Accessibility of glucose 6-phosphate: phosphohydrolase to antibody attack in modified microsomal vesicles

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Received 25 May 1982

Glucose 6-phosphatase *Microsomal membrane* *Detergent* *Sonication* *Antibodies*

1. INTRODUCTION

Recent investigations supported the existence of 2 components of the endoplasmic reticulum participating in the process of glucose 6-phosphate hydrolysis: the glucose 6-phosphate-specific transporter that mediates the movement of the substrate from the cytoplasmic membrane surface into the lumen and the unspecific phosphohydrolase on the luminal side of the membrane [1,2]. However, this model has not been generally accepted; especially the proposed molecular arrangement of the glucose 6-phosphatase components within the membrane is contradictory [3–7]. Furthermore, immunological studies [6] have suggested that the glucose 6-phosphate: phosphohydrolase is presumably not freely accessible on the luminal surface which, however, is one of the prerequisites of the substrate-transport hypothesis as described in [1,2]. Therefore, we have reinvestigated the transverse topology of the glucose 6-phosphatase by detailed immunological studies on detergent-modified and mechanically disrupted microsomes. These findings support our preliminary concept and demonstrate that, indeed, the glucose 6-phosphate:phosphohydrolase is not attached to the luminal membrane surface, but buried within the microsomal membrane.

Enzymes: glucose 6-phosphatase, EC 3.1.3.9; IDPase, EC 3.6.1.6.

2. MATERIALS AND METHODS

Microsomes were prepared as in [8] from livers of rats (female Wistar rats AF/Hannover, 180–200 g body wt), fasted for 15 h.

Antibodies against native rat liver microsomes were raised in 3-month-old male rabbits (yellow-silver strain) [6], isolated from the antiserum by chromatography on a DEAE–Sephadex A-50 column (2 × 40 cm) in 0.1 M sodium phosphate buffer (pH 7.4) [9] and purified by gel filtration on a Sephadex G-200 column (2.5 × 100 cm), using 0.1 M Tris–HCl (pH 7.4). Immunoglobulins, prepared from sera of non-immunized rabbits were used as experimental controls.

Ultrasonic treatment of microsomes (6 mg protein in 3 ml 0.1 M Tris–HCl (pH 7.4)) was performed with the micro-tip of a Branson sonicator (model S-125) at grade 3 for 5 *s* intervals in a thermostatically controlled chamber. To decrease the temperature from 5°C after each sonication, intervals of 55 s were used for cooling with methanol (0°C) [6].

Detergent-modified microsomal vesicles were prepared by treatment of microsomes (2 mg protein in 0.1 M Tris–HCl (pH 7.4)) with Triton X-114 for 10 min at 0°C, final detergent conc. of 0.03% or 0.05% (w/v) to give a Triton/microsomal protein ratio of 0.15 or 0.25 (w/w) in a final volume of 1.0 ml.

The specific enzyme activities (mU/mg protein) are expressed as P_i released and were measured at 15°C as described: glucose 6-phosphatase [10]; IDPase [11].

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Immunoelectrophoresis was performed as in [12]. Protein was assayed by the biuret method in [13].

All experiments were run ≥3 times with similar results of each. In the figures the results of 1 typical expt are shown.

3. RESULTS AND DISCUSSION

3.1. *Detergent-modified microsomal vesicles*

Reversible modifications within the hydrophobic part of the microsomal membranes including the formation of openings, undoubtedly occur when microsomes are exposed to low detergent concentrations [14–16]. In view of these observations, the question arises, whether detergent-induced openings in the membranes will be large enough to enable a passage of antibodies into the vesicular cavities where they can specifically interact with luminal exposed antigens.

