Conformational Change of the Catalytic Subunit of Glucose-6-phosphatase in Rat Liver during the Fetal-to-Neonatal Transition*

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The glucose-6-phosphatase system was investigated in fetal rat liver microsomal vesicles. Several observations indicate that the orientation of the catalytic subunit is different in the fetal liver in comparison with the adult form: (i) the phosphohydrolase activity was not latent using glucose-6-phosphate as substrate, and in the case of other phosphoesters it was less latent; (ii) the intravesicular accumulation of glucose upon glucose-6-phosphate hydrolysis was lower; (iii) the size of the intravesicular glucose-6-phosphate pool was independent of the glucose-6-phosphatase activities; (iv) antibody against the loop containing the proposed catalytic site of the enzyme inhibited the phosphohydrolase activity in fetal but not in adult rat liver microsomes. Glucose-6-phosphate, phosphate, and glucose uptake could be detected by both light scattering and/or rapid filtration method in fetal liver microsomes; however, the intravesicular glucose-6-phosphate and glucose accessible spaces were proportionally smaller than in adult rat liver microsomes. These data demonstrate that the components of the glucose-6-phosphatase system are already present, although to a lower extent, in fetal liver, but they are functionally uncoupled by the extravesicular orientation of the catalytic subunit.

Hepatic glucose-6-phosphatase system catalyzing the last step of both gluconeogenesis and glycogenolysis plays a pivotal role in the maintenance and regulation of blood glucose levels (1, 2). Accordingly, a huge increase in the activity of the enzyme can be observed after birth (3, 4). This developmental change forms the liver to be a gluconeogenic organ and serves the adaptation of the organism to the feeding-starvation cycles in the postnatal life. The proper timing of this event is of vital importance; in several cases very low glucose-6-phosphatase activities could be detected in the liver of newborns died in sudden infant death syndrome (5); also, the mean activity of the enzyme could be observed in nongluconeogenic organs and tissues, including fetal liver. Diminished latency, altered substrate specificity, different kinetic properties, or unusual lability of the enzyme could be observed in nongluconeogenic organs expressing glucose-6-phosphatase (17), esophagus (18, 19), adult adrenal (20), pancreatic β-cells (21), skeletal muscle (22), astrocytes (23), etc. Moreover, even the enzyme from the nuclear envelope of adult hepatocytes (24) or the microsomal enzyme prepared from glucocorticoid-treated animals (3, 25) exhibited moderate latency. A recent study indicated that the intravesicular glucose accumulation upon glucose-6-phosphate hydrolysis, a characteristic feature of adult liver microsomes, was absent in microsomes from newborn mice (25). These observations altogether clearly show that the arrangement of the glucose-6-phosphatase system as it can be seen in the endoplasmic reticulum of the adult liver is a special situation supported by developmental and local factors. Therefore, experiments were undertaken to investigate the presence and the characteristics of the phosphohydrolase and the related transport activities in fetal rat liver microsomes and their change around the birth.

EXPERIMENTAL PROCEDURES

Preparation of Rat Liver Microsomes—Fetal rat liver microsomes from 18-day-old fetuses and adult rat liver microsomes from male Sprague-Dawley rats (180–230 g) were prepared according to Ref. 26. Microsomal fractions were resuspended in a buffer (buffer A) contain-
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TABLE I

| Peptide | Sequence from the human glucose-6-phosphatase protein | Residue |
|---------|------------------------------------------------------|---------|
| A       | CSHIHSYIANNLSLKKY                                  | 196–210 |
| B (N-terminal) | CMMVLDIFGQSTHY                                      | 5–18   |
| C (C-terminal)   | CLAQVGLQPHKKSLL                                    | 345–357 |
| D       | CLSRILYAAHFPHQ                                     | 168–181 |

ing: 100 mM KCl, 20 mM NaCl, 1 mM MgCl₂, 20 mM Mops, pH 7.2. The suspensions were rapidly frozen and maintained under liquid nitrogen until used. Intactness of microsomal vesicles was checked by investigating osmotically induced changes in microsomal vesicle size upon the addition of the nonpermeant compound sucrose by the light scattering technique. Microsomes used in this study showed a sustained shrinking upon sucrose addition (see Fig. 1). Microsomal protein concentrations were determined by biuret reaction using bovine serum albumin as standard.

