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A DIRECT EVIDENCE FOR DEFECT IN GLUCOSE-6-PHOSPHATE TRANSPORT SYSTEM IN HEPATIC MICROSOMAL MEMBRANE OF GLYCOGEN STORAGE DISEASE TYPE IB

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SUMMARY: Uptake of glucose-6-phosphate by microsomes of hepatocyte in rats, human controls and patients with glycogen storage disease type Ia and Ib was studied. In rat the uptake of glucose-6-phosphate increased rapidly and reached to a plateau, but mannose-6-phosphate was not accumulated. These findings indicate that a glucose-6-phosphate specific transport system exists in the microsomal membrane. In human controls and patients with glycogen storage disease type Ia the uptake of glucose-6-phosphate was clearly observed. On the other hand, no accumulation of it was detected in a patient with glycogen storage disease type Ib. These data provide a direct evidence of the defect in the glucose-6-phosphate transport system of hepatic microsomal membrane in glycogen storage disease type Ib.

In 1978 we reported the possibility that the pathogenesis of glycogen storage disease type Ib (GSD Ib) was a defect in glucose-6-phosphate (G6P) transport system in hepatic microsomal membrane from the analysis of glucose-6-phosphate phosphohydrolase (G6Pase, E.C.3.1.3.9.) activity in liver homogenates treated with or without detergent (1,2). Subsequently several reports have been published to support our study (3-5). Also we reported other two cases, the one was a brother of first case and the other seemed to be the first case of a partial defect in G6P transport system (6,7). However, there has been no report that clearly demonstrated the existence of microsomal G6P trans-

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port system by direct method which measures the uptake of G6P by microsomes even in animal experiments. Under these circumstances, we developed a method to investigate the uptake of G6P by hepatic microsomes.

The purpose of this paper is to clarify the pathogenesis of GSD Ib by investigating the uptake of G6P by hepatic microsomes of rats, human controls and patients with GSD Ia and Ib.

MATERIALS AND METHODS

Case The patient Y.M. was an 8-month-old boy, whose parents were first cousins. He was born after an uneventful pregnancy and full term normal delivery. At 2-month-old of age hepatomegaly was noticed. Therefore he was admitted to Shinshu University Hospital for further examinations of hepatomegaly. On admission his liver was 5 cm palpable below the right costal margin. The fasting blood glucose level was 42 mg/dl. The laboratory findings revealed hyperlactic acidemia, hyperlipidemia, hyperuric acidemia, elevation of GOT and GPT and marked granurocytopenia. Furthermore, the result of glucose or glucagon loading tests suggested GSD Ib. At 8-month-old of age, open liver biopsy was performed to confirm the diagnosis. The glycogen content in liver tissue was 10.8% of wet weight. Table 1 summarizes the results of G6Pase activity and pyrophosphate phosphohydrolase activity in freshly prepared liver specimens from patient Y.M. and two controls by the method of Narisawa et al. (7). From these data, he was finally diagnosed GSD Ib.

Measurement of G6P and M6P uptake by hepatic microsomes Liver specimens were obtained from patient Y.M., two patients with GSD Ia and 3 controls with informed consent from patients or their parents. In animal experiments, albino Wister rats weighing approximately 250 g were fasted for 48 h before sacrifice. Liver microsomes were prepared as described by Kamath (8).

Incubation medium (final volume 0.5 ml) contained 40 mM cacodylate buffer, pH 6.5, 1 mM G6P or mannose-6-phosphate (M6P) and microsomes (about 200 μg of protein). As a tracer, 0.5 μCi of [^{14}C] G6P (NEN, Boston) or [^{14}C] M6P (NEN, Boston, specially ordered) was used, respectively.

The incubation mixture was kept at 30°C. The reaction was stopped by diluting with 20-fold excess of cold 0.25 M sucrose solution supplemented with 50 mM Tris-Cl buffer (pH 7.5), 25 mM KCl, 5 mM $MgCl_2$ and 8 mM $CaCl_2$ at any time from 0 to 5 min. The microsomes were immediately collected on a washed membrane filter (TOYO ROSHI Co., LTD., Type TM-80P 24 mm in diameter, Tokyo) and washed twice with 5 ml of the above sucrose solutions. The filter membrane was dried at room temperature. The radioactivity on filter membrane was counted by liquid scintillation counter (Intertec, SL30). Protein assay on a filter membrane was done by Lowry method (9) after the same procedure without tracer. Recovery rate of protein on membrane filter was approximately 70%. All experiments were made duplicated. The uptake at each time was estimated after subtracting the count of 0 time from that of each time and expressed as nmoles/mg protein.

