Nutritional regulation of hepatic glucose-6-phosphate dehydrogenase

Transient activation of transcription

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Hepatic glucose-6-phosphate dehydrogenase (G6PDH; EC 1.1.1.49) is subject to nutritional and hormonal regulation. Previous work has shown that increased amounts of mRNA encoding G6PDH can account for the increase in enzyme activity. The results of this study demonstrate that transcription of the G6PDH gene is transiently elevated after ingestion of a high-carbohydrate diet. However, the increased rate of transcription cannot totally account for the increased G6PDH mRNA. The half-life of the G6PDH mRNA appears to be about 4–5-fold higher during ingestion of a high-carbohydrate diet. Thus increased transcription as well as mRNA stability are each partially responsible for the nutritional regulation of G6PDH mRNA.

INTRODUCTION

Glucose-6-phosphate dehydrogenase (G6PDH) is often regarded as the product of a typical 'housekeeping' gene, since it has been found in all cell types and organisms analysed thus far. Sequence analysis of the promoter region of several 'housekeeping' genes has revealed that these genes lack certain structural features such as a 'TATA or CAAT box' that are usually found in genes transcribed by RNA polymerase II (Melton *et al.*, 1986; Reynolds *et al.*, 1984), implying that the 'housekeeping' genes may not be transcriptionally regulated. Indeed, Carneiro & Schibler (1984) demonstrated that accumulation of mRNA for ten putative 'housekeeping' genes was regulated post-transcriptionally.

G6PDH is needed in each cell for the production of NADPH and for control of carbon flow through the pentose phosphate pathway. However, it is well established that G6PDH expression in liver is subject to nutritional and hormonal control, and that highest extents of expression are correlated with elevated rates of lipogenesis (Berdanier & Shubeck, 1979; Volpe & Vagelos, 1976). In previous studies, we have established that nutritional or hormonal regulation of G6PDH is the result of changes in the degrees of G6PDH mRNA abundance (Kletzien *et al.*, 1985; Fritz *et al.*, 1986), except for changes resulting from manipulations of thyroid hormone (Fritz & Kletzien, 1987). Increasing the mRNA could result from increased transcription or a post-transcriptional event such as stabilization of the mRNA. In the study reported here, we have used transcriptions assays *in vitro* to assess the activity of the hepatic G6PDH gene as a function of time after refeeding of a high-carbohydrate diet. In addition, we analysed the kinetics of accumulation and degradation of mRNA encoding G6PDH during the same time frame. The results of this study establish that carbohydrate feeding

transiently stimulates gene expression at the transcriptional level in liver, but stabilization of the mRNA clearly plays a major role also.

EXPERIMENTAL

Materials

Rats (Sprague–Dawley) were obtained from Hilltop Farms, Scottsdale, PA, U.S.A. Guanidine thiocyanate was purchased from Fluka A.G. (Switzerland). DNAase I was from Cooper Biomedical. All restriction enzymes or nucleases were from either Bethesda Research Laboratories or Pharmacia. Nitrocellulose filters (BA85) were purchased from Schleicher and Schuell. Radiolabelled UTP and dCTP were from New England Nuclear. All other chemicals were of the highest purity available from Pharmacia, Bethesda Research, Sigma Chemical Co. or Boehringer Mannheim. A G6PDH genomic clone (pGDT1) containing 9.4 kb of the G6PDH gene cloned into the pGEM vector (ProMega) was used as the probe for the newly transcribed G6PDH sequences. This cloned DNA contains all of the gene (12.2 kb in total length) except intron 1, exons 1 and 2, and the 5' control region. The b-actin cDNA was generously given by Dr. D. Cleveland, Johns Hopkins University.

Animals

Male rats weighing approx. 200 g were housed in individual cages. Animals were deprived of food for 48 h and then fed on a fat-free high-carbohydrate diet, consisting of (by wt.) 75.5% sucrose, 20.5% casein and 4% vitamin/mineral mixture, for up to 72 h. In all experiments animals were maintained on a 12 h-light/12 h-dark cycle with free access to water. Animals were monitored for food consumption to be certain that they began to feed shortly after food was presented. Animals

Abbreviation used: G6PDH, glucose-6-phosphate dehydrogenase.