Uptake Measurements—Liver microsomes (1 mg protein/ml) were incubated in buffer A containing 0.2–30 mM glucose-6-phosphate plus [¹⁴C]glucose-6-phosphate (8–10 μCi/ml) or 0.2 mM glucose-1-phosphate plus [¹⁴C]glucose-1-phosphate (8–10 μCi/ml) or 1 mM glucose plus [³H]glucose (9 μCi/ml) or 1 mM sucrose plus [¹⁴C]sucrose (1 μCi/ml) at 22 °C. In a series of incubations, the pore-forming antibiotic alamethicin (0.05 mg/ml) was added to distinguish the intravesicular and the extravesicular compartment.

Results:

Transport of Glucose-6-phosphate, Glucose, and Phosphate in Fetal Rat Liver Microsomal Vesicles—The intactness of the microsomal vesicles and their permeability toward the substrates and products of the glucose-6-phosphatase system was investigated by the light scattering technique. Both fetal and adult microsomes were practically impermeable to glucose, sucrose, which excluded the possibility of the nonspecifically increased permeability. Glucose-6-phosphate was able to enter both types of microsomal vesicles, but the rate of entry was somewhat lower in the fetal microsomes. No major differences could be observed in the permeation of glucose and phosphate (Fig. 1). Other hexose phosphates, which are the substrates of the catalytic subunit, did not permeate the microsomal membrane, their addition caused a prolonged shrinking both in adult and fetal microsomes with the exception of glucose-1-phosphate, which was slowly permeable only in fetal vesicles (Table II).

Transport activities were also assessed using radiolabeled compounds and a rapid filtration method. Fetal rat liver microsomes incubated in the presence of various concentrations of glucose-6-phosphate (plus [¹⁴C]glucose-6-phosphate as a tracer) rapidly accumulated glucose-6-phosphate + glucose reaching a steady-state level in 2 min. More than 90% of the radioactivity associated to the microsomes was releasable by the pore-forming alamethicin, indicating the intravesicular accumulation. In contrast to adult microsomes, fetal vesicles did not accumulate the isotope over the equilibrium. Separate measurement of glucose-6-phosphatase and glucose revealed that both compounds were present in the intraluminal space of the organelle; however, the rate of the intravesicular glucose was very low compared with that of adult microsomes and hardly reached the detection limit (Table III). Upon glucose-1-phosphate addition (which is a poor substrate of the phosphohydrolase, see Table II), the difference in the intravesicular isotope accumulation was much less expressive between fetal and adult microsomes, due to the very low glucose production (Table III).

1 The abbreviation used is: Mops, 4-morpholinepropanesulfonic acid.
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The uptake was attributable to intravesicular accumulation, because the majority of radioactivity (>90%) could be released by permeabilizing the vesicles with the pore-forming alamethicin. The steady-state level of intravesicular glucose was stable for at least 2 h. In the steady-state phase of glucose uptake, the apparent intravesicular glucose space, which was calculated by dividing the intravesicular glucose content (nmol/mg protein) with the extravesicular glucose concentration (nmol/μL), was 0.23 ± 0.04 (μL/mg protein) at 1 mM glucose concentration. This apparent space was about 50% of that observed in adult rat liver microsomes by using the same method (Table III).

Because the intravesicular glucose-6-phosphate, glucose-1-phosphate, and glucose accessible spaces of fetal liver microsomes were lower than the corresponding maximal values obtained in adult microsomes, we have estimated the total intravesicular space measuring the sucrose accessible space of microsomes. Sucrose, a poorly permeant compound, reached a steady-state level of uptake at 2 h of incubation. The value of the intravesicular sucrose space was also 2-fold higher in adult rat liver microsomes (Table III).

