At the beginning, most of the activity was found in the supernatant fraction, but after incubation, almost all the activity was recovered in the particulate fraction. The synthetase in the supernatant fraction from non-incubated cells was little responsive to G6P, whereas that in the particulate fraction from incubated cells was activated 12-fold by 10 mM G6P. The latter fraction was washed twice by suspending in the sucrose-EDTA medium and centrifuging at 105,000 X g for 60 min. After this procedure, -G6P/+G6P activity ratio fell to 0.04. The synthetase of the supernatant fraction from non-incubated cells was sedimentable between 25 and 55 % saturation of $(NH_4)_2SO_4$. After being precipitated twice, the enzyme showed a -G6P/+G6P activity ratio of 0.81. Dialysis did not affect the activity ratio.

The UDP-glucose concentration dependence with and without G6P was studied with these two enzyme preparations (Fig. 1). Like the synthetase D from

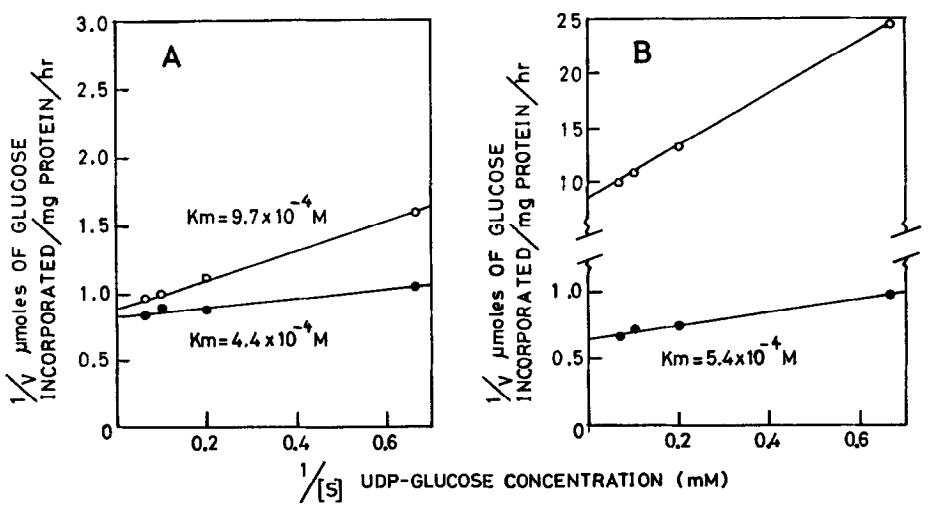

Fig. 1. Reciprocal plot of the UDP-glucose concentration dependence with glycogen synthetase of $(NH_4)_2SO_4$-precipitated fraction from non-incubated cells (A) and of washed particulate fraction from incubated cells (B). No G6P, o—o ; with 10 mM G6P, ●—● .

muscle (Villar-Palasi et al., 1966), the washed particulate fraction from incubated cells showed little activity when G6P was absent. The $(NH_4)_2SO_4$-precipitated fraction of non-incubated cells behaved like the synthetase I from muscle (Villar-Palasi et al., 1966): G6P decreased the K_m for UDP-glucose but did not alter the V. Evidently, the synthetase of hepatoma, being almost totally in an I form at the beginning, was converted to a D form with the progress of incubation. In Table I, minute activity found in the particulate fraction from non-incubated cells showed a G6P dependence higher than that of supernatant fraction. By washing in the sucrose-EDTA medium once, the -G6P/+G6P activity ratio of the particulate fraction fell from 0.62 to 0.40, thereby indicating a preferential binding of the synthetase D to glycogen.

In the experiment shown in Fig. 2, glycogen-deficient hepatoma cells were incubated with U-^{14}C-glucose. Although glucose uptake and lactate formation were almost linear up to 85 min, glycogen formation as determined by the incorporation of ^{14}C-glucose into glycogen ceased after 45 min of incubation. The cessation of glycogen formation appears to coincide with the disappearance of synthetase I.

