Protein disulphide-isomerase of chick-embryo tendon

Barbara E. BROCKWAY and Robert B. FREEDMAN

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

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1. Protein disulphide-isomerase can be partially purified from the high-speed-supernatant fraction of extensively disrupted chick-embryo tendon tissue. 2. The catalytic properties of the preparation resemble those of the enzyme from mammalian liver. 3. Gel electrophoresis and isoelectric focusing show the enzyme to be very acidic, with $pI 4.4 \pm 0.3$. 4. Gel filtration indicates an M_r for the active enzyme of 140 000. 5. The enzyme can be partially purified by preparative gel filtration or isoelectric focusing, but its limited stability has prevented purification to homogeneity; active fractions from both gel filtration and isoelectric focusing show two major polypeptide components by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis. 6. The major polypeptides present in partially purified preparations have M_r 45 000 and 55 000; the latter band co-distributes with the enzyme activity in fractionations by both gel filtration and isoelectric focusing. 7. The subcellular location of the enzyme cannot be established from work on homogenates of whole tissue, which are extensively disrupted. In homogenates from isolated tendon cells, the enzyme is located in a vesicle fraction that is excluded from Sepharose 2B but is of low density and can only be sedimented at very high speeds. This fraction is identified as deriving from the endoplasmic reticulum on the grounds of marker-enzyme studies and electron microscopy.

Protein disulphide-isomerase (PDI), an enzyme that catalyses thiol–disulphide oxidoreductions and interchanges in protein and hence catalyses formation of the most stable disulphide pairing, has been found in a wide range of sources. The structure and catalytic properties of the enzyme have been most widely studied in mammalian liver. Thus the enzyme has been purified to homogeneity from bovine liver (De Lorenzo *et al.*, 1966; Hawkins & Freedman, 1976; Lambert & Freedman, 1983a), rat liver (Ohba *et al.*, 1977, 1981; Mills *et al.*, 1983) and mouse liver (Roth & Koshland, 1981), and the structural findings indicate a close degree of homology between the enzymes from these sources. Work with either liver microsomal preparations or partially purified preparations from liver has shown that the enzyme can act on a wide range of protein substrates: small single-domain proteins such as ribonuclease and pancreatic trypsin inhibitor, multi-domain proteins such as albumin and multi-chain proteins such as immunoglobulins (for review see Freedman & Hillson, 1980).

Since the discovery of the enzyme (Goldberger *et*

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

al., 1963; Venetianer & Straub, 1963), it has been proposed that it participates in the biosynthesis of disulphide-bonded proteins, ensuring rapid formation of the 'correct' disulphide pairing (Anfinsen, 1973). The liver enzyme has the appropriate catalytic properties and subcellular location to perform this role. But the best evidence supporting this proposal comes from studies on a number of other, less versatile, tissues, in which correlations have been established between the amount of synthesis of disulphide-bonded proteins and the amount of PDI activity. Thus Roth & Koshland (1981) in a study of a number of cultured mouse lymphoid-cell lines established that the cell lines actively secreting immunoglobulins had activities of the enzyme 10–100-fold greater than those of unstimulated B-cells or non-immunoglobulin-producing T-cells; in the actively secreting lines, PDI constituted up to 0.8% of the total extractable cellular protein. In chick-embryo tissues, procollagen is the major disulphide-bonded protein synthesized. We have shown that in this system the enzyme is found at greatest specific activities in tendon, cartilage, bone etc., the tissues most active in procollagen synthesis (Brockway *et al.*, 1980); a good quantitative correlation is found between the tissue distri-

bution of PDI and those of lysyl hydroxylase and prolyl 4-hydroxylase, enzymes that modify nascent collagenous polypeptides and are regarded as good markers of procollagen synthesis. Developmental correlations have also been found. In the wheat endosperm, PDI is found throughout seed development, but its activity rises to a maximum about 40 days after anthesis, in the period of maximum synthesis of the disulphide-bonded storage proteins (Freedman *et al.*, 1983). In chick-embryo bone, the specific activity is maximal at day 15 of development, which corresponds to the time of maximal specific procollagen synthesis and maxima in the activities of the hydroxylases (Brockway *et al.*, 1980).

In view of these results it seemed important to establish whether the molecular properties of the mammalian liver enzyme were typical and whether they corresponded to those of the enzyme in tissues where the physiological role was more clearly indicated. In the present paper we report some basic characteristics of PDI from chick-embryo tendon tissue.