Glucose-6-phosphatase Activity in Fetal Rat Liver Microsomal Vesicles—The total phosphohydrolase activity in fetal microsomes was less than 5% of the adult value (Tables II and IV). According to Western blot analysis, the amount of the enzyme protein was less than in adult microsomes, yet it was clearly detectable (Fig. 4). The latency of the enzyme was almost absent; permeabilization of the vesicles elevated the activity slightly, whereas in adult microsomes more than 50% of the activity was latent (Table II). The difference in glucose-6-phosphatase activities obtained by the addition of various concentrations of glucose-6-phosphate did not affect the extent of the intravesicular glucose-6-phosphate accessible space (Fig. 2).

Hydrolysis of other phosphoesters (10 mM) by fetal microsomes showed diminished latency compared with adult microsomes (Table II). Very moderate latency was observed in the case of glucose-1-phosphate and fructose-6-phosphate, whereas the phosphohydrolase activity toward mannose-6-phosphate, deoxylucose-6-phosphate, and glucosamine-6-phosphate remained latent in two-thirds. The eventual conversion of the hexose phosphates to glucose-6-phosphate during the incubation (which has been reported in brain microsomes, for example; Ref. 23) was checked by measuring the glucose-6-phosphate content at 0 and 30 min of incubation. We have found low glucose-6-phosphate contents, presumably due to the impurity of chemicals. The highest value, in the case of fructose-6-phosphate, was 0.04 mM at the start of incubation (0.4% of the added fructose-6-phosphate) and 0.07 mM at 30 min. Other incubates contained less than 0.01 mM glucose-6-phosphate, a value that did not increase during the incubation (data not shown).

Inhibition of the Phosphohydrolase Activity by Antibodies Against Different Sections of Glucose-6-phosphatase—Native fetal and adult microsomal vesicles were preincubated in the presence of antibodies raised against different sections of glucose-6-phosphatase: the N- and C-terminal parts, an external loop, and an internal loop containing the His residue that belongs to the active site of the enzyme. After 50 min of preincubation 10 mM glucose-6-phosphate was added, and the glucose-6-phosphatase activities were measured. In adult microsomes neither of the antibodies affected the phosphohydrolase activity. On the contrary, in fetal microsomes antibodies A and D, raised against two loops supposed to be orientated toward the cytosol and the lumen in adult liver, strongly inhibited the enzyme (Table IV).

DISCUSSION

The results of the present study demonstrate that the components of the hepatic glucose-6-phosphatase system are already present in the fetal liver, although at a lower extent. In

![Fig. 1. Influx of glucose-6-phosphate, glucose, and phosphate into liver microsomal vesicles evaluated by a light scattering technique.](image)
Microsomal vesicles (1 mg protein/ml) were incubated in buffer A in the presence of various phosphoesters (10 mM) for 30 min (fetal) or 5 min (adult) at room temperature. Phosphate production was measured. Permeabilized microsomes were treated with 50 μg/ml alamethicin. Data are the means ± S.D. of three to six experiments. The influx rates of the phosphoesters (25 mM) into microsomal vesicles expressed as half-time of the light scattering signal are also shown (n = 2–5).