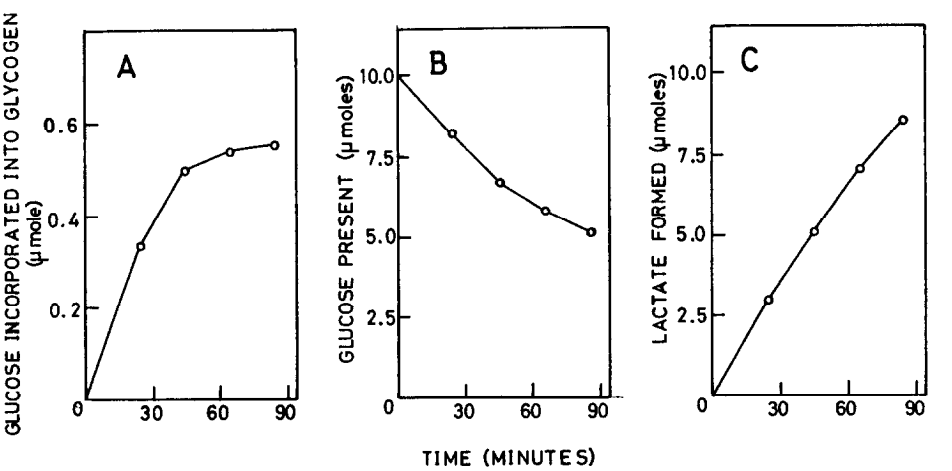

Fig. 2. Course of glycogen formation (A), glucose uptake (B) and lactate formation (C) in freshly harvested hepatoma cells. The packed cells (0.1 ml) were incubated at 37° in 2 ml of Ca^{++} -free Krebs-Ringer phosphate buffer (pH 7.4) containing 10 mM U-^{14}C-glucose. Glucose was determined by the method of Somogyi (1952) and lactate by the method of Barker and Summerson (1941).

Discussion: An inverse relationship has been noted between glycogen content and I/I+D ratio of glycogen synthetase in rat muscle (Danforth, 1965) and HeLa cells (Alpers, 1966). In the present work, a total conversion of synthetase I to D associated with glycogen synthesis was demonstrated by incubating the glycogen-deficient hepatoma cells in a glucose-containing medium. Although several I to D conversion systems have been reported with muscle homogenates (Friedman and Larner, 1963; Belocopitow et al., 1967), the system described here is unique in that it functioned in whole cell concomitantly with the redistribution of enzyme from soluble to particulate fraction. The preferential binding of the synthetase D to glycogen may play a role in this system. The binding may stabilize the synthetase D or it may shift the equilibrium toward D formation. The picture is consistent with an inactivation of glycogen synthetase by its product, glycogen, and explains why glycogen formation in hepatoma cells slows down with time. The affinity of glycogen synthetase for polymerized glycogen may thus determine the capacity of tissue to store glycogen. One might speculate further that a low glycogen content of transplantable hepatomas (Sweeney et al., 1963) is attributed to a higher affinity of hepatoma synthetase for glycogen as compared to the liver enzyme.

REFERENCES

Alpers, J.B., J. Biol. Chem., 241, 217 (1966).
Barker, S.B. and Summerson, W.H., J. Biol. Chem., 138, 534 (1941).
Belocopitow, E., Garcia Fernandez, Maria del Carmen, Birnbaumer, L. and Torres, H.N., J. Biol. Chem., 242, 1227 (1967).
Danforth, W.H., J. Biol. Chem., 240, 588 (1965).
Friedman, D.L. and Larner, J., Biochemistry, 2, 669 (1963).
Somogyi, M., J. Biol. Chem., 195, 19 (1952).
Steiner, D.F., Younger, L. and King, J., Biochemistry, 4, 740 (1965).
Sweeney, M.J., Ashmore, J., Morris, H.P. and Weber, G., Cancer Res., 23, 995 (1963).
Tsuiki, S. and Saheki, R., in Morris, H.P. and Hayaishi, O. (eds.), "Gann Monograph 1", Japanese Cancer Association, Tokyo, p. 117 (1966).
Villar-Palasi, C., Rossel-Perez, M., Hizukuri, S., Huijing, F. and Larner, J., Methods Enzymol., 8, 374 (1966).