maleate. uptake of 2 mM-[U-14C]serine fell to 106 ± 31 (3) μmol/h per mg, i.e., 55% of controls.

Lauteburg & Mitchell (1979) showed that hepatic GSH turnover is increased in the presence of amino acids. We have shown that inhibition of γ-glutamyltransferase decreases uptake of amino acids and that depletion of intracellular GSH causes a decrease in amino acid uptake by hepatocytes. These facts strongly support the hypothesis that the γ-glutamyl cycle is functioning in the hepatocyte.

The fact that γ-glutamyltransferase activity and amino acid uptake are increased by dexamethasone may be an indication that the γ-glutamyl cycle is subject to hormonal control in the hepatocyte. Indeed we have shown that this is the case in mammary gland (Viña et al., 1981a,b).

Glutathione S-transferases AA and B possess a common antigenic determinant

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Department of Biochemistry, Trinity College, Dublin 2, Ireland

Glutathione S-transferases exists in multiple forms in rat and human liver (Jakoby, 1978). Recent work has shown that glutathione S-transferase B is a heterodimer composed of α- and c-subunits of molecular weight 22000 and 25000 respectively, whereas glutathione S-transferase AA appears to be a homodimer of c-subunits (Hayes et al., 1979, 1980; Scully & Mantle, 1980, 1981a). This conclusion was based on gel-filtration estimates of native molecular weights and sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of the purified proteins. We have presented evidence, based on partial-proteolysis experiments, that the two c-subunits of the AA-form are identical with the c-subunit of the B-form (Scully & Mantle, 1981b). The presence of a common c-subunit in forms AA and B suggests that antibodies raised to either form would cross-react with both.

To test this, antibodies were raised in rabbits to glutathione S-transferases AA and B purified from rat liver as previously described (Scully & Mantle, 1981a). Antisera raised to either form cross-reacted with both forms AA and B, giving lines of identity in Ouchterlony plates, confirming that they possess a common antigenic determinant, which we assume to be the c-subunit.

We thank the Medical Research Council of Ireland for financial support.

Modified procedure for rapid purification of protein disulphide-isomerase from bovine liver

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Biological Laboratory, University of Kent, Canterbury, Kent CT2 7NJ, U.K.

Protein disulphide-isomerase (EC 5.3.4.1) is one of a group of enzymes catalysing thiol-protein disulphide interchange (Freedman, 1979). Studies on this enzyme have been hampered by lengthy, low-yielding purification procedures. The original method (De Lorenzo et al., 1966) involves acetone extraction of bovine liver microsomal fractions, precipitation with (NH₄)₂SO₄, three ion-exchange-chromatography steps and one gel filtration. Using essentially this method, Hawkins & Freedman (1976) purified the enzyme to a final specific activity of 543 units/g of protein (for definition of a unit see Ibbetson & Freedman, 1976) with 3.7% yield. A modification of this method including a thiopropyl-Sepharose covalent chromatography step (Hillson & Freedman, 1980a) resolved the enzyme from glutathione:insulin transhydrogenase, a related activity, but did not improve either the specific activity or the yield of the final product.

Carmichael et al. (1977) introduced a new procedure for purification of a thiol:protein disulphide oxidoreductase, and Hillson & Freedman (1980b) showed that this method resolved protein disulphide-isomerase from glutathione:insulin transhydrogenase. Protein disulphide-isomerase purified by this method had a final activity of only 68 units/g and a yield of less than 3% (Hillson, 1979).

However, further modifications to the method of Carmichael et al. (1977) have now produced a cheap, reproducible and rapid procedure for the preparation of protein disulphide-isomerase with high specific activity (over 900 units/g) and in yields approaching 30%. Data from a typical preparation are shown in Table 1. The method is as follows. Batches (500 g) of bovine liver are homogenized in 2 vol of phosphate buffer (0.1 M, pH 7.5) containing EDTA (5 mM) and Triton X-100 (1%/v/v). (The final detergent:protein ratio is substantially higher than that used by Carmichael et al., 1977.) The homogenate is centrifuged (18000g, 40 min) and the supernatant heated to 54°C for 15 min. The heat-denatured supernatant is re-centrifuged, fractionated by (NH₄)₂SO₄ precipitation (55–85% satd.) and dialysed against citrate buffer (25 mM, pH 5.3). The non-dialysable material is applied to a CM-Sephadex C-50 column equilibrated in the citrate buffer, and protein disulphide-isomerase activity is eluted in the void volume. The final fractionation is on a DEAE-Sephadex column eluted with a linear 0–0.7 M-NaCl gradient in phosphate buffer (20 mM, pH 6.3); the second of three protein peaks contains protein disulphide-isomerase with a specific activity routinely over 900 units/g.