Table 1 Activities of glucose-6-phosphate phosphohydrolase and pyrophosphate phosphohydrolase (μmoles/h/mg protein) of fresh liver from patient Y.M. and controls

	Intact microsomes[1]	Disrupted homogenate	Latency (%)[2]
Glucose-6-phosphate phosphohydrolase			
Patient Y.M.	0.02	5.06	99.6
Control 1	2.12	3.21	34.0
Control 2	2.65	4.19	36.8
Pyrophosphate phosphohydrolase			
Patient Y.M.	2.25	7.10	68.3
Control 1	1.44	5.63	74.4
Control 2	2.16	7.40	70.8

1. Calculated using the equation of Arion et al. (1980)

2. Latency is defined as

$$\left[1 - \frac{\text{activity in intact microsome}}{\text{activity in disrupted homogenate}}\right] \times 100$$

RESULTS

At first the G6P and M6P uptake by microsomes of rat hepatocyte was investigated (Fig. 1). When 1 mM of G6P was added to the incubation mixture, the uptake was rapidly and linearly increased until 90 seconds. Then it reached to a plateau. On the other hand, the uptake of M6P was almost negligible at any time from 1 min to 5 min.

The results on human hepatic microsomes were shown in Fig. 2. As regard to controls and patients with GSD Ia the uptake of G6P by microsomes was observed significantly, although the uptake in patients with GSD Ia was lower than that in controls. On the contrary, the patient with GSD Ib showed negligible uptake of G6P. Also the uptake of M6P in controls was not detected (result was not shown).

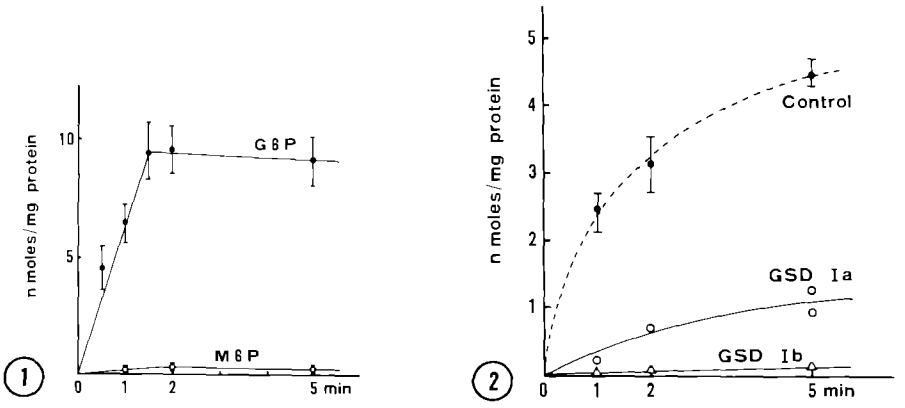

Fig. 1 G6P and M6P uptake by hepatic microsomes of rats. Incubation medium (pH 6.5) contained 40 mM cacodylate buffer, 200 μg of microsomal protein, either 1 mM of G6P or M6P and 0.5 μCi of [^{14}C] G6P or [^{14}C] M6P, respectively. Data are shown as mean ± SD (n=8).

Fig. 2 G6P uptake by hepatic microsomes of human controls and patients with GSD Ia and Ib. Incubation medium (pH 6.5) contained 40 mM cacodylate buffer, 200 μg of microsomal protein, 1 mM G6P and 0.5 μCi [^{14}C] G6P. Controls are shown as mean ± SE (n=3). Data of GSD Ia include 2 patients of GSD Ia. GSD Ib is the patient Y.M..

DISCUSSION

The existence of transport system specific for G6P in hepatic microsomal membrane was strongly speculated by Arion et al. in the study on G6Pase activating system (10,11). Furthermore, the possibility of the defect on this transport system was shown as the pathogenesis of GSD Ib by us (1,2). However, there has been no clear evidence that directly demonstrated the existence of G6P transport system even in animal experiments. Under these circumstances, we developed a membrane filter method to investigate the direct uptake of G6P by microsomes. The uptake of G6P by hepatic microsomes in rats was rapidly and linearly increased and reached to the plateau, as shown in Fig. 1. In the other hand, M6P was not accumurated in microsomes at any time through 5 minutes. These findings indicate that the G6P specific transport system exists in microsomal membrane of rat liver and M6P is not the substrate of this transport system. In case of human (Fig. 2), G6P uptake by hepatic microsomes was

observed in controls and patients with GSD Ia. On the other hand, hepatic microsomes of the patient with GSD Ib did not accumurate G6P. In human controls no accumuration of M6P was observed in hepatic microsomes. From these data, we concluded that the G6P specific transport system exists in human hepatic microsomal membrane and GSD Ib is due to a defect of this G6P transport system.

The reason why the uptake of G6P in patients with GSD Ia was lower than that of controls may be due to that in GSD Ia microsomes are already filled with G6P because of defect in G6Pase. Indeed, in preliminary experiment, the content of G6P in liver tissue of patient with GSD Ia was found to be high as compared with that of controls (unpublished data).

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