Following a mild treatment of microsomal membranes by 0.05% (w/v) Triton X-114, microsomal IDPase which was found to be attached to the luminal surface [11] is partly released from the vesicular cavities (table 1). In contrast, under the same experimental conditions glucose 6-phosphatase completely remained bound to the detergent-modified membranes (table 1). When Triton-treatment is performed in the presence of saturated amounts of antibodies against native rat liver microsomes the inosine-5′-diphosphate hydrolysis is decreased up to 31% (fig.1a), indicating that the residual IDPase activity which remained bound to the luminal membrane side after treatment by Triton X-114, is unaffected. Consequently, a reaction between antibodies and microsomal antigens exposed on the inner surface of the detergent-modified membranes could not have taken place. In contrast, glucose 6-phosphate hydrolysis of detergent-modified microsomes in the presence of antibodies is completely

Table 1

Membrane-bound activity of glucose 6-phosphatase and IDPase in native, detergent-modified or sonicated microsomes

Microsome preparation	Membrane-bound act. (% total act.)	
	G-6-Pase	IDPase
Native	98.4 ± 1.5	97.1 ± 2.2
Detergent modified	96.4 ± 3.5	33.6 ± 4.7
Sonicated	96.2 ± 2.3	19.2 ± 2.0

Native, detergent-modified (0.05% (w/v) Triton X-114) or sonicated microsomes (6 mg protein in 3 ml 0.1 M Tris–HCl (pH 7.4), respectively) were centrifuged for 1 h at 105 000 × g_{max} (Spinco-ultracentrifuge, rotor 30); the enzyme activities were then determined in the pellet (membrane-bound activity) and supernatant. Data are means ± SEM ($n = 5$)

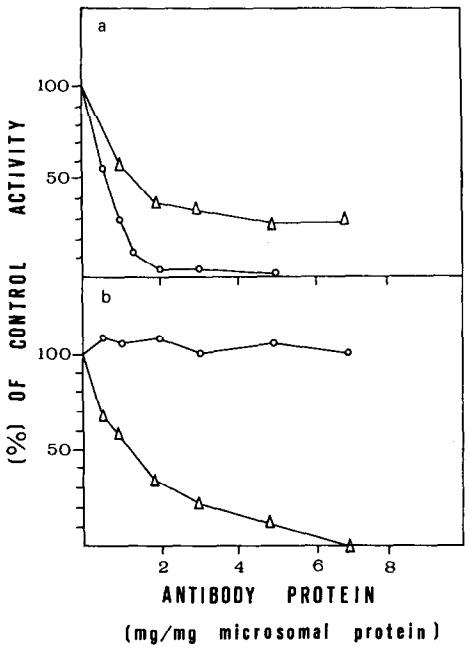

Fig.1. Effects of antibodies on glucose 6-phosphatase and IDPase activities in detergent-modified or sonicated microsomes. (a) Detergent-modified microsomes: Microsomes were modified by 0.05% (w/v) Triton X-114 as in section 2, then preincubated for 1 h at 30°C and 15 h at 0°C with increasing amounts of antibodies or immunoglobulins of non-immunized rabbits (controls). 100% activity corresponds to 120 (○) and 220 (△) mU/mg protein, respectively. The detergent-binding capacity of added antibodies and immunoglobulins was saturated before preincubation [6]. (b) Sonicated microsomes: Microsomes were sonicated as in section 2 in the presence of increasing amounts of antibodies or immunoglobulins of non-immunized rabbits (controls), then preincubated for 1 h at 30°C and 15 h at 0°C. 100% activity corresponds to 68 (○) and 588 (△) mU/mg protein:(○) Glucose 6-phosphatase: (△) IDPase.

