PHENOBARBITAL-INDUCED INCREASE OF THE HEXOSE 6-PHOSPHATE DEHYDROGENASE ACTIVITY

Samuel H. Hori and Takayuki Takahashi
Department of Zoology, Faculty of Science, Hokkaido University, Sapporo, Japan

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SUMMARY: The intracellular and intramicrosomal distributions of hexose 6-phosphate dehydrogenase were examined in the livers of normal and starved rats before and after phenobarbital treatment. The results have revealed that the administration of phenobarbital (75 mg/kg body weight, 4 days) causes a significant increase of the hexose 6-phosphate dehydrogenase activity only in smooth-surfaced, but not in rough-surfaced endoplasmic reticulum, with concomitant increases of the activities of NADPH-cytochrome c reductase and aminopyrine demethylase. Starvation prior to the phenobarbital treatment enhanced the effect of phenobarbital on the microsomal enzymes.

INTRODUCTION: G6P dehydrogenase has been known to exist almost exclusively in cytosol (1,2). However, recent studies demonstrated its existence in a large particulate fraction (3-9) and in microsomes (10-13). Ohno et al. (10) designated this microsomal enzyme as H6P dehydrogenase inasmuch as it was found to be as active on Gal6P as on G6P. Subsequent works on H6P dehydrogenase have revealed that this enzyme is resistant to inhibitors of cytosol G6P dehydrogenase, has a broad substrate and coenzyme specificity, is not precipitated by antibody against cytosol G6P dehydrogenase and is probably identical with the enzyme formerly known as glucose dehydrogenase (14-20). The physiological role of this enzyme is still unclear, but it is possible that one of the roles of this enzyme is to supply reduced NADP and NAD for the microsomal oxidase system involved in drug and steroid metabolism (16,17). If this holds, H6P dehydrogenase

Abbreviations: G6P, glucose 6-phosphate; Gal6P, galactose 6-phosphate; H6P, hexose 6-phosphate; SER and RER, smooth- and rough-surfaced endoplasmic reticulum.

might be induced by phenobarbital, a potent inducer of drug metabolizing enzymes located in SER (21-23). In order to test this possibility, an attempt was made to examine the change of H6P dehydrogenase activity in hepatic microsomes before and after phenobarbital treatment, which is dealt with in the present communication.

MATERIALS AND METHODS: Male Wistar rats weighing 150-200 g were given daily intraperitoneal injections (10 a.m.) of Na phenobarbital at a dose of 75 mg per kg body weight during 4 days, starved overnight and killed by decapitation. Some rats were starved for 2 days before the phenobarbital treatment. Control rats were starved overnight or for 2 days. All rats were killed at 8:30 a.m.

Livers were rinsed in cold 0.25 M sucrose, blotted with filter paper, weighed and minced with scissors. The minced tissues were homogenized with 3 volumes of 0.25 M sucrose and centrifuged at 950 x g for 10 min (nuclear fraction). The pooled supernatant was centrifuged successively at 9,000 x g for 30 min and 144,000 x g for 60 min to obtain mitochondrial and microsomal fractions, respectively. Each fraction was washed once with 4 volumes of 0.25 M sucrose, suspended in 1.5 volumes of 20 mM phosphate buffer, pH 6.4 and sonicated for 3 min using a Umeda sonicator.

Subfractionation of the microsomal fraction was carried out by the method of Rothschild (24) as follows: 3 ml of the 9,000 x g supernatant were layered over 1 ml of 0.31 M sucrose and centrifuged for 13 hours at 68,000 x g using a swinging bucket rotar (Hitachi RPS-50) in order to sediment RER. The resulting supernatant was transferred into a new tube and centrifuged at 144,000 x g for 60 min to sediment SER. It was proved electronmicroscopically that the SER fraction contained no RER and that the RER fraction contained only a very few SER fragments and mitochondria. The fractions were then sonicated for 1 min with 2 ml of 20 mM phosphate buffer, pH 6.4.

G6P dehydrogenase and H6P dehydrogenase were assayed spectrophotometrically. Reaction mixture consisted of 0.7 ml of glycine-NaOH buffer (I=0.2, pH 10.0), 0.6 μmoles NADP, 6 μmoles G6P or Gal6P and enzyme in a final volume of 1.0 ml. Reaction was started by the addition of substrate and extinction changes at 340 nm were measured at 21 ± 1°C for 10 min. G6P dehydrogenase activity was corrected for 6-phosphogluconate dehydrogenase activity by the method of Glock and McLean (1).