### Table II

**Uptake and hydrolysis of glucose-6-phosphate in fetal rat liver microsomal vesicles**

Lever microsomes (1 mg/ml) were incubated in buffer A containing 0.2 mM glucose-6-phosphate (in some experiments glucose-1-phosphate) plus [14C]glucose-6-phosphate (5–10 μCi/ml). In a series of incubations the pore-forming antibiotic alamethicin (0.05 mg/ml) was added to distinguish the intravesicular and the bound radioactivity. The alamethicin releasable (i.e., intravesicular) portion of radioactivity is shown. At the steady-state level of uptake (5–10 min), samples (0.1 ml) were rapidly filtered and washed; the intravesicularly accumulated glucose deriving from glucose-6-phosphate (glucose-1-phosphate) hydrolysis during the measurement of glucose-6-phosphate uptake was measured parallelly as described under “Experimental Procedures.” For the measurement of intravesicular glucose and sucrose spaces, 1 mM glucose plus [3H]glucose (9 mCi/ml) or 1 mM sucrose plus [14C]glucose (1 μCi/ml) were incubated with microsomal vesicles for 2 h at 22 °C. At the end of the incubation the intravesicular radioactivity was assessed as described for glucose-6-phosphate. Apparent intravesicular isotope spaces were calculated according to the formula: apparent intravesicular space (nmol/mg protein) = intravesicular accumulation (nmol/mg protein)/concentration of the added compound (nmol/ml). The same measurements were also executed on adult rat liver microsomes. The abbreviations used in the table are: G6P, glucose-6-phosphate; G1P, glucose-1-phosphate; NM, not measurable.

| Hexose phosphates | Fetal rat liver microsomes | Adult rat liver microsomes |
|-------------------|---------------------------|---------------------------|
|                   | Phosphohydrolase activity | Latency | T1/2 of influx |
|                   | Native | Permeabilized | | nmol/min/mg protein | % | s | nmol/min/mg protein | % | s |
| Glucose-6-phosphate | 13.2 ± 1.6 | 13.2 ± 1.6 | 10 | 28 ± 5 | 149 ± 14 | 303 ± 18 | 51 | 17 ± 3 |
| Glucose-1-phosphate | 2.9 ± 0.1 | 3.1 ± 0.1 | 5 | 57 ± 13 | 1.9 ± 0.6 | 45 ± 4 | 96 | >300 |
| Mannose-6-phosphate | 4.4 ± 0.2 | 14.2 ± 1.8 | 69 | >300 | 13.4 ± 0.4 | 279 ± 15 | 95 | >300 |
| Fructose-6-phosphate | 4.8 ± 0.1 | 14.2 ± 0.4 | 66 | >300 | 25.7 ± 1.9 | 266 ± 16 | 90 | >300 |
| Glucosamine-6-phosphate | 5.8 ± 1.1 | 18.0 ± 2.8 | 67 | >300 | 19.5 ± 1.0 | 327 ± 20 | 94 | >300 |

### Table III

**Intravesicular accumulation upon addition of 0.2 mM glucose-6-phosphate**

| Hexose phosphates | Fetal rat liver microsomes | Adult rat liver microsomes |
|-------------------|---------------------------|---------------------------|
|                   | nmol/mg protein | nmol/mg protein |
| G6P + glucose | 0.16 ± 0.03 (5) | 1.73 ± 0.13 (4)* |
| G6P | 0.11 ± 0.03 (5) | 0.33 ± 0.09 (9)* |
| Glucose | 0.046 ± 0.035 (5) | 1.40 ± 0.10 (4)* |

| Hexose phosphates | Fetal rat liver microsomes | Adult rat liver microsomes |
|-------------------|---------------------------|---------------------------|
|                   | nmol/mg protein | nmol/mg protein |
| G1P + glucose | 0.13 ± 0.01 (3) | 0.30 ± 0.07 (3) |
| Glucose | NM | NM |

### Table IV

**Calculated intravesicular isotope spaces**

| Hexose phosphates | Fetal rat liver microsomes | Adult rat liver microsomes |
|-------------------|---------------------------|---------------------------|
|                   | µmol/mg protein | µmol/mg protein |
| G6P + glucose | 0.80 ± 0.15 (5) | 8.65 ± 0.65 (4)* |
| G6P | 0.57 ± 0.15 (5) | 1.65 ± 0.45 (4)* |
| G1P | 0.65 ± 0.05 (3) | 1.50 ± 0.35 (3) |
| Sucrose | 1.23 ± 0.13 (3) | 2.83 ± 0.24 (3) |
| Glucose | 0.24 ± 0.04 (4) | 0.43 ± 0.09 (6)* |