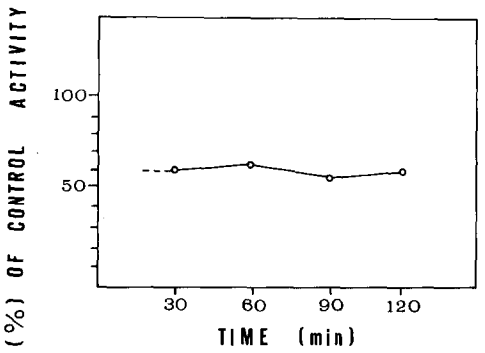

Fig.2. Effect of antibodies on glucose 6-phosphatase activity in microsomes, modified by 0.03% Triton X-114. Microsomes were modified by 0.03% (w/v) Triton X-114 as in section 2, then preincubated for the times indicated at 30°C and 15 h at 0°C with 14 mg protein of antibodies or immunoglobulins of non-immunized rabbits (controls), respectively. 100% activity corresponds to 75 mU/mg protein. The detergent-binding capacity of added antibodies and immunoglobulins was saturated before preincubation [6].

inhibited (fig.1a). Therefore, it would be imperative to assume that modifications of the microsomal membranes by low Triton concentrations which enables a passage of IDPase molecules (M_r 110 000, see fig.3b) out of but not of immunoglobulins (M_r 160 000) into the vesicles cavities, give rise to an accessibility of the glucose 6-phosphate:phosphohydrolase from the cytoplasmic membrane surface. This accessibility of the phosphohydrolase results in a complete inhibition of the enzyme when antibodies are present.

This presentation of the antibody attack on glucose 6-phosphatase in detergent-modified microsomes is supported by results obtained with microsomes pretreated with 0.03% (w/v) Triton X-114. Preincubation of these Triton-treated rat liver microsomes with saturated amounts of antibodies decreases the glucose 6-phosphate hydrolysis only up to 60%, compared to controls (fig.2). Prolonged preincubation, expected to favour a diffusion of antibodies through the openings of the detergent-modified membranes does not increase the rate of inhibition. Therefore, it seems more than likely that antibodies attack the glucose 6-phosphatase from the cytoplasmic surface of the detergent-modified membrane rather than from the luminal on is

3.2. *Mechanically disrupted microsomal vesicles*

In contrast to Triton X-114-treatment, resulting in reversible modifications within the membranes by incorporation of small amounts of detergent [14–16], sonication causes a simple but effective breakage of the vesicles [8,17,18]. As shown in table 1, ultrasonic treatment of microsomes results in a great loss of IDPase activity from the vesicular lumen; only ~20% remained bound to the membrane. The most striking finding, however, is that

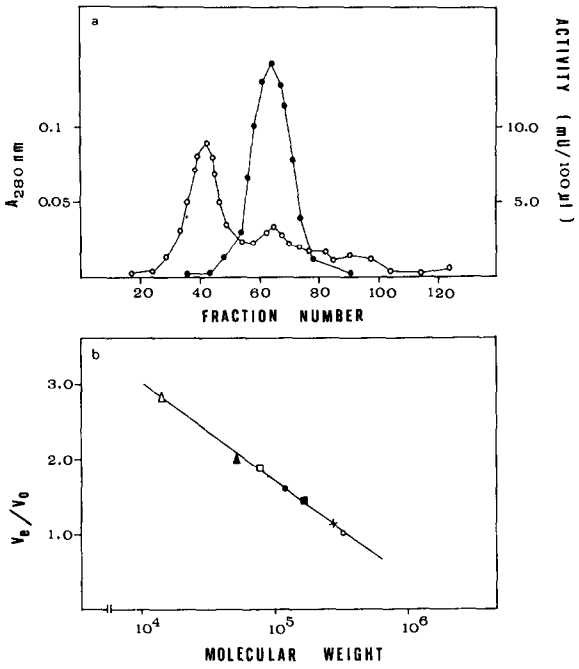

Fig.3. Gel filtration of the supernatant from sonicated microsomes. (a) Microsomes were sonicated as in section 2, centrifuged for 1 h at 105 000 × g_{max} (Spinco-ultracentrifuge, rotor 30) and the gel filtration of the supernatant (0.3 mg protein in 3.0 ml 0.1 M Tris–HCl, pH 7.4) was performed on a Sephadex G-200 column (2.5 × 100 cm, total vol. 441.6 ml, void volume 148.0 ml, fraction vol. 3.7 ml), using 0.1 M Tris–HCl (pH 7.4). The elution of the proteins (○) was monitored at 280 nm. The IDPase activity (●) was determined in 100 µl of each of the collected fractions at 37°C. (b) For the determination of M_r-values the column has been calibrated with (M_r): cytochrome *c* (13 500) (△); hexokinase dimer (50 000) (▲); bovine serum albumin (69 000) (□); immunoglobulin (160 000) (■); and aminopeptidase (276 000) (X). V_e, elution volume; V_o, void volume.