The activities of G6P dehydrogenase and H6P dehydrogenase in the fractions containing both enzymes are calculated as follows:

G6P dehydrogenase activity at pH 10.0= $\frac{0.71\ A - B}{0.69}$; H6P dehydrogenase activity at pH 10.8= $\frac{A - \text{G6P dehydrogenase activity}}{0.6}$

where A is the activity with G6P and B is the activity with Gal6P at pH 10.0. This is based on the following findings with the enzymes purified according to the method of Hori and Sado (20): G6P dehydrogenase, optimal pH, 10.0, activity with Gal6P is 2.2% of that with G6P at pH 10.0. H6P dehydrogenase, optimal pH, 10.8, activity with Gal6P is 71% of that with G6P at pH 10.0 which in turn is 60% of the activity with G6P at pH 10.8.

The oxidative demethylation activity was assayed using aminopyrine as substrate according to the method of Orrenius (25). With this technique, the rate of oxidative demethylation of aminopyrine was linear with varying amounts of microsomes (up to 2.5 mg of protein). The NADPH-cytochrome c reductase activity was assayed at 22 ± 1°C by the method of Horecker(26), except a NADPH-generating system which consisted of 5 mM dl-isocitrate, 10 μM $MnCl_2$ and isocitrate dehydrogenase (type IV, Sigma), 0.62 units. The rate of cytochrome c reduction was linear with time and with enzyme amounts (at least up to 0.4 mg protein). Protein was determined by the method of Lowry et al. (27).

RESULTS AND DISCUSSION: Table 1 shows the intracellular distri-

TABLE 1. Intracellular distributions of G6P dehydrogenase and H6P dehydrogenase in the livers of normal, starved and phenobarbital-treated rats.

Fraction	Treatment	No. of rats	Protein mg/g liver	H6P dehydrogenase units/μg protein	G6P dehydrogenase units/μg protein
Nuclei	None	(9)	27.1 ± 0.9	1.4 ± 0.2	2.1 ± 0.5
	Starved	(4)	32.1 ± 1.3	1.0 ± 0.2	1.6 ± 0.2
	Phenobarbital	(6)	24.9 ± 1.4	1.5 ± 0.2	2.5 ± 0.2
	Starv.+Pheno.	(5)	27.0 ± 0.7	1.5 ± 0.2	4.5 ± 0.4 ↑↑
Mitochondria	None	(9)	37.6 ± 1.1	1.8 ± 0.1	3.1 ± 0.3
	Starved	(4)	36.6 ± 1.7	2.1 ± 0.3	2.1 ± 0.1
	Phenobarbital	(6)	36.2 ± 1.2	2.0 ± 0.2	4.4 ± 0.4 ↑↑
	Starv.+Pheno.	(5)	34.8 ± 1.4 ↓	2.6 ± 0.2 ↑↑	13.7 ± 0.6 ↑↑
Microsome	None	(9)	41.4 ± 1.1	15.8 ± 0.7	2.7 ± 0.2
	Starved	(4)	40.1 ± 2.3	14.2 ± 0.6 ↓	2.4 ± 0.3
	Phenobarbital	(6)	49.1 ± 0.8 ↑↑	16.7 ± 0.5	3.3 ± 0.3 ↑
	Starv.+Pheno.	(5)	43.9 ± 1.0 ↑↑	19.5 ± 0.6 ↑↑	8.6 ± 1.6 ↑↑
Cytosol	None	(9)	71.5 ± 2.3	2.5 ± 0.3	33.2 ± 3.3
	Starved	(4)	64.4 ± 3.2 ↓	2.0 ± 0.3	22.8 ± 1.4 ↓↓
	Phenobarbital	(6)	68.9 ± 2.9	2.9 ± 0.6	37.3 ± 1.5
	Starv.+Pheno.	(5)	71.7 ± 3.7	5.6 ± 0.8 ↑↑	128.8 ± 6.5 ↑↑

Each value is the mean ± SEM. The direction and number of arrows indicates the direction of change and degree of significance of the differences relative to the control value. Fisher's P values are shown as: ↑, $P < 0.05$; ↑↑, $P < 0.01$.

TABLE 2. Intramicrosomal distributions of H6P dehydrogenase, NADPH-cytochrome c reductase and aminopyrine demethylase in the livers of normal, starved and phenobarbital-treated rats.