Materials and methods

Eggs were supplied by Golden Produce, Lydd, Kent, U.K. Eagle's minimal medium and 10% (v/v) foetal-calf serum were from Flow Laboratories, trypsin solution (2.5%) was supplied by Gibco, and collagenase (*Clostridium histolyticum*, type I) was from Sigma Chemical Co. Soya-bean trypsin inhibitor, UDP (yeast, type I, sodium salt), glucose 6-phosphate (crystalline, monosodium salt) and *p*-nitrophenyl phosphate (crystalline disodium salt) were from Sigma Chemical Co. Other materials were from sources previously described (Hawkins & Freedman, 1976; Hillson & Freedman, 1980; Kaderbhai & Freedman, 1980; Lambert & Freedman, 1983a).

Preparation of soluble enzyme

Fertile eggs were incubated in a Westernette incubator in a moist atmosphere at 37°C for 17 days. The embryos were removed and decapitated, and tendon tissue was removed from the legs. The tissue was transferred to ice-cold buffer. When the intact tissue was to be homogenized it was placed in TKM buffer (50 mM-Tris/HCl buffer, pH 7.5, containing 25 mM-KCl and 5 mM-MgCl₂), rinsed in this buffer and then chopped finely with a scalpel blade in 2 vol. of this buffer containing 1 mM-phenylmethanesulphonyl fluoride. The suspension was then homogenized by 15 × 1 s bursts in the Ultra-Turrax homogenizer, followed by 20 strokes in a motor-driven Teflon/glass Potter-Elvehjem homogenizer. The crude homogenate was centrifuged at 1000 *g*_{av.} for 10 min and the supernatant

was retained; the pellet was resuspended in 1 vol. of TKM buffer containing 1 mM-phenylmethanesulphonyl fluoride and then taken through the complete homogenization procedure. The pellet from the low-speed spin of this homogenate was again resuspended and homogenized. Finally the three low-speed supernatants were combined and centrifuged first at 10000 *g*_{av.} for 20 min and then at 105000 *g*_{av.} for 1 h; in both cases the pellets were discarded. The high-speed supernatant was used without further treatment in some studies, but in most cases (NH₄)₂SO₄ (0.35 g/ml) was added slowly, precipitated proteins were removed by centrifugation at 10000 *g*_{av.} for 10 min, and the supernatant was dialysed overnight at 4°C against three changes of 5 litres of 50 mM-NH₄HCO₃. The dialysed material was freeze-dried and stored at -20°C.

Assay of PDI activity

PDI was assayed by its ability to re-activate 'scrambled' ribonuclease, with the use of TKM buffer and by following the method of Lambert & Freedman (1983a). For assays of multiple or low-activity samples (e.g. gel slices or column fractions), instead of monitoring of the appearance of ribonuclease activity at a number of time points, the PDI activity was determined from ribonuclease activity at a single time point, usually 30 min after the beginning of an assay.

Electrophoretic techniques

SDS/polyacrylamide-gel electrophoresis was performed with Laemmli (1970) buffers as described by McIntosh & Freedman (1980). Gels were stained with Coomassie Blue R-250 or by the silver method of Wray *et al.* (1981). Isoelectric focusing was performed in 6% (w/v) polyacrylamide gels containing 2% (w/v) Ampholines; both wide-range (pH 3.5-10) and narrow-range (pH 4-6) Ampholines, and mixtures in various proportions, were used. Gels were pre-focused for 30 min before the sample was loaded. The samples for isoelectric focusing contained 0.5% (w/v) Ampholines, and sucrose was added to increase sample density. Gel filtration was performed on a 1 cm × 25 cm column of Sephadex G-200 in 50 mM-sodium phosphate buffer, pH 7.5, containing 25 mM-KCl and 5 mM-MgCl₂. The sample was loaded in 0.5-1 ml of this buffer, and the column was eluted at a flow rate of 3 ml/h.

