Identification of protein disulphide-isomerase as a major acidic polypeptide in rat liver microsomal membranes

E. N. Clare MILLS, Nigel LAMBERT and Robert B. FREEDMAN
Biological Laboratory, University of Kent, Canterbury, Kent, CT2 7NJ, U.K.

(Received 13 December 1982/Accepted 8 April 1983)

Protein disulphide-isomerase was purified to homogeneity from rat liver by a rapid high-yielding procedure. Structural properties of the pure enzyme were very similar to those of the bovine liver enzyme purified by the same method. The purified rat liver enzyme was subjected to two-dimensional gel electrophoresis in the presence and in the absence of microsomal membranes, and shown to co-electrophorese with a major acidic polypeptide clearly identifiable in the two-dimensional electrophoretic profile of microsomal membranes. This identification was confirmed by peptide 'mapping' of the pure enzyme and of the defined spot from a two-dimensional electrophoresis gel.

Protein disulphide-isomerase (PDI), which catalyses the thiol-dependent isomerization of disulphide bonds in proteins, has been detected in rat liver microsomal membranes (Goldberger *et al.*, 1963, 1964; Williams *et al.*, 1968; Ibbetson & Freedman, 1976; Drazic & Cottrell, 1977; Ohba *et al.*, 1977, 1981). Recently the enzyme has been purified from rat liver microsomal fraction, and partially characterized (Ohba *et al.*, 1981). Quantitative immunoprecipitation suggested that this enzyme accounted for 1–2% of total microsomal protein (Ohba *et al.*, 1981).

We have developed a rapid and high-yielding purification of PDI from bovine liver (Lambert & Freedman, 1982, 1983a). The structural properties of the pure bovine enzyme are very similar to those described for the rat liver enzyme purified by Ohba *et al.* (1981). We have therefore investigated whether the method developed for the bovine enzyme will purify PDI from rat liver, with advantages in speed and yield over the conventional method. In the present paper we describe the purification of rat liver PDI by this more convenient method, we confirm the similarity in structural properties of the rat and bovine liver enzymes and we describe the identification of PDI as a prominent acidic polypeptide in two-dimensional gel-electrophoretic profiles of rat liver microsomal membranes. Some of the data have

been presented in preliminary form elsewhere (Freedman *et al.*, 1983).

Experimental

Livers from 20 male Sprague–Dawley rats were used for the enzyme purification. The procedure was exactly that described previously (Lambert & Freedman, 1983a) for the purification from bovine liver. PDI activity was assayed as described previously (Lambert & Freedman, 1983b). Protein was determined by the Folin–Lowry method, with bovine serum albumin as standard.

Microsomal membranes were prepared from livers of male Sprague–Dawley CD rats as described previously (Kaderbhai & Freedman, 1980).

Gel-electrophoresis methods were as described by Kaderbhai & Freedman (1980). SDS/polyacrylamide-gel electrophoresis was performed on 10%-acrylamide gels, except with peptide mixtures from proteinase digests, which were analysed on 15% gels. Two-dimensional gel electrophoresis was performed by two methods developed by Kaderbhai & Freedman (1980) and Vlasuk & Walz (1980). Gels to be stained with Coomassie Blue were fixed overnight in 10% (v/v) acetic acid/25% (v/v) propan-2-ol before staining in 0.25% Coomassie Blue R-250 in 50% (v/v) methanol/10% (v/v) acetic acid. Gels to be stained with silver were fixed in 50% (v/v) methanol overnight before treatment by the procedure of Wray *et al.* (1981).

Peptide 'mapping' was performed by the method of Cleveland *et al.* (1977).

Abbreviations used: PDI, protein disulphide-isomerase; SDS, sodium dodecyl sulphate.

Results and discussion

Rat liver was homogenized in Triton X-100, and the solubilized PDI was purified exactly as described previously for the enzyme from bovine liver (Lambert & Freedman, 1983a). Table 1 gives details of the purification. The final preparation had a specific activity of 950 units/g. The preparation was homogeneous by isoelectric focusing and by SDS/polyacrylamide-gel electrophoresis, with pI 4–4.5 and a subunit M_r of 57000 ± 2000 (see Fig. 2 below). The activity and structural properties of the homogeneous rat liver enzyme are very similar to those of the bovine enzyme. This similarity was confirmed by peptide 'fingerprint' analysis. Fig. 1 shows the time courses of digestion of pure PDI from the two sources by *Staphylococcus aureus* proteinase; the peptide patterns show some differences, but clearly have many major bands in common, indicating a high degree of structural relatedness.