Fraction	Treatment	No. of rats	Protein mg/g liver	H6P dehydrogenase units/μg protein	Reductase ΔE_{550}/min/ μg protein	Demethylase mμmoles formaldehyde/min/ mg protein
Total microsome	None	(9)	41.4 ± 1.1	15.8 ± 0.7	93 ± 11	3.5 ± 0.3
	Starved	(4)	40.1 ± 2.3	14.2 ± 0.6 ↓	142 ± 37	3.5 ± 0.3
	Phenobarbital	(6)	49.1 ± 0.8 ↑↑	16.7 ± 0.5	173 ± 12 ↑↑	7.2 ± 0.2 ↑↑
	Starv.+Pheno.	(5)	48.9 ± 1.0 ↑↑	19.5 ± 0.6 ↑↑	277 ± 9 ↑↑	7.3 ± 0.4 ↑↑
RER	None	(9)	23.2 ± 0.6	15.7 ± 0.5	59 ± 9	1.5 ± 0.2
	Starved	(4)	19.9 ± 0.6 ↓	15.7 ± 1.9	85 ± 15 ↑	1.7 ± 0.3
	Phenobarbital	(6)	21.0 ± 0.6	16.1 ± 0.6	76 ± 7 ↑	2.6 ± 0.3 ↑↑
	Starv.+Pheno.	(5)	23.1 ± 0.7	17.8 ± 0.7 ↑↑	133 ± 3 ↑↑	3.7 ± 0.3 ↑↑
SER	None	(9)	16.7 ± 1.1	9.5 ± 0.8	88 ± 16	4.4 ± 0.3
	Starved	(4)	18.0 ± 1.4	11.2 ± 1.8	153 ± 39 ↑	3.6 ± 0.3
	Phenobarbital	(6)	23.4 ± 1.5 ↑↑	12.9 ± 0.7 ↑↑	207 ± 10 ↑↑	8.9 ± 0.5 ↑↑
	Starv.+Pheno.	(5)	23.0 ± 0.6 ↑↑	16.1 ± 0.9 ↑↑	261 ± 16 ↑↑	9.5 ± 0.5 ↑↑
Recovery*	None		96.4	81.0	71.2	76.0
	Starved		94.5	89.7	78.3	70.0
	Phenobarbital		90.4	77.6	75.2	72.2
	Starv.+Pheno.		94.3	81.7	67.1	85.6

Each value is the mean ± SEM. The direction and number of arrows indicates the direction of change and degree of significance of the differences relative to the control value. Fisher's P values are shown as: ↑, $P < 0.05$; ↑↑, $P < 0.01$.

* Recovery= $\frac{\text{RER + SER}}{\text{total microsome}}$ x 100.

butions of G6P dehydrogenase and H6P dehydrogenase. It indicates that the two enzymes are predominantly located in the cytosol and microsomal fractions, respectively.

Phenobarbital treatment caused a significant increase in the amount of microsomal proteins, while the change in the microsomal H6P dehydrogenase activity was slight (6% increase, $P > 0.05$). However, the effect of phenobarbital on this enzyme became evident when the enzyme was assayed with the submicrosomal fractions, as shown in Table 2; i.e., about a 37% increase of the specific activity was observed in SER without any change in RER. Since the amount of protein also increased 40%, the increase in enzyme activity amounted to 90% when expressed in units per g liver.

The activities of NADPH-cytochrome c reductase and of aminopyrine demethylase were also increased in SER in good agreement with previous data (25). Starvation prior to the phenobarbital treatment intensified the effect of phenobarbital on the three microsomal enzymes. In this case, the H6P dehydrogenase activity was also increased in the cytosol fraction. On the other hand, starvation alone had no stimulative effect on the H6P dehydrogenase activity, although it enhanced the NADPH-cytochrome c reductase activity in both SER and RER.

The cytosol G6P dehydrogenase activity was low in starved rats and was increased by the phenobarbital treatment of starved rats, but not of fed rats. This is consistent with the previous data that refeeding stimulates, but phenobarbital alone has no effect on the hepatic G6P dehydrogenase activity (28,29,30). The G6P dehydrogenase activity was also increased in the mitochondrial and microsomal fractions by the phenobarbital treatment of fed and starved rats. It is uncertain, however, whether this increase would suggest an increased adsorption of cytosol G6P dehydrogenase onto the particulates or a net increase of the enzyme intrinsic to these organelles.

As is clear in the above experiments, a significant increase of H6P dehydrogenase activity occurs in SER concomitantly to the induction of NADPH-

cytochrome c reductase and aminopyrine demethylase after phenobarbital treatment, with no change in the cytosol G6P dehydrogenase activity. It is thus possible to assume that H6P dehydrogenase may be built in the microsomal membrane as a member of the electron transport system, thus involving drug metabolism. That this enzyme is resistant to several inhibitors of cytosol G6P dehydrogenase favors the above view (13,17,20).

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