* Data taken from Ref. 12.

a Data taken from Ref. 40.

accordance with earlier studies the catalytic subunit of the enzyme can be demonstrated by Western blotting, although the microsomal phosphohydrolyrase activity in fetal liver is less than the 5% of the adult one. The transporters belonging to the system (T1, T2, and T3) are also present, because microsomal transport of glucose-6-phosphate, glucose, and phosphate can also be detected by uptake measurements. However, the intraluminal glucose-6-phosphate and glucose spaces are 40–50% of the values observed in adult rat liver microsomal vesicles. These alterations might suggest that the corresponding transporters are present in a lower percentage of fetal microsomal vesicles. More probably, they can reflect the differences in the protein content and/or vesicle size between fetal and adult microsomal preparations. This latter possibility is supported by the facts that (i) the light scattering behavior of fetal and adult microsomes is similar in the presence of glucose or glucose-6-phosphate and (ii) the intravesicular sucrose space, which is nearly as extensive as the intravesicular water space (32), is proportionally lower in fetal microsomes.
agitation. The incubations were started with the addition of 10 mM glucose (1% of the incubation volume) for 50 min at room temperature under continuous agitation in the presence of each of the four anti-peptide antibodies. The phosphohydrolase activity was detected by monitoring the decrease in extravesicular glucose-6-phosphate. The phosphohydrolase activity was detected by measuring glucose or phosphate production. Data are the means ± S.D. of four experiments.

**Table IV**

**Effect of antibodies against various sections of glucose-6-phosphatase on its catalytic activity in fetal and adult rat liver microsomes**

Native microsomes (0.35 mg protein/ml) were preincubated buffer A in the presence of each of the four anti-peptide antibodies (1% of the incubation volume) for 50 min at room temperature under continuous agitation. The incubations were started with the addition of 10 mM glucose-6-phosphate. The phosphohydrolase activity was detected by measuring glucose or phosphate production. Data are the means ± S.D. of 4–9 measurements.

| Antibodies | Glucose-6-phosphatase activity (nmol/min/mg protein) | Fetal rat liver microsomes |
|------------|----------------------------------------------------|-----------------------------|
| None       | 259 ± 5                                            | 4.9 ± 0.5                   |
| A          | 260 ± 8                                            | 2.9 ± 0.9*                  |
| B          | 278 ± 10                                           | 4.7 ± 0.5                   |
| C          | 280 ± 16                                           | 5.1 ± 0.6                   |
| D          | 274 ± 9                                            | 2.8 ± 0.7*                  |

*Significantly different from control, *p* < 0.05.

**Fig. 3. Glucose uptake by fetal rat liver microsomal vesicles.** Microsomes were incubated in the presence of 1 mM glucose plus [3H]glucose as tracer and intravesicular glucose contents were measured as detailed under “Experimental Procedures.” The data are the means ± S.D. of four experiments.

**Fig. 4. Immunoblot analysis of fetal and adult rat liver microsomes.** 5 and 10 µg (adult, lanes 1 and 2) or 10 µg (fetal; lane 3) of microsomal protein was loaded. The arrow indicates a 38-kDa molecular mass.

Beyond the quantitative differences, fetal liver microsomes also differ qualitatively. Permeabilization of the microsomal vesicles by alamethicin slightly increases the phosphohydrolase activity, i.e., the latency of the enzyme is minimal. Using other substrates than glucose-6-phosphate, we have also observed reduced latency that is accompanied with a reduced substrate specificity. The intravesicular accumulation of glucose due to glucose-6-phosphate hydrolysis is hardly measurable, in accordance with the observation of Annabi and van de Werve (25). The intravesicular glucose-6-phosphate space, which is in inverse ratio to the extravesicular glucose-6-phosphate concentration and the subsequent glucose-6-phosphatase activity in adult rat liver microsomes (12), is stable independently of extravesicular glucose-6-phosphate concentrations. It suggests that in fetal rat liver microsomes glucose-6-phosphatase does not utilize an intravesicular substrate pool.