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that did not begin to eat within 1 h were not used in these experiments. Animals were killed either after 48 h of food deprivation or after 5–72 h of carbohydrate refeeding, by decapitation. In one set of experiments, animals were deprived of food for 48 h, refed on a high-carbohydrate diet for 48 h, and then killed at various times after food was removed from the animals. Livers were quickly excised and perfused with ice-cold phosphate-buffered saline (Fritz *et al.*, 1986). Samples of each liver were taken for assay of G6PDH activity (Kletzien *et al.*, 1985), isolation of nuclei and extraction of RNA.

RNA isolation and blot analysis

Total cellular RNA was isolated from whole liver by the guanidine thiocyanate procedure described previously (Fritz & Kletzien, 1987). Cytoplasmic RNA was isolated by minor modifications of previously published methods (Favoloro *et al.*, 1980). Nuclear RNA was isolated from purified nuclei by the guanidine thiocyanate method. For analysis of G6PDH sequences present in RNA, dot-blot hybridizations were carried out. RNA was denatured with formaldehyde and blotted on to nitrocellulose with a vacuum-manifold apparatus (Schleicher and Schuell). Hybridization reactions were performed as described previously (Fritz *et al.*, 1986). Hybridization probes were based on the G6PDH cDNA sequence (Kletzien *et al.*, 1985) and were either nick-translated cDNA or strand-specific probes from Klenow-catalysed sequencing reactions. Blots were exposed to X-ray film and the signals quantified with an integrating soft-laser densitometer (Zeineh). Some blots were analysed by cutting out the dots and measuring the bound radioactivity by scintillation counting.

Measurement of RNA transcription

Transcription of the G6PDH gene was measured by transcription 'run-on' assays in isolated nuclei. Nuclei were isolated from whole liver as follows. Minced tissue (1 g wet wt./6 ml of buffer) was suspended in homogenizing buffer [15 mM-Tris (pH 7.5)/250 mM-sucrose/5 mM-MgCl₂/5 mM-dithiothreitol] and homogenized in a Potter-Elvehjem glass/Teflon homogenizer. Nuclei were collected by low-speed centrifugation and rehomogenized in the same buffer with the addition of 0.5% Nonidet P-40. The nuclei were then purified by centrifugation through a 3.5 ml pad of 30% (w/v) sucrose in homogenization buffer (20000 *g* for 20 min). The purified nuclei were resuspended in storage buffer [50 mM-Tris (pH 8)/45% (w/v) glycerol/5 mM-MgCl₂/0.1 mM-EDTA] and stored at -70 °C.

For the 'run-off' transcription assay, nuclei (8×10^7) were incubated in 20% glycerol/25 mM-Tris (pH 8)/2 mM-MgCl₂/150 mM-KCl/1 mM-dithiothreitol containing 1 mM-ATP, 0.5 mM-CTP, 0.5 mM-GTP, 8 mM-phosphocreatine, 40 µg of creatine kinase/ml, RNasin (50 units/ml) and [α -³²P]UTP. RNA synthesis was terminated after 30 min, and nuclei were treated with DNAase I (20 µg/ml) and proteinase K (100 µg/ml), and 50 µg of tRNA/ml was added. The RNA was extracted twice with phenol/chloroform (1:1, v/v) and precipitated in ethanol.