The final degree of purification of rat liver PDI (270-fold) supports the immunological result obtained by Ohba *et al.* (1981), showing that PDI accounts for 0.37% of total extractable protein in rat liver and for 2% of the protein of microsomal membranes. This result, and the knowledge of the enzyme's isoelectric point and subunit M_r , suggested that PDI might be identified with one of the major acidic polypeptides recognizable in two-dimensional electrophoretic profiles of this fraction. Fig. 2 shows experiments in which pure rat PDI was subjected to two-dimensional electrophoresis in the absence and in the presence of rat liver microsomal fraction. Two electrophoretic procedures were used. The co-electrophoresis, in both systems, of pure PDI with a single major polypeptide of rat liver microsomal membranes (labelled 'A' in Kaderbhai & Freedman, 1980) strongly suggests that this prominent polypeptide is rat liver PDI.

The identity of this spot with PDI was confirmed by the method of Cleveland *et al.* (1977). Fig. 3 shows a comparison of the peptide 'fingerprints' of pure rat liver PDI and of material from the corresponding spot from a two-dimensional electrophoretic separation of rat liver microsomal fraction. The 'fingerprints' are identical within the resolution of the gel. Thus this spot can be definitely identified as PDI. This identification makes possible labelling and other studies on the topology etc. of PDI in the endoplasmic reticulum. The prominence of this spot emphasizes the abundance of PDI in rat liver microsomal membranes.

The evidence here for the abundance of PDI and for its conservation in structure suggest that the enzyme plays an important role in the endoplasmic reticulum of liver cells. Freedman *et al.* (1983) have summarized the evidence from the enzyme's distribution and other properties, that it is involved in

Table 1. *Purification of PDI from rat liver (220 g)*

The purification protocol was exactly that described in Lambert & Freedman (1983a) for the purification of the enzyme from bovine liver. The specific activities of the rat liver preparation at each stage of purification correspond closely to those observed for the bovine liver preparation (cf. Table 1 in Lambert & Freedman, 1983a).

	Protein (g)	PDI activity (units)	Specific activity (units/g)
Homogenate	35.8	125.3	3.5
18 000 g supernatant	28.6	114.4	4.0
Heat-treatment supernatant	7.15	99.4	14.0
55–85%-satn.-(NH ₄) ₂ SO ₄ fraction	2.47	102.0	41.0
CM-Sephadex, pH 5.3 eluate	0.17	31.0	181
DEAE-Sephacel, pH 6.3 eluate	0.02	22	~1000

Fig. 1. *Comparison of peptide 'fingerprints' of rat and bovine liver PDI*

Samples of the homogeneous enzymes were made up to 0.5 mg/ml in 0.125 M-Tris/HCl buffer, pH 6.8, containing 0.5% (w/v) SDS, 10% (v/v) glycerol and 0.0001% Bromophenol Blue, and boiled for 3 min. *S. aureus* V8 proteinase [0.5 mg/ml in 0.125 M-Tris/HCl buffer, pH 6.7, containing 1 mM-EDTA, 0.1% (w/v) SDS and 20% (v/v) glycerol] was added to a final concentration of 0.025 mg/ml, and then samples containing 0.015 mg of PDI were removed at $t = 0$ min (tracks 1 and 6), $t = 10$ min (tracks 2 and 7), $t = 20$ min (tracks 3 and 8), $t = 60$ min (tracks 4 and 9) and $t = 90$ min (tracks 5 and 10). The samples were immediately adjusted to 10% (v/v) 2-mercaptoethanol and 0.2% (w/v) SDS, mixed well and boiled for 3 min. After cooling, the samples were stored at -20°C overnight before analysis by SDS/polyacrylamide-gel electrophoresis. Tracks 1–5, rat PDI; tracks 6–10, bovine PDI. The gel is stained with Coomassie Blue.