The differences between fetal and adult glucose-6-phosphatase system can be explained in several ways. The nonspecific increase of membrane permeability in fetal microsomal vesicles is out of the question on the basis of normal sucrose response in light scattering and the presence of intravesicular pools. The altered (diminished) specificity of glucose-6-phosphate transporter and/or the presence of other transporters responsible for the permeation of other substrates may also be supposed. However, other hexose-6-phosphates are not permeable on the basis of light scattering experiments. Furthermore, the altered balance of transport and hydrolysis, i.e., the very low phosphohydrolase activity together with the quasi normal transport processes, may cease the rate-limiting property of glucose-6-phosphate transport, which is characteristic of adult microsomes. This explanation can account for the diminished latency of the system in the presence of glucose-6-phosphate and the low intravesicular glucose accumulation upon glucose-6-phosphate hydrolysis. In itself it is not enough to explain the decreased latency of phosphohydrolase toward other phosphohydrases. On the basis of the results we suppose that the changed orientation of the proposed active site of glucose-6-phosphatase can be responsible for the altered features of glucose-6-phosphatase system in fetal rat liver microsomes. This feature of the enzyme is not without precedents: multiple topological orientations linked with different functions has been reported in the case of other membrane proteins (34–36). The premise of the presence of a phosphohydrolase oriented with its catalytic site toward the cytosolic surface of the endoplasmic reticulum membrane is compatible with the reduced latency, the relative substrate specificity in native microsomes, the phosphohydrolase-independent intravesicular glucose-6-phosphate pool, and the small intravesicular glucose accumulation.

The inhibitory effect of an antibody against the loop containing the proposed active site of the enzyme, which was present only in the fetal microsomal vesicles, strongly supports this hypothesis. Because glucose-6-phosphate transport is present in fetal microsomes, it means that the transport and hydrolysis are at least partially uncoupled. Due to the altered orientation, the enzyme can act as a rather nonspecific phosphohydrolase or, alternatively, can catalyze the inverse reaction (37), i.e., it may replace the glucokinase activity missing in the neonatal period (38).

It has been observed previously that the latency of the glucose-6-phosphatase system (and other microsomal enzymes with different time course; Ref. 39) increases rapidly around the birth. It may be caused by the change of orientation of the enzyme or by the replacement of the fetal isozyme with an adult form. In the first case it can be supposed that the folding of the protein converts it into a more active, more specific, intralumenally oriented form. The activation of preformed proteins by folding together with the increased transcription of glucose-6-phosphatase gene may serve the rapid accommodation to the new gluconeogenic/glycogenolytic role of the liver after the birth.

**REFERENCES**

1. Nordlie, R. C., and Sukalski, K. A. (1985) in *The Enzymes of Biological Membranes* (Martonosi, A. N., ed) pp. 349–398, Plenum Press, New York
2. Chen, Y. T., and Burchell, A. (1995) in *The Metabolic Basis of Inherited Disease* (Scriver, C. R., Beaudet, A. L., Sly, S. W., and Valle, D., eds) pp. 953–965, McGraw-Hill, New York
3. Burchell, A., and Leakey, J. E. A. (1988) *Biol. Neonate* 54, 107–115
4. Burchell, A., Gibb, L., Waddell, I. D., Giles, M., and Hume, R. (1990) *Clin. Chem.* 36, 1635–1637
5. Burchell, A., Bell, J. E., Busuttil, A., and Hume, R. (1989) *Lancet* 2, 291–294
6. Hume, R., and Burchell, A. (1993) *Arch. Dis. Child.* 68, 202–204
7. Girard, J., and Pegrario, J.-P. (1998) *Biochem. Soc. Trans.* 26, 69–74