The newly transcribed RNA was hybridized to DNA immobilized on to nitrocellulose filters. Plasmid DNA (including pGEM as control for non-specific hybridization) was linearized, alkali-denatured, spotted on to the

filters and baked (Greenberg & Ziff, 1984). Pre-hybridization was carried out in 50% formamide/5 × Denhardt's/5 × SSPE (1 × SSPE = 180 mM-NaCl/10 mM-sodium phosphate (pH 7.7)/1 mM-EDTA)/0.1% SDS containing 100 µg of tRNA/ml. Hybridization was carried out in the same solution with 5×10^7 c.p.m. of labelled RNA added for 2–3 days at 42 °C. After hybridization, the filters were washed and treated with RNAases A and T1. The filters were exposed to X-ray film and the intensity was measured by densitometry. The filters were then cut, and the specifically bound RNA was eluted and counted for radioactivity with a liquid-scintillation counter. The efficiency of hybridization was monitored by use of a ³H-cRNA produced from a G6PDH cDNA, encompassing 470 bases near the 3' end of the mRNA, cloned into the pGEM vector.

RESULTS AND DISCUSSION

Analysis of G6PDH gene transcription and mRNA amounts

The rate of G6PDH gene transcription and the amount of G6PDH mRNA in liver were determined after feeding of a high-carbohydrate diet to rats that had been fasted for 48 h. The data in Fig. 1 show that transcription increased sharply after a lag of at least 5 h after refeeding rats on a high-carbohydrate diet. The transcription rate was substantially elevated when measured at 10 h (4.4-fold increase) or 15 h (3.1-fold increase), but fell to less than a 2-fold increase when measured at later times. The time course of accumulation of mRNA encoding G6PDH after refeeding of a high-carbohydrate

Fig. 1. Time course of the effect of a high-carbohydrate diet on G6PDH gene transcription and mRNA content

G6PDH gene transcription (○) and mRNA encoding G6PDH (●) were measured in the livers of rats fed on the high-carbohydrate diet for various periods of time. Each value represents the mean value from three to five rats, except the 5h time point, which represents the average from two rats. From each rat that was killed, liver samples were taken for isolation of nuclei for transcription studies and total RNA was isolated for analysis of G6PDH mRNA abundance. The values are expressed as percentages of the maximum induction and are derived directly from the data presented in Table 1.

diet also showed a lag of at least 5 h before the concentration began to increase (Fig. 1). The highest accumulation was reached at 24 h (16.5-fold increase) and then declined when measured at both 48 h (12-fold increase) and 72 h (9.4-fold increase). Thus the time course of the changes in concentration of hepatic G6PDH mRNA paralleled that of transcription, and the transient nature of the transcription increase is reminiscent of similar results observed for type L pyruvate kinase after refeeding of a high-carbohydrate diet (sucrose, glucose or fructose) to fasted rats (Vaulont *et al.*, 1986; Munnich *et al.*, 1987). However, the 4.4-fold increase in G6PDH gene transcription is not sufficient to account for the observed increase in G6PDH mRNA at either the peak value or the values at 48 or 72 h. Therefore, an additional mechanism must also contribute to the increased concentration of G6PDH mRNA after refeeding animals on a high-carbohydrate diet.

Determination of G6PDH mRNA half-life

Previous studies have shown that mRNA encoding several different proteins increases in stability after hormonal treatment or dietary manipulation (Goldman *et al.*, 1985; McKnight & Palmiter, 1979). Therefore we estimated G6PDH mRNA half-life during the accumulation phase from a plot of the difference in the peak induced amount of mRNA and the amounts at various times during the accumulation phase versus time after refeeding of the high-carbohydrate diet (Schimke *et al.*, 1964). The data in Fig. 2(a) gave estimates of 9–11 h for G6PDH mRNA half-life. Similar half-life estimates were carried out by analysing G6PDH mRNA concentrations after withdrawal of the high-carbohydrate diet from rats. The half-life estimates during the decay phase were about 1.5–2 h (Fig. 2b), although there is a lag of 3–4 h before mRNA stability decreases sharply. The lag may reflect the time needed for digestion of food already present in the stomach.

Analysis of the b-actin mRNA during the same time frames showed no difference in the half-life, thereby establishing that the dietary manipulation does not have a general effect on all mRNA species (results not shown). Although this method of estimating mRNA half-life is indirect, it is evident that a major change in hepatic G6PDH mRNA half-life occurs as a result of dietary manipulation.

