PRESENCE OF TWO DIFFERENT TYPES OF PROTEIN-DISULFIDE ISOMERASE ON CYTOPLASMIC AND LUMINAL SURFACES OF ENDOPLASMIC RETICULUM OF RAT LIVER CELLS

Hiroko Ohba, Tomoyuki Harano, and Tsuneo Omura

Department of Biology, Faculty of Science, Kyushu University, Fukuoka 812, Japan

Received May 20,1977

SUMMARY: The presence of two types of protein-disulfide isomerase in rat liver microsomes was demonstrated by their different susceptibility to detergent treatment. The activity detectable with intact microsomes was strongly inhibited by low concentrations of Triton X-100. Higher concentrations of the detergent caused significant stimulation of the enzyme activity, which was latent in intact microsomes.

The detergent-labile enzyme was purified from the acetone powder of microsomes. Judging from the effect of the antibody, which was prepared against the purified enzyme, on the enzyme activities of intact and detergent-treated microsomes, the detergent-labile enzyme is located on the cytoplasmic surface of endoplasmic reticulum, whereas another type of the enzyme, which is latent in intact microsomes, is located on the luminal surface.

Protein-disulfide isomerase activity in animal tissues is mainly associated with microsomes (1,2). The role of this enzyme in the folding of newly synthesized polypeptide chains to attain their specific conformations was suggested by Goldberger et al. (1).

In hepatic or pancreatic cells, where large amounts of secretory proteins are synthesized, the nascent peptides of secretory proteins are discharged from ribosomes into the lumen of endoplasmic reticulum, whereas other portion of nascent peptides are released into the cytoplasm to become cell constituents (3). Thus the folding of nascent peptides takes place in two different subcellular compartments, the lumen of endoplasmic reticulum and cytoplasm, which situation suggests the existence of protein-disulfide isomerase activity on both sides of endoplasmic reticulum membrane. However, the intramembranous distribution of microsomal protein-disulfide isomerase(s) has not been studied so far.

In this study, the presence of two different types of protein-disulfide

ISSN 0006-291X

isomerase in rat liver microsomes was revealed from their different susceptibility to Triton X-100. One of them was highly purified and its antibody was prepared. The different nature of these two protein-disulfide isomerases and their location on different sides of microsomal membrane were confirmed by the use of the antibody.

MATERIALS AND METHODS: Liver microsomes were prepared from adult male Sprague-Dawley rats as described previously (4). Protein-disulfide isomerase activity was assayed by the method of de Lorenzo et al (5) with some modifications. The reaction mixture contained 35 μg of randomly crossed RNase (5), 0.5 μmoles of EDTA, 0.05 μmoles of 2-mercaptoethanol, and enzyme in 0.5 ml of 0.2 M Tris-HCl (pH7.8). Incubation was carried out at 37°C for 15 minutes, and reaction was stopped by adding 0.05 ml of 0.1 M N-ethylmaleimide. Ten μl of the incubation mixture was taken out to assay the regenerated RNase activity by the method of Fletcher and Hash (6). One unit of the isomerase produced 1.0 increment in the optical density at 260 nm per minute at 25°C in the RNase assay. Protein was determined by the method of Lowry et al. (7) using bovine serum albumin as the standard.

The antiserum against protein-disulfide isomerase was prepared by immunizing a white rabbit with purified enzyme preparations. Immunoglobulin G fraction was prepared from the serum by ammonium sulfate fractionation and column chromatography with DEAE-cellulose (DE-52, Wattman) (8). A control immunoglobulin G fraction was obtained from unimmunized rabbits. Ouchterlony agar double diffusion tests were performed as described previously (4).

Bovine pancreatic RNase was obtained from Boehringer Mannheim GmbH. Other chemicals were of analytical grade.

RESULTS: Purification of a protein-disulfide isomerase from microsomes. We noticed that about half of protein-disulfide isomerase activity was released from microsomes by the washing with 150 mM KCl containing 20 mM Tris-HCl (pH 7.8) and 0.2 mM EDTA. The activity which remained associated with the washed microsomes was not further released by repeating the washing with the same medium.