Isolation and disruption of tendon cells

For isolation of tendon cells, the dissected tissue was placed in Krebs modified balanced salts solution (111.2 mM-NaCl, 15.7 mM-Na₂HPO₄, 5.4 mM-KCl, 4.0 mM-NaHCO₃, 1.6 mM-KH₂PO₄ and 1.3 mM-MgCl₂) containing 13 mM-glucose, and

washed in 2 vol. of this buffer before being transferred to the digestion medium. For 20 g of tendon tissue the digestion medium comprised 45 ml of Eagle's minimal essential medium, 5 ml of trypsin solution (2.5%) and 120 mg of collagenase. Digestion was carried out in agitated flasks at 37°C for 1 h under a constant stream of water-saturated CO₂. The digest was filtered through two thicknesses of lens tissue and centrifuged at 600g_{av.} for 6 min. The cell pellet was resuspended in Krebs modified balanced salts solution supplemented with 13 mM-glucose, 10% (v/v) foetal-calf serum and 0.25% (w/v) soya-bean trypsin inhibitor and washed twice in this buffer. The cells were finally resuspended in TKM buffer containing 0.25 M-sucrose. Cells were homogenized in 2 ml of this buffer in the motor-driven Potter-Elvehjem homogenizer; undisturbed cells were pelleted as before, resuspended in 2 ml of buffer and rehomogenized. The procedure was repeated once more. Finally the three low-spin supernatants were combined, before being subjected to further fractionation.

Subcellular fractionation

Several differential-centrifugation protocols were used in studies of the subcellular location of PDI activity. Details are given in the text and Figure legends as appropriate. For isopycnic centrifugation, a homogenate prepared in TKM buffer containing 0.25 M-sucrose from isolated tendon cells was centrifuged at 600g_{av.} for 5 min and then at 10000g_{av.} for 10 min. The supernatant was layered over a sucrose gradient in TKM buffer. In the case of the experiment in Fig. 4, 2 ml of supernatant in TKM containing 0.25 M-sucrose [equivalent to 8.5% (w/v) sucrose] was layered over 10 ml of a 9–25% (w/v) sucrose gradient in TKM buffer, and a further 10 ml of a 4–8% (w/v) sucrose gradient in TKM buffer was applied above the sample. The gradient was then spun at 25000 rev./min in an MSE PrepSpin 50 centrifuge with a 3 × 25 ml swing-out rotor (65000g_{av.}) for 18 h. Fractions (0.6 ml) were collected from the bottom. Sucrose concentrations were determined from refractive index measured on a Gallenkamp Abbé refractometer. Gel filtration on Sepharose 2B was performed as previously described (Hawkins & Freedman, 1979; Kaderbhai & Freedman, 1980).

Marker assays

Methods for assay of markers were: protein, Lowry *et al.* (1951), with crystalline bovine serum albumin as standard; DNA, Burton (1956); RNA, Munro & Fleck (1966); succinate dehydrogenase, Earl & Korner (1965); acid phosphatase, Lansing *et al.* (1967); uridine diphosphatase, Plaut (1963); glucose-6-phosphatase, Baginski *et al.* (1974).

Fig. 1. Dependence of chick-embryo tendon PDI activity on the concentration of 'scrambled' ribonuclease. PDI activities were determined, as described in the Materials and methods section, at various concentrations of 'scrambled' ribonuclease. The sample was the supernatant obtained from a high-speed (105000g_{av.}, 60 min) centrifugation of a homogenate of tendon tissue. The continuous line is calculated from the Michaelis-Menten equation with parameters $K_m = 40 \mu\text{g/ml}$, $V_{max} = 2.5 \text{ units/g}$.

Electron microscopy was performed as previously described (Hawkins & Freedman, 1979).

Results and discussion

Isolation and stability of partially purified chick-embryo tendon PDI

In previous work we have shown that PDI activity is found in homogenates from a wide range of chick-embryo tissues; the greatest activity was found in tendon homogenates (Brockway *et al.*, 1980). When tendon tissue is disrupted by extensive homogenization in the Ultra-Turrax blender followed by the Potter-Elvehjem homogenizer, almost all the activity is solubilized, by the criterion that it is not sedimented after centrifugation at 105000g_{av.} for 1 h. This high-speed supernatant is a rich source of isomerase activity, but the enzyme is very unstable, even when stored frozen or in the presence of phenylmethanesulphonyl fluoride or 7-amino-1-chloro-3-tosylamidoheptan-2-one ('TLCK'). Greater stability can be achieved after partial purification by (NH₄)₂SO₄ fractionation; the enzyme is soluble in solution containing 350 g of (NH₄)₂SO₄/l (equivalent to 58% saturation), which precipitates approx. 70% of the protein in the high-speed supernatant. This step gives a 6-fold purification of the enzyme with respect to