Fig. 2. Two-dimensional electrophoresis of rat liver microsomal fraction and purified rat liver PDI
 (a) Microsomal fraction (approx. 0.25 mg of microsomal protein); (b) PDI (0.01 mg); (c) microsomal fraction (approx. 0.25 mg of protein) + PDI (0.01 mg); (d) microsomal fraction (approx. 0.13 mg of protein); (e) PDI (approx. 0.01 mg); (f) microsomal fraction (0.13 mg of protein) + PDI (0.01 mg). Gels (a)–(c) were resolved by a procedure based on that of Kaderbhai & Freedman (1980); gels (d)–(f) were resolved by a procedure based on that of Vlasuk & Walz (1980). The gels are stained with Coomassie Blue.

Fig. 3. Identification of PDI by peptide 'fingerprint' Track 1: a sample of pure rat PDI (0.01 mg) was subjected to SDS/polyacrylamide-gel electrophoresis, stained with Coomassie Blue and a gel slice containing the protein band was cut out. Track 2: a suspension of rat liver microsomal fraction (0.25 mg of protein) was resolved by two-dimensional gel electrophoresis as in Fig. 2(a). The putative PDI spot (marked with an arrow in Fig. 2a) was punched out. The slice and spot were then treated as outlined by Cleveland *et al.* (1977). They were loaded into wells of a polyacrylamide slab gel, 0.01 μ g of *Staphylococcus aureus* V8 proteinase was layered over each slice and electrophoresis was started. When the dye front reached the end of the stacking gel, the current was switched off for 20 min to allow proteolysis to proceed. The gel is silver stained.

formation of disulphide bonds in protein biosynthesis.

This work was supported by Science and Engineering Research Council Studentships: a Quota Studentship to N. L. and a CASE Studentship (in association with Shell Research Ltd., Sittingbourne, Kent, U.K.) to E. N. C. M. We are grateful to Dr. M. A. Kaderbhai for useful discussions.

References

- Cleveland, D. W., Fischer, S. G., Kirschner, M. W. & Laemmli, U. K. (1977) *J. Biol. Chem.* **252**, 1102–1106
- Drazic, M. & Côttré, R. C. (1977) *Biochim. Biophys. Acta* **484**, 476–485
- Freedman, R. B., Brockway, B. E., Forster, S. J., Lambert, N., Mills, E. N. C. & Roden, L. T. (1983) in *Functions of Glutathione: Biochemical, Physiological, Toxicological and Clinical Aspects* (Larsson, A., Orrenius, S., Holmgren, A. & Mannervik, B., eds.), pp. 271–282, Raven Press, New York
- Goldberger, R. F., Epstein, C. J. & Anfinsen, C. B. (1963) *J. Biol. Chem.* **238**, 628–635
- Goldberger, R. F., Epstein, C. J. & Anfinsen, C. B. (1964) *J. Biol. Chem.* **239**, 1406–1410
- Ibbetson, A. L. & Freedman, R. B. (1976) *Biochem. J.* **159**, 377–384
- Kaderbhai, M. A. & Freedman, R. B. (1980) *Biochim. Biophys. Acta* **601**, 11–22
- Lambert, N. & Freedman, R. B. (1982) *Biochem. Soc. Trans.* **10**, 113–114
- Lambert, N. & Freedman, R. B. (1983a) *Biochem. J.* **213**, 225–234
- Lambert, N. & Freedman, R. B. (1983b) *Biochem. J.* **213**, 235–243
- Ohba, H., Harano, T. & Omura, T. (1977) *Biochem. Biophys. Res. Commun.* **77**, 830–836
- Ohba, H., Harano, T. & Omura, T. (1981) *J. Biochem. (Tokyo)* **89**, 889–900
- Vlasuk, G. P. & Walz, F. G. (1980) *Anal. Biochem.* **105**, 112–120
- Williams, D. J., Gurari, D. & Rabin, B. R. (1968) *FEBS Lett.* **2**, 133–135
- Wray, W., Boulikas, T., Wray, V. P. & Hancock, R. (1981) *Anal. Biochem.* **118**, 197–203