Acetone powder was prepared from the washed microsomes and extracted with 100 mM Tris-HCl (pH 7.8)-100 mM KCl. The extract was applied on a column of DEAE-cellulose, and the enzyme was eluted with a linear concentration gradient of KCl from 100 to 350 mM. The active fraction was then chromatographed on a column of Sephadex G-150 (Pharmacia) using 5 mM potassium phosphate buffer (pH 6.8) as the elution medium. The enzyme was further purified by the hydroxylapatite column chromatography (linear concentration gradient of potassium phosphate buffer,pH 6.8, from 20 to 200 mM), and by gel filtration

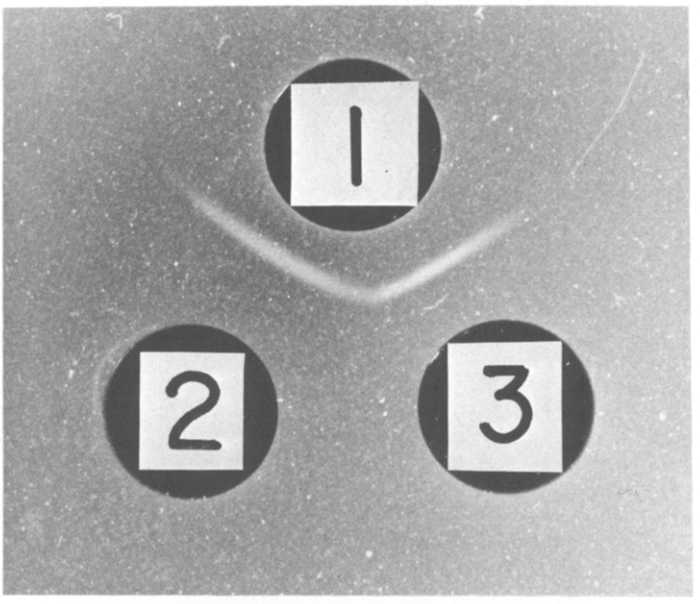
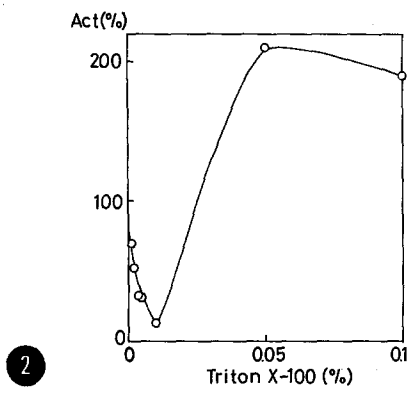


Fig.1 Ouchterlony double diffusion test of protein-disulfide isomerase.
The double diffusion test was run in 1.2 % agar in 50 mM potassium phosphate buffer (pH 7.5) containing 0.9 % NaCl at 25°C. Wells number 1,2, and 3 contained 800 μg of antibody, 90 μg (0.2 unit) of acetone powder extract, and 13 μg (0.2 unit) of purified enzyme, respectively.

with Sephadex G-100 (5mM potassium phosphate buffer, pH 6.8). The specific activity of the final preparation was 130 fold higher over KCl-washed microsomes, and the recovery of enzyme activity was about 5 %. The homogeneity of the purified enzyme was confirmed by the polyacrylamide gel electrophoresis in the presence of 0.1 % sodium dodecylsulfate, which was carried out as described by Weber and Osborn (9).

The purified enzyme and the crude extract of the acetone powder of microsomes gave a fused single precipitation line when examined by the Ouchterlony agar double diffusion test with the antibody against the purified enzyme (Fig.1). KCl-supernatant of microsomes also gave a single precipitation line with the antibody which fused with the precipitation line of the purified enzyme.