the initial homogenate, with a 40% recovery. This enriched fraction can be stored after dialysis and freeze-drying, and the enzyme activity in this form has a half-life of 20 days. This is sufficient to allow preliminary characterization, but insufficient to allow further extensive purification, in view of the limited amount of material available. A batch of 200 eggs yields approx. 20 g of tendon tissue, which after $(\text{NH}_4)_2\text{SO}_4$ fractionation produces approx. 20 mg of protein containing (initially) approx. 0.2 unit of enzyme activity. Neither tendon tissue, nor homogenates nor partially purified extract could be stock-piled without rapid loss of activity.

Kinetic properties

Initial activity studies on chick tissue PDI were performed with assay conditions identical with those developed for work with the mammalian liver enzyme. To check the appropriateness of these for the chick tendon enzyme, the variation in activity with substrate concentrations and pH were studied. Fig. 1 shows the dependence of activity in the high-speed supernatant on the concentration of 'scrambled' ribonuclease. Both in this case and with the 'partially purified' material, the activity shows saturation at substrate concentrations in the range 100–200 $\mu\text{g}/\text{ml}$ and half-maximal activity is observed at approx. 30 $\mu\text{g}/\text{ml}$ (approx. 2.5 μM , or 10 μM in disulphide bonds). This is comparable with the value of $s_{0.5} = 2 \mu\text{M}$ found for the purified bovine liver enzyme (Lambert & Freedman, 1983b). The variation of activity with dithiothreitol concentration showed a maximum at approx. 10 μM -dithiothreitol (the maximum was found at values between 6 μM and 15 μM for different preparations). The standard value for assay of the bovine enzyme is 10 μM , and inhibition at higher concentration has been noted (Lambert & Freedman, 1983b). The pH-dependence of the chick-embryo tendon enzyme shows a maximum at pH 7.5–7.8, which again is close to the value found for the bovine liver enzyme (Hillson & Freedman, 1980; Lambert & Freedman, 1983b). The enzyme is extremely sensitive to inhibition by Cd^{2+} , with 50% inhibition at concentrations below 10 μM , as for the enzyme from bovine liver (Hillson & Freedman, 1980) and that from wheat endosperm (L. T. Roden & R. B. Freedman, unpublished work).

Structural properties

Gel filtration of the partially purified material on Sephadex G-200 showed that PDI activity was eluted at a volume identical with that of lactate dehydrogenase (M_r 145 000). Calibration with a series of standard proteins with M_r values ranging from 12 500 to 450 000 indicated an M_r value for the active enzyme of $140\,000 \pm 20\,000$.

Polyacrylamide-gel electrophoresis followed by assay of gel sections indicated that the enzyme was anionic at pH 6.5, and this acidity was confirmed by isoelectric focusing. From seven independent experiments with a number of different ampholyte compositions, a pI value of 4.4 ± 0.3 was obtained.

Fractions from gel filtration and gel sections from polyacrylamide-gel electrophoresis and isoelectric focusing were subjected to SDS/polyacrylamide-gel electrophoresis. The results are shown in Fig. 2, together with plots of enzyme activity resolved by each of these techniques. The active fractions, in each case, contain two major polypeptide components, of M_r approx. 45 000 and approx. 55 000. The former band is found in a large number of fractions and its presence is not correlated with enzyme activity; the latter co-distributes with enzyme activity. Hence we conclude that the polypeptide of M_r 55 000 is a constituent of chick-embryo tendon PDI. The polypeptide of M_r approx. 45 000 may be a proteolytic degradation product.

Subcellular fractionation

When tendon homogenates, prepared as above by vigorous Ultra-Turrax and Potter-Elvehjem homogenization, were filtered and subjected to differential centrifugation, more than 60% of the extracted protein was found in the high-speed (105 000 g_{av} , 60 min) supernatant fraction. PDI activity was found to be distributed between the high-speed pellet and supernatant fractions, which would normally be designated 'microsomal' and 'cytosol' fractions. The distribution of PDI closely matched that of uridine diphosphatase (see Fig. 3a). This co-distribution was found with a number of different centrifugation protocols. The PDI activity found in the high-speed-supernatant fractions was not truly soluble; when such a supernatant was spun at 250 000 g_{av} for 18 h, 59% of the PDI activity was recovered in a firm pellet (comparable values for glucose-6-phosphatase and uridine diphosphatase were 51% and 40%).