G6PDH activity, mRNA, nuclear RNA and transcription

The data presented in Table 1 show the relationship between G6PDH enzyme activity, G6PDH mRNA, G6PDH RNA sequences in the nucleus and transcription of the G6PDH gene after feeding of a high-carbohydrate diet to fasted rats. The time course of accumulation of enzyme activity lags behind the increase in G6PDH mRNA and reached highest values after the animals were on the diet for 48–72 h, consistent with previous studies (Berdanier & Shubeck, 1979). Although the increase in G6PDH mRNA appears to account for the increase in G6PDH activity which was observed in a previous study when only a single time point after refeeding was used (Kletzien *et al.*, 1985), changes in the rate of enzyme protein synthesis or degradation may also play an important role (Fritz & Kletzien, 1987; Stumpo & Kletzien, 1984). The concentration of G6PDH mRNA sequences in the nucleus closely follows the rate of

Fig. 2. Effect of feeding or food deprivation on G6PDH mRNA abundance in liver

In the experiment depicted in (a), rats were deprived of food for 48 h and then fed on the high-carbohydrate diet. At the indicated times after refeeding, rats were killed and RNA was isolated from the livers. RNA was also isolated from rats that had been refed for 24 h. G6PDH mRNA abundance was measured over a 5-fold concentration range, and values were calculated from the slope of concentration versus signal intensity. The data in (a) are a semi-logarithmic plot of the difference (Δ) in concentration of G6PDH mRNA at the peak accumulation (24 h) minus that at the indicated times. Each value represents the mean from three or four rats, except for the 5 h value, which is the average from two rats. In the experiment depicted in (b), rats were deprived of food for 48 h and then fed on the high-carbohydrate diet for 48 h. Food was then removed, the animals were killed at the indicated times, and RNA was isolated and assayed for G6PDH mRNA content as described above. RNA was also isolated from animals that had been fasted for 48 h. The data in (b) are a semi-logarithmic plot of the difference in concentration of G6PDH mRNA at the indicated times after food deprivation minus that in the 48 h-fasted animal. Each value represents the average from two rats, except the 12 h time point, which is from one rat. Determination of the half-life for b-actin mRNA under conditions in either (a) or (b) revealed no difference in half-life (22–26 h).

transcription of the G6PDH gene, both in the time course of the respective increases and in the actual extent of the increases at each time point. Thus the highest amount of nuclear G6PDH sequences was observed at 10 h after the animals were on the high-carbohydrate diet, which is when the highest rate of transcription was observed. That the cellular G6PDH mRNA reached its highest concentration 10–15 h after the peak of transcription strongly argues for a role of mRNA stabilization in the dietary induction of hepatic G6PDH. Although the indirect method that we used to measure mRNA half-life may give values which might not reflect the absolute values *in vivo*, it is evident that the half-life of the G6PDH mRNA can be influenced greatly by the nutritional state of the animal. Thus the 4–5-fold increase in mRNA half-life and the 4.5-fold increase in transcription appear to be adequate to account for the increase in cellular G6PDH mRNA.

Table 1. Regulation of G6PDH during dietary induction

Rats were starved for 48 h and refed on a high-sucrose diet for 5–72 h before being killed. Livers were removed and perfused, and samples were taken for enzyme activity, mRNA isolation and isolation of nuclei. Isolated nuclei were used in transcription 'run-on' assays using the 9.4 kb fragment (pGDT1) of the G6PDH gene as probe for the newly transcribed RNA. The transcription rate (p.p.m.) was calculated as follows:

$$\frac{\text{c.p.m. (pGDT1 - pGEM)}}{\text{c.p.m. (} \times 10^6 \text{) of total RNA}} \times \frac{100}{\text{efficiency}} \times \frac{12200}{9400}$$

where 12200 is the length in nucleotides of the gene and 9400 is the number of nucleotides represented in pGDT1. Hybridization efficiency averaged 40–45%. Transcription rate for the b-actin gene did not change when measured in rats fasted for 48 h or refed for 10, 24 or 72 h (174, 168, 183 and 191 p.p.m. respectively) G6PDH mRNA and nuclear RNA abundance values are expressed in integrator units. Each value represents the mean $\pm$ S.E.M. for three to five rats, except the 5 h values, which represents the averages for two rats.