Detergent treatment of microsomes and purified enzyme. The activity of



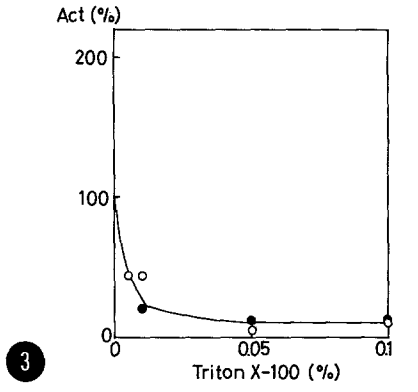


Fig 2 (left) Effect of Triton X-100 on the protein-disulfide isomerase activity of microsomes. A fixed amount of microsomes (300 μg protein, 0.3 unit/mg) was used in the assay in the presence of various concentrations of Triton X-100.

Fig.3 (right) Effect of Triton X-100 on the activities of KCl-supernatant and purified enzyme. KCl-supernatant (800 μg protein, 0.7 unit/mg) and purified enzyme (3μg, 16 unit/mg) were assayed in the presence of various concentrations of Triton X-100. o—o, purified enzyme; ●—●, KCl-supernatant.

microsome-bound protein-disulfide isomerase was greatly affected by the presence of Triton X-100 in the incubation mixture (Fig.2). Low concentrations of Triton X-100 strongly inhibited the enzyme activity, while higher concentrations of the detergent (above 0.05%) caused significant increase of the enzyme activity. This apparent biphasic response of microsome-bound protein-disulfide isomerase to Triton X-100 indicated the presence of two types of the enzyme having different susceptibilities to the detergent.

The activity of the purified enzyme was strongly inhibited by the detergent (Fig.3). Inactivation was observed in the low concentrations of the detergent as in the case of microsomes, but activation in the high concentrations of the detergent was not observed. The protein-disulfide isomerase activity of intact microsomes was possibly due to the enzyme molecules exposed on the outside surface of microsomal vesicles, whereas the activation of the enzyme activity at higher detergent concentrations was caused by the exposure of hidden enzyme molecules which were located in the inside of microsomes.

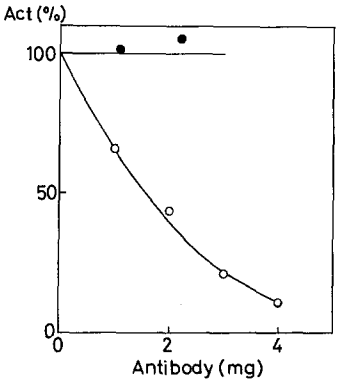


Fig.4 Inhibition of purified enzyme by the antibody. Partially purified enzyme (150 μg, 1.0 unit/mg) was assayed in the presence of various amounts of the antibody. o—o, antibody; •—•, control immunoglobulin.

Judging from the effect of the detergent, the purified enzyme was identical with the outside-located enzyme of microsomes. The activity of the KCl-supernatant was also strongly inhibited by Triton X-100 (Fig.3).

Enzyme inhibition by the antibody. The rabbit antibody against the purified enzyme almost completely inhibited the activity of partially purified enzyme (Fig.4). Control immunoglobulin did not inhibit the enzyme activity. The antibody also inhibited the enzyme activity of intact microsomes (Table I), which suggested the immunochemical identity of the purified enzyme with the membrane-bound enzyme located on the outside surface of the microsomes.

However, in the presence of 0.1 % Triton X-100, the enzyme activity of microsomes was little affected by the presence of the antibody. The inside-located enzyme is immunochemically different from the outside-located enzyme.

The enzyme activity in KCl-supernatant was inhibited by the antibody, which observation supported the conclusion obtained by the Ouchterlony agar double diffusion test and the detergent treatment that the enzyme released from microsmes by KCl-washing was identical with the purified enzyme.

DISCUSSION: The presence of protein-disulfide isomerase activity on both sides of microsomal membrane is favourable for the hypothesis (1) that the

Table I

Inhibition by the antibody of protein-disulfide isomerase activities of intact microsomes, Triton X-100-treated microsomes, and KCl-supernatant.