These data suggest that PDI in chick-embryo tendon homogenates is associated with a particulate fraction, and marker-enzyme results support the assumption that this is derived from the endoplasmic reticulum. However, the endoplasmic reticulum markers were associated with particles either much smaller or less dense than conventional microsomal vesicles. Marker studies with DNA, succinate dehydrogenase and acid phosphatase suggested that the vigorous homogenization required to disrupt this tough tissue was frustrating a proper subcellular fractionation. To circumvent this, tendons were digested with collagenase to produce a suspension of tendon cells that was then disrupted with the Potter-Elvehjem

Fig. 2. Resolution of chick-embryo tendon PDI activity by electrophoresis and gel-filtration

Samples of crude tendon PDI [freeze-dried $(\text{NH}_4)_2\text{SO}_4$ fractions from high-speed supernatant fractions of whole-tissue homogenates] were resolved by gel filtration (a and b), polyacrylamide-gel electrophoresis (c and d) and isoelectric focusing (e and f). Gel filtration and isoelectric focusing were performed as described in the Materials and methods section; polyacrylamide-gel electrophoresis was performed at pH 6.8 on 12.5%-(w/v)-acrylamide gels. Gel slices from the electrophoretic separations and fractions from gel filtration were analysed by SDS/polyacrylamide-gel electrophoresis (a, c and e) and assayed for PDI activity (b, d and f). The gel from SDS/polyacrylamide-gel electrophoresis of fractions from gel filtration and polyacrylamide-gel electrophoresis were silver-stained (see the Materials and methods section). In (f) the pH gradient established during isoelectric focusing is also shown. The numbers above the SDS/polyacrylamide-gel electrophoretograms and below the plots of enzyme activity represent fraction or slice number. M_r standards for SDS/polyacrylamide-gel electrophoresis were bovine serum albumin, ovalbumin, soya-bean trypsin inhibitor and lysozyme. * indicates the polypeptide of M_r 55000 referred to in the text.

Fig. 3. Co-distribution of PDI and uridine diphosphatase activities in chick-embryo tendon tissue

Homogenates from whole tendon tissue (a) and from isolated tendon cells (b) were subfractionated by differential centrifugation. (a) Tendon tissue was homogenized with the Ultra-Turrax and Potter-Elvehjem homogenizers as described in the Materials and methods section and spun at $500g_{av.}$ for 15 min to remove undisrupted tissue. The homogenate was then spun successively at $2000g_{av.}$ for 15 min, $18000g_{av.}$ for 20 min and $250000g_{av.}$ for 360 min. (b) Tendon cells were obtained by digestion of the tissue and homogenized as described in the Materials and methods section. The homogenate was spun successively at $600g_{av.}$ for 5 min, $10000g_{av.}$ for 10 min and $105000g_{av.}$ for 60 min. The pellets were resuspended in TKM buffer containing 0.25 M-sucrose, and in both cases the three resuspended pellets and the final supernatant were assayed for protein content and PDI and uridine diphosphatase activities. The ordinate represents the specific activity of each fraction relative to that of the homogenate, and the abscissa represents the protein content of each fraction as a percentage of the total protein recovered in the four fractions.

homogenizer only. When this homogenate was fractionated by a conventional differential-centrifugation protocol, both PDI and uridine diphosphatase were again found mainly in the high-

Fig. 4. Distribution of PDI activity after isopycnic centrifugation of a chick-embryo tendon post-mitochondrial supernatant

The procedure is described fully in the Materials and methods section. The fractions were frozen and thawed before being assayed for PDI activity. Sample densities at 4°C were determined from the sucrose concentrations by using standard Tables (Dawson *et al.*, 1969).

speed-supernatant fraction (Fig. 3b). When a post-mitochondrial supernatant from such a fractionation was subjected to isopycnic centrifugation on a sucrose gradient (Fig. 4) it was found that the maximum of PDI activity occurred at an unexpectedly low buoyant density (1.05; 11.6% sucrose).