Rats	G6PDH activity (munits/mg)	Relative abundance		Transcription (p.p.m.)
		G6PDH mRNA	G6PDH nuclear RNA	
Fasted	3.3 $\pm$ 0.3	3.9 $\pm$ 0.2	1.61 $\pm$ 0.18	13 $\pm$ 3
Refed (5 h)	3.2	4.1	1.55	14.9
Refed (10 h)	3.5 $\pm$ 0.3	8.3 $\pm$ 0.6	7.11 $\pm$ 1.12	57.3 $\pm$ 5
Refed (15 h)	6.8 $\pm$ 0.4	23.1 $\pm$ 1.5	4.54 $\pm$ 0.91	40.3 $\pm$ 3
Refed (24 h)	16.4 $\pm$ 1.2	64.8 $\pm$ 6.1	3.11 $\pm$ 0.27	24.2 $\pm$ 6
Refed (48 h)	56.4 $\pm$ 2.8	48.3 $\pm$ 1.8	2.23 $\pm$ 0.31	19.5 $\pm$ 2
Refed (72 h)	61.6 $\pm$ 3.9	37.7 $\pm$ 3.2	1.85 $\pm$ 0.20	18.7 $\pm$ 4

Transient activation of gene transcription after re-feeding of a high-carbohydrate diet has also been observed for pyruvate kinase, a protein which also is considered to have primarily a 'housekeeping' function. Vaulont *et al.* (1986) demonstrated that feeding fasted rats on a high-sucrose diet similar to that used in our study caused maximal induction of hepatic pyruvate kinase transcription at 12 h after refeeding, and the rate of transcription declined to less than 10% of the maximum by 72 h. Further studies by that group (Munnich *et al.*, 1987) have established that refeeding rats with either fructose or glucose will transiently activate pyruvate kinase transcription. Many of the molecular details have now come to light as to how this regulation is accomplished for pyruvate kinase. First, the type L and type M isoenzymes are encoded by completely different genes (Noguchi *et al.*, 1986, 1987) and are expressed in a tissue-specific manner. Second, the type L gene encodes two forms of pyruvate kinase (type L and type R), which differ in composition at the N-terminal end (Noguchi *et al.*, 1987). Thus all terminally differentiated tissues exhibit pyruvate kinase activity, as is expected for a 'housekeeping' enzyme, but only in liver is the enzyme hormonally/nutritionally responsive and inducible at the transcriptional level. Although it is premature to say whether a similar situation exists for G6PDH, the data presented here establish that the hepatic G6PDH gene is transcriptionally regulated. However, in contrast with pyruvate kinase, the G6PDH gene is thought to be present in only a single copy (X-linked) per cell. Therefore, if tissue-specific hormonally responsive expression does occur from a single gene, then perhaps the mechanism responsible involves different transcription start sites. In this regard, controversy exists in the literature as to the sequence at the N-terminal end for human G6PDH (Takizawa *et al.*, 1986; Persico *et al.*, 1986). It is possible that both groups may be correct, in that G6PDH

produced in red blood cells could have a different N-terminal end from that in another tissue if the transcription start sites were different for different G6PDH mRNAs. Indeed, multiple transcription start sites appears to be characteristic of the 'housekeeping' genes (Melton *et al.*, 1986).

At present it is unclear as to what are the specific mediators of G6PDH gene transcription on the high-carbohydrate diet. The sugars themselves, metabolites of the sugars or hormones secreted in response to the nutritional manipulations must all be considered candidates. The effect of a high-carbohydrate diet on both gene transcription and mRNA stabilization has also been observed for other enzymes involved in lipogenesis, including fatty acid synthase (Back *et al.*, 1986) and malic enzyme (Goldman *et al.*, 1985). The use of highly defined tissue-culture systems and chimeric gene constructs by using the putative control regions of the lipogenic genes should facilitate progress in this area.

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