The entire procedure takes only 5 days to perform, and compares very favourably with earlier methods (Hawkins & Freedman, 1976; Hillson & Freedman, 1980a,b), with almost ten times the yield and approaching twice the specific activity in the final fraction. The purification data of Hawkins & Freedman


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(1976) show that 70% of the total protein disulphide-isomerase activity was lost in the preparation of microsomal fraction. By extracting the total liver homogenate with Triton X-100, the new method bypasses this lengthy and apparently wasteful subcellular fractionation. In addition, the initial isomerase activity of the Triton extract is 50% greater than that of the conventional sucrose homogenate; this detergent effect has been observed previously (Obba et al., 1977; Freedman et al., 1978).

The purified enzyme migrates as one band on sodium dodecyl sulphate/polyacrylamide-gel electrophoresis with a $M_\text{r}$ of $57000 \pm 2000$, in both the presence and absence of $\beta$-mercaptoethanol, in agreement with earlier estimates (Hawkins & Freedman, 1976; Hillson & Freedman, 1980a,b). Gel filtration, under non-denaturing conditions gave $M_\text{r} = 107000 \pm 5000$, suggesting that the native enzyme is a homodimer. The rat liver enzyme has also been shown to be dimeric by this method (Obba et al., 1981). The $pI$ in the presence of $8M$-urea was $4.2 \pm 0.1$, and the total carbohydrate content, as determined by the phenol/$H_2SO_4$ reaction (Dubois et al., 1956), was 0.5–1%.

Preliminary kinetic studies confirm the results of previous work (Hillson & Freedman, 1980a). This rapid, high-yielding preparative procedure will allow more detailed molecular characterization of the enzyme and comparisons between it and other preparations catalysing thiol-protein disulphide oxidoreductions.


Freedman, R. B. (1979) FEBS Lett. 97, 201–210


Table 1. Representative purification of protein disulphide-isomerase from 1 kg of bovine liver

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Protein (mg)</th>
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<th>Sp. activity (units/mg)</th>
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<td>18000g Supernatant</td>
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<td>Heat-denatured supernatant</td>
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<td>55–85% (NH₄)₂SO₄ fractionation</td>
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Protein disulphide-isomerase is distinct from the liver microsomal protein capable of inactivating cytosol enzymes by thiol-disulphide interchange

ROBERT B. FREEDMAN and PAUL GARDEN
Biological Laboratory, University of Kent, Canterbury, Kent CT2 7NJ, U.K.

Francis & Ballard (1980a) showed that liver microsomal fractions are capable of inactivating several cytosolic enzymes, and isolated a microsomal 'inactivation factor' which they proposed might play a role in intracellular protein degradation. They studied the action of purified 'inactivation factor' on cytosolic glucose 6-phosphate dehydrogenase and concluded that the inactivation involved thiol-disulphide interchange (Francis & Ballard, 1980b). Several enzyme activities catalysing thiol-disulphide interchange are known (Freedman, 1979), but the best studied being protein disulphide-isomerase (Hillson & Freedman, 1980a,b), which may play a role in the formation of disulphide bonds in protein biosynthesis (Freedman & Hillson, 1980). Here we examine the relationship between protein disulphide-isomerase and the 'inactivation factor' of Francis & Ballard (1980a,b).

Rat liver cytosol and microsomal fractions were prepared as described by Francis & Ballard (1980a,b). Highly purified protein disulphide-isomerase (>500 units/g) was prepared from bovine liver by a modification (N. Lambert, unpublished work) of the standard method (Hillson & Freedman, 1980b). Inactivation incubations were set up as follows: 180 $\mu$L of cytosol preparation, 20 $\mu$L of Triton X-100 (1%), 20 $\mu$L of 0.5 M-Tris/HCl buffer, pH 8.0, plus further additions in 50 mM-Tris/HCl, pH 7.6, plus this buffer to a final volume of 500 $\mu$L. The final concentration of cytosol protein was 4.25 mg/ml. Incubation was at 37°C for up to 300 min. At zero time, and at various times thereafter, a 50 $\mu$L sample was withdrawn for the assay of lactate dehydrogenase (Kornberg, 1955), fructose 1,6-bisphosphate (Horecker, 1955) activities. Protein was determined by the method of Lowry et al. (1951).

Lactate dehydrogenase activity was stable in all the conditions studied, and is not considered further. Fructose 1,6-bisphosphatase (Clark et al., 1973) and glucose 6-phosphate dehydrogenase (Kornberg & Horecker, 1955) activities. Protein was determined by the method of Lowry et al. (1951).

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