In view of this evidence that PDI was associated with a particulate fraction that was not easily sedimentable, post-mitochondrial supernatants from homogenates of isolated tendon cells were fractionated by gel filtration on Sepharose 2B, a technique that has been shown to give good resolution of liver microsomal and cytosolic fractions (Tangen *et al.*, 1973; Hawkins & Freedman, 1979; Kaderbhai & Freedman, 1980). Turbid material was eluted at the void volume, preceding a broad band of soluble proteins. Electron microscopy of the turbid fraction revealed the presence of small membranous vesicles (Fig. 5). PDI activity was associated mainly with this void-volume material. Table 1 summarizes the distribution of PDI and a number of enzymes in a two-stage subcellular fractionation-differential centrifugation to give 'nuclear' pellet, 'mitochondrial' pellet and 'post-mitochondrial supernatant' fractions, followed by gel filtration to resolve the 'post-mitochondrial supernatant' fraction into 'microsomal' and 'cytosol' fractions. After differential centrifugation, succinate dehydrogenase and acid phosphatase are found predominantly in the 'mitochondrial' pellet frac-

Fig. 5. *Electron-microscopic appearance of the vesicle fraction obtained by gel filtration of chick-embryo tendon-cell homogenate*

Tendon cells were isolated by digestion, and homogenized as described in the Materials and methods section. The homogenate was fractionated as described in the legend to Table 1. The turbid fractions from Sepharose 2B eluted at the void volume were prepared for electron microscopy as previously described (Hawkins & Freedman, 1979).

tion, whereas RNA, glucose-6-phosphatase and PDI are predominantly in the 'post-mitochondrial supernatant'; these three markers are then found predominantly in the void-volume fraction after gel filtration. Gel filtration of a chick-embryo liver post-mitochondrial supernatant gave similar results.

It can be concluded that PDI in chick-embryo tendon cells is associated with the endoplasmic reticulum by the criteria that it co-distributes with markers of the endoplasmic reticulum and not with those for other organelles, that it is associated with particles that are excluded by Sepharose 2B, and that by electron microscopy the particles are

vesicular with the dimensions and appearance typical of vesicles derived from the endoplasmic reticulum.

Comparison of chick-embryo tendon enzyme with that from mammalian liver

It has not been possible to purify the chick-embryo tendon enzyme to homogeneity or to carry out detailed structural characterization. Nevertheless the chick-embryo tendon enzyme appears closely homologous to mammalian liver enzyme in most respects. It shows similar steady-state kinetics towards both substrates, an identical pH optimum and similar high sensitivity to inhibition

Table 1. *Subcellular fractionation of chick-embryo tendon-cell homogenate*

Tendon cells were isolated by digestion, and homogenized as described in the Materials and methods section. The homogenate was centrifuged at 2000g_{av} for 5 min to produce pellet 1, and the supernatant was then spun again at 10000g_{av} for 10 min to produce pellet 2 and a post-mitochondrial supernatant. A 5 ml sample of post-mitochondrial supernatant was then loaded on to a column of Sephadex G-200 (void volume 14 ml) and eluted with TKM buffer containing 0.25 M-sucrose. Fractions eluted around the void and total column volumes were collected. The activities of markers in pellets 1 and 2 and post-mitochondrial supernatant are expressed as percentages of the total recovered in these three fractions, and the activities in the void-volume and total-volume fractions as percentages of the total recovered in these two fractions.

Marker	Activity (% of total)			Activity (% of total)	
	Pellet 1	Pellet 2	Post-mitochondrial supernatant	Void-volume fraction	Total-volume fraction
Protein	28	46	26	72	28
Succinate dehydrogenase	23	77	0	Not determined	
Acid phosphatase	27	49	24	Not determined	
RNA	24	37	39	86	14
Glucose-6-phosphatase	22	23	55	67	33
PDI	14	9	77	82	18

by Cd²⁺. Like the mammalian liver enzyme and that from developing wheat endosperm (Roden *et al.*, 1982), the chick-embryo tendon enzyme is located in the endoplasmic reticulum. This enzyme is extremely acidic, with an isoelectric point (4.4 ± 0.3) very close to that of the homogeneous bovine liver enzyme (4.2 ± 0.1) (Lambert & Freedman, 1983a). The polypeptide identified from SDS/polyacrylamide-gel electrophoresis of active fractions as a component of chick-embryo tendon PDI has M_r 55 000, compared with the 57 000 found for the polypeptide M_r of the homogeneous preparations from bovine liver and rat liver (Lambert & Freedman, 1983