Analysis of Glucose-6-Phosphate Translocase and Hexose-6-Phosphate Phosphohydrolase, the Two Obligatory Components of Microsomal Glucose-6-Phosphatase System, in Rat Liver

Keiko Hata, Kunimi Kikuchi, Keiya Tada* and Shigeru Tsuki

Biochemistry Laboratory, Research Institute for Tuberculosis and Cancer, Tohoku University and *Department of Pediatrics, Tohoku University School of Medicine, Sendai 981

Hata, K., Kikuchi, K., Tada, K. and Tsuki, S. Analysis of Glucose-6-Phosphate Translocase and Hexose-6-Phosphate Phosphohydrolase, the Two Obligatory Components of Microsomal Glucose-6-Phosphatase System, in Rat Liver. Tohoku J. exp. Med., 1988, 155 (2), 173-181 — A membrane filter procedure developed by Igarashi et al. (1984) for the measurement of glucose 6-phosphate uptake by the microsomes has been demonstrated to be a good method for assaying glucose-6-phosphate translocase, an obligatory component of the microsomal glucose-6-phosphatase system. When glucose-6-phosphate translocase was assayed in developing and diabetic rat livers independently of hexose-6-phosphate phosphohydrolase, another obligatory component of the glucose-6-phosphatase system, the two activities were found to undergo alterations, whose profiles, however, were quite distinct from each other. The profile of the microsomal glucose-6-phosphatase activity resembles the profile of the phosphohydrolase activity rather than that of the translocase activity, suggesting that the phosphohydrolase may be rate-limiting at least under these conditions. AH-109A, a strain of transplantable rat ascites hepatoma, was found to lack both glucose-6-phosphate translocase and hexose-6-phosphate phosphohydrolase activities. —— rat liver; glucose-6-phosphatase; glucose-6-phosphate translocase; hexose-6-phosphate phosphohydrolase; glycogen storage disease Ib

Glucose-6-phosphatase (EC 3.1.3.9) is a rate-limiting enzyme in gluconeogenic and glycogenolytic pathways (Nordlie et al. 1968). The enzyme is a two-component system consisting of glucose-6-phosphate-specific translocase, which translocates glucose 6-phosphate into the cisternal space of the microsomes, and

Received April 8, 1988; revision accepted for publication May 17, 1988.
This work was supported by Grants-in Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan.
relatively non-specific phosphohydrolase, which is localized in the cisternal surface of the microsomal membrane and hydrolyzes hexose 6-phosphate (Arion et al. 1976, 1980). In human, in addition to glycogen storage disease due to a defect in the phosphohydrolase (type Ia), patients that are defective in glucose-6-phosphate translocase (Type Ib) are known (Narisawa et al. 1978; Tada et al. 1985), thereby indicating the two components being genetically distinct.

Glucose-6-phosphatase activity is known to be regulated in response to various physiological and pathological stimuli (Jakobsson and Dallner 1968; Nordlie et al. 1968; Hanson and Nordlie 1970; Zoccoli et al. 1982). Whether the two components of the glucose-6-phosphatase system are subject to common or independent regulation has yet to be elucidated. In order to answer this question, we attempted to assay the translocase and the phosphohydrolase activities of rat liver independently under a variety of conditions.

Materials and Methods

Animals

Fetal, neonatal and adult rats of the Wistar strain were used. Adult male rats weighed 150–200 g and were maintained on a commercial rat diet from Oriental Yeast Co. (Tokyo) ad libitum. Insulin-deficient rats were prepared by injecting with 50 mg/kg body weight of streptozotocin as described by Kikuchi et al. (1983). Ascites hepatoma cells (AH-109A) were harvested from the peritoneal cavity of male Donryu rats 5 days after implantation.