	Specific Activity (unit/mg protein)		
	Ms	Ms+Triton X-100	KCl-sup
None	0.32	0.47	1.35
Control IgG	0.32	0.49	1.35
Antibody	0.0	0.41	0.23

KCl-supernatant was concentrated by membrane filter. KCl-washed microsomes (118 μg protein) in the presence or absence of 0.1 % Triton X-100, and concentrated KCl-supernatant (31 μg protein) were assayed with 800 μg of the antibody or 1100 μg of control immunoglobulin. Final volume of the incubation mixture was 0.2 ml.

enzyme is functional in the folding of newly synthesized polypeptides. The enzyme on the outside surface of microsomal vesicles could be distinguished from the inside enzyme by their different susceptibility to Triton X-100.

Some portion of the enzyme activity on the outside surface of microsmes was released by the washing with 150 mM KCl. The enzyme released by the washing was immunochemically identical with the microsome-bound enzyme, which was purified in this study. The extent of release of the enzyme activity by the washing was not much affected by increasing the KCl concentration, nor by including EDTA in the washing medium. There is no plausible explanation at present why some portion of microsome-bound enzyme is easily released by the washing with KCl solution, while other portion remaines attached to the microsomes even after repeated washings.

The activities of the purified enzyme and intact microsomes were both strongly inhibited by low concentrations of Triton X-100, which observation agreed with the report of Goldberger et al (1) that the protein-disulfide isomerase activity of beef liver microsomes was inactivated by deoxycholate.

We noticed, however, significant activation of microsomal protein-disulfide isomerase activity at higher concentrations of Triton X-100, which was explained by the unmasking of the activity of inside-located enzyme.

Several investigators (10,11,12) reported the molecular similarity and the overlapping substrate specificity of "glutathione-insulin transhydrogenase" with protein-disulfide isomerase, both of which were present in liver micorosomes. The former activity was activated by various treatments affecting the membrane structure (13), and could be purified starting from deoxycholate-solubilized rat liver microsomes (14). The inside-located protein-disulfide isomerase is possibly identical with "glutathione-insulin transhydrogenase".

REFERENCES:

1. Goldberger.R.F., Epstein,C.J., and Anfinsen,C.B. (1963) J.Biol.Chem., 238, 628-635.
2. Venetianer,P., and Straub,F.B. (1963) Biochim.Biophys.Acta, 67, 166-168.
3. Savatini,D.D., and Kreibich,G. (1976) Enzymes of Biological Membrane, Vol.2, ed.by Marponosi,A., Plenum Press, New York, pp.531-579.
4. Ito,A., Kuwahara,T., Mitsunari,Y., and Omura,T. (1977) J.Biochem.,81, 1531-1541.
5. De Lorenzo,F., Goldberger,R.F., Steers,E.,Jr., Givol,D., and Anfinsen, C.B. (1966) J.Biol.Chem., 241, 1562-1567.
6. Fletcher,P.L.,Jr., and Hash,J.H. (1972) Biochemistry, 11, 4274-4280.
7. Lowry,O.H., Rosebrough,N.J., Farr,A.L., and Randall,R.J. (1951) J.Biol. Chem., 193, 265-275.
8. Utsumi,S., and Kraush,F. (1965) Biochemistry, 4, 1766-1779.
9. Weber,K., and Osborn,M. (1969) J.Biol.Chem., 244, 4406-4412.
10. Varandani,P.T. (1974) Biochim.Biophys.Acta, 371,577-581.
11. Ansorge,S., Bohley,P., Kirschke,H., Langner,J., Marquardt,I., Wiederanders,B., and Hanson,H. (1973) FEBS Lett., 37, 238-240.
12. Hawkins,H.C.,and Freedman,R.B. (1976) Biochem.J., 159, 385-393.
13. Varandani,P.T. (1973) Biochim.Biophys.Acta, 304, 642-659.
14. Ansorge,S., Bohley,P., Kirshke, H., Langner,J., Wiederanders,B., and Hanson,H. (1973) Eur.J.Biochem., 32, 27-35.