both membrane-bound and released IDPase activity are inhibited when sonication is performed in the presence of antibodies (fig.1b). Thus it should be assumed that mechanical disruption of microsomes by ultrasonic treatment enables an entry of antibodies into the vesicular cavities during the fragmentation process. Since glucose 6-phosphate hydrolysis is not inhibited under the same experimental conditions, it seems unlikely that the glucose 6-phosphate:phosphohydrolase is also accessible, attached on the inner side of the microsomal membranes, as postulated in [1,2].

Fig.3a shows the Sephadex G-200 elution profile of the supernatant obtained by centrifugation of sonicated microsomes at 105 000 × g_{max}. Measured at 280 nm, sonication results in a loss of high M_r proteins from the microsomal vesicles. The minor peak of protein, containing IDPase activity, was determined to be M_r 110 000 (fig.3b). The major protein peak, eluted in volumes corresponding to M_r 340 000 (fig.3b), contains plasma proteins which are generally accepted to be located also within the microsomal vesicles [19,20]. The plasma proteins are identified by immunoelectrophoretic analysis in fig.4. Two precipitin lines are observed in the reaction between the proteins of the major peak and antiserum from rabbit raised to rat plasma proteins. Furthermore, one of the precipitin lines corresponds to the precipitate developed in an immunoelectrophoretogram of the proteins from the major peak and antiserum raised to rat plasma C1 complement M_r found to be slightly >340 000 M_r [21].

These data demonstrate that IDPase and plasma proteins having a higher M_r-value than immunoglobulins are released from the vesicles. We interpret these results as additional evidence that, vice versa, antibodies should have opportunity to reach the inner membrane surface of the vesicles opened during prolonged sonication in the presence of immunoglobulins. Since, however, the hydrolysis of glucose 6-phosphate is not affected under the conditions used for the inhibition experiments, again, the phosphohydrolase cannot be exposed on the luminal surface of the microsomal membrane but is buried within the hydrophobic part of the bilayer, as suggested in [6] and expected for a phospholipid-dependent [6,22] integral membrane protein.

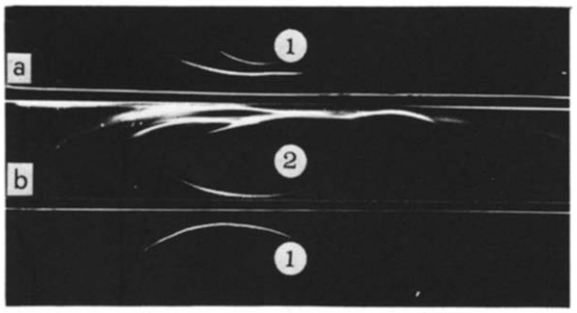

Fig.4. Immunoelectrophoretic analyses of proteins released by sonication of microsomes. Electrophoresis was run for 90 min at pH 8.2 in 1% (w/v) agarose, 7 mA/gel with (1) proteins from the major peak (10 μg/10 μl eluate) and (2) rat serum (600 μg/10 μl serum) in the wells. After electrophoresis antiserum (100 μl) was raised to rat plasma proteins (a) or C1 complement (b) from rabbits was placed in the trough.

ACKNOWLEDGEMENTS

The authors thank Mrs B. Junker and Mrs R. Kannler for their excellent technical assistance. This investigation was supported by the Deutsche Forschungsgemeinschaft (Schu 54/1 and Schu 54/7).

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