Preparation of the microsomal fraction

The following preparative operations were conducted at 0-4°C. Rats were exsanguinated. The liver was quickly excised and homogenized for 1 min in 4 vol. of 0.25 M sucrose/0.5 mM EDTA/10 mM Hepes, pH 7.4, using a Teflon pestle homogenizer. The homogenate was centrifuged at 5,000 x g for 15 min, and the microsomes were precipitated from the supernatant by centrifugation at 105,000 x g for 1 hr. The microsomes were then suspended in an appropriate volume of 0.25 M sucrose/1 mM EDTA adjusted to pH 7.4 and used. The microsomal fraction was also prepared from AH-109A cells: the procedure was the same except that the cells were homogenized in 2 vol. of distilled water then mixed with 2 vol. of 0.5 M sucrose/1 mM EDTA/20 mM Hepes, pH 7.4.

Enzyme assays

When glucose-6-phosphatase was being assayed, 0.1 ml of the microsomal fraction (60–100 μg in protein) was added with 0.3 ml of 83.3 mM sucrose/0.33 mM EDTA/33 mM glucose 6-phosphate/33 mM sodium cacodylate, pH 6.5, and the mixture was incubated for 5 min at 37°C. The termination of the reaction, centrifugation of the reaction mixture and assay of inorganic phosphate released were carried out as described by Baginski et al. (1974).

Hexose-6-phosphate phosphohydrolase was assayed as follows. Fifty μl of the microsomal fraction (0.7–0.9 mg in protein) was incubated at 4°C in the presence of 0.2% (W/V) sodium deoxycholate. After 30 min, 1.0 ml of 0.25 M sucrose was added, and 0.1 ml of the resulting mixture was assayed for glucose-6-phosphate hydrolysis as described above.

Glucose-6-phosphate translocase was assayed using the conditions described by Igarashi et al. (1984). Thirty μl of the microsomal fraction was mixed with 10 μl of 200 mM sodium cacodylate, pH 6.5, and the mixture was kept at 30°C for 1 min. The mixture was then added with 10 μl of 25 mM [1-14C] glucose 6-phosphate (0.5 μCi) and incubated at 30°C. After a few minutes, the reaction was stopped by adding 1 ml of cooled 0.25 M sucrose/50
mM Tris-HCl, pH 7.5/25 mM KCl/5 mM MgCl₂/8 mM CaCl₂, and the microsomes were immediately collected on Whatman GF/A filter membrane (2.5 cm in diameter). The filter membrane was then washed with the above stop solution, dried at room temperature and counted for radioactivity.

Other assay method

Protein was assayed by the method of Bradford (1976).

Chemicals

Glucose 6-phosphate was purchased from Boehringer (Mannheim, FRG) and mannose 6-phosphate, glucosamine 6-phosphate and streptozotocin from Sigma (St. Louis, MO, USA). [1-¹⁴C] Glucose 6-phosphate was obtained from New England Nuclear (Boston, MA, USA). Other reagents and solvents were of analytical grade.

Results

Assays of glucose-6-phosphatase and hexose-6-phosphate phosphohydrolase activities

In confirmation of the previous report (Arion et al. 1976), intact rat liver microsomes hydrolyzed glucose 6-phosphate but not mannose 6-phosphate (Fig. 1). When the microsomes were pretreated with sodium deoxycholate, however, the hydrolysis of mannose 6-phosphate arose and was increased with increasing concentrations of the detergent until it reached a level comparable to that for glucose 6-phosphate, which was little affected by the detergent (Fig. 1). While the activity of intact microsomes is due to microsomal glucose-6-phosphatase
system, microsomes maximally disrupted by deoxycholate exhibits only the phosphohydrolytic activity of the glucose-6-phosphatase system. This phosphohydrolase is best designated as “hexose-6-phosphate phosphohydrolase” since, in addition to glucose 6-phosphate, it is also capable of hydrolyzing mannose 6-phosphate (Fig. 1) and glucosamine 6-phosphate (data not shown) provided that circumstances allow them to access to the enzyme.

Assay of glucose-6-phosphate translocase

Previously, Igarashi et al. (1984) used a membrane filter technic to investigate the uptake of glucose 6-phosphate by intact rat liver microsomes at 30°C. The uptake started quickly; it continued linearly as least for 90 sec, indicating that within that period, microsomal hydrolysis of glucose 6-phosphate was only negligible. Using the same technic, we not only confirmed their observation (Fig. 2A) but also showed that under the conditions that satisfy proportionality to time, glucose 6-phosphate uptake can also be proportional to protein (microsome) concentration even though confined within narrow limits (Fig. 2B). The membrane filter technic was therefore adopted to assay the translocase component of microsomal glucose-6-phosphatase system with no/little interference by hexose-6-phosphate phosphohydrolase. Since the assay was made at 30°C, the results obtained were not directly comparable with the phosphohydrolase activities determined at 37°C. At 37°C, microsomal uptake of glucose 6-phosphate occurred too quickly to be determined accurately.

Fig. 2. Dependence of the microsomal glucose-6-phosphate translocase activity on incubation time (A) and the amount of enzyme protein (B). In A, the translocase was assayed for various lengths of time using 1.0 mg/ml of microsomal protein. In B, the assay was conducted for 1 min using various amounts of microsomal protein. Other assay conditions were as described in text.
Inhibition of glucose-6-phosphate translocase by pyridoxal phosphate

In order to verify microsomal uptake of glucose 6-phosphate described above being due to the translocase component of microsomal glucose-6-phosphatase system, we have studied how the uptake is affected by pyridoxal phosphate. Pyridoxal phosphate has been shown by Gold and Widnell (1976) to inhibit microsomal glucose-6-phosphatase but not hexose-6-phosphate phosphohydrolase (Fig. 3A). Fig. 3B shows that microsomal uptake of glucose 6-phosphate as determined by the above membrane filter method is progressively inhibited by increasing concentrations of pyridoxal phosphate; the inhibition was even more striking than that of glucose-6-phosphatase shown in Fig. 3A. These results clearly show that microsomal uptake of glucose 6-phosphate described here is an obligatory step for glucose-6-phosphatase system. The protein responsible for this uptake should be designated as “glucose-6-phosphate translocase” since it is incapable of translocating mannose 6-phosphate (Igarashi et al. 1984), which is a good substrate for hexose-6-phosphate phosphohydrolase (Fig. 1).

Development alterations in glucose-6-phosphate translocase and hexose-6-phosphate phosphohydrolase

Hepatic glucose-6-phosphatase is an enzyme system, whose activity is almost negligible during most of the fetal life; a significant level of this enzyme system appears just around the birth and a precipitous rise occurs again on the initial few days of extrauteric life (Burch et al. 1963; Yeung et al. 1967). We have made a similar observation (Fig. 4). To obtain further insights into the nature of the developmental alterations in glucose-6-phosphatase, the activities of glucose-6-phosphate translocase and hexose-6-phosphate phosphohydrolase have been assayed independently as a function of age (day) of the rat. Fig. 4 shows that the
two activities are increased with liver development but in markedly different manner. The behavior of the phosphohydrolase activity was similar to that of the glucose-6-phosphatase activity; it was first detected around birth and rose sharply reaching a maximum at the fifth postnatal day which was almost 3 times the adult level; a gradual decline to the adult level then followed. The level of the translocase, on the other hand, rose rather steadily but slowly and was still lower than the adult level even at 3 weeks after the birth.

Fig. 4. Developmental alterations in the activities of glucose-6-phosphatase (○), hexose-6-phosphate phosphohydrolase (●) and glucose-6-phosphate translocase (▲).

Fig. 5. Effect of administration of streptozotocin on the activities of glucose-6-phosphatase (A), hexose-6-phosphate phosphohydrolase (B) and glucose-6-phosphate translocase (C). The values are mean of 3 experiments.
**Diabetic alterations**

Glucose-6-phosphatase is also known to respond to various nutritional and hormonal stimuli (Nordlie et al. 1968). Since the highest stimulation of glucose-6-phosphatase observed thus far occurs in animals rendered diabetic (Jakobsson and Dallner 1968), the effect of administration of streptozotocin on the activities of glucose-6-phosphate translocase and hexose-6-phosphate phosphohydrolase were studied. Fig. 5 (B and C) shows that while phosphohydrolase activity starts to rise within 24 hr from streptozotocin, translocase activity is unchanged for the first 24 hr then rapidly rises in the second 24 hr. It is of interest that here too, glucose-6-phosphatase activity (Fig. 5A) resembles phosphohydrolase activity but not translocase activity.

**Neoplastic alterations**

In rat liver, microsomal glucose-6-phosphatase is lost with the progress of hepatocarcinogenesis (Weber et al. 1964). Nothing is known, however, about the activities of glucose-6-phosphate translocase and hexose-6-phosphate phosphohydrolase in rat hepatomas. When AH-109A hepatoma was assayed, not only glucose-6-phosphatase activity but also the translocase and phosphohydrolase activities were almost negligible, thereby indicating that both of the two glucose-6-phosphatase components are responsible for the neoplastic disappearance of glucose-6-phosphatase activity. For all of these enzyme activities, there was little difference between the livers of male adult Donryu and Wistar rats.

**DISCUSSION**

Glucose-6-phosphatase is known to respond to a variety of physiological and pathological stimuli (Nordlie et al. 1968; Greengard 1971). In the present work, we have found a procedure, by which glucose-6-phosphate translocase, an obligatory component of microsomal glucose-6-phosphatase system, can be assayed with little interference by hexose-6-phosphate phosphohydrolase, another obligatory component for the system. This has enabled us to investigate the response of the two activities to various stimuli independently. However, the assay conditions are so remote that only qualitative comparison appears to be feasible.

When translocase and phosphohydrolase activities were independently assayed on microsomes from developing and diabetic livers, the two activities were found to undergo alterations but not necessarily in parallel. For instance, the level of phosphohydrolase at the fifth postnatal day was 2.8 times that in adult animals, whereas the level of translocase at the same day was much lower (less than 50%) than the adult level (Fig. 4). The activity of phosphohydrolase was found to be much increased when it was determined at 24 hr from the administration of streptozotocin; at that time, however, the activity of translocase was not yet increased at all (Fig. 5).
The substrate specificity of microsomal glucose-6-phosphatase system is determined by glucose-6-phosphate translocase. Thus mannose 6-phosphate and glucosamine 6-phosphate cannot be hydrolyzed by intact microsomes. It is, however, still uncertain which of the two components, translocase and phosphohydrolase, constitutes the rate-limiting step of microsomal glucose-6-phosphatase system. Zoccoli et al. (1982) took the glucose-6-phosphatase activity of intact microsomes as a measure of the translocase activity. These authors as well as Arion et al. (1980) stressed that the translocase reaction is the rate limiting step. In our hands, however, the difference in glucose-6-phosphatase activity between intact and disrupted microsomes (Fig. 1) was too small to verify their conclusion. Moreover, Figs. 4 and 5 demonstrate that the developmental and diabetic profiles of microsomal glucose-6-phosphatase activity closely resemble the corresponding profiles of phosphohydrolase activity but are rather remote from the profiles of translocase activity. It seems therefore likely that at least under the conditions described, intracisternal hydrolysis of glucose 6-phosphate rather than its transport into the cisternal space may be the rate-limiting step for microsomal glucose-6-phosphatase system. Obviously, however, more studies including the solubilization and purification of the two enzyme components are needed to fully elucidate their regulatory mechanisms.

Acknowledgments

We thank Mr. K. Konno and Mrs. C. Ito for their skilful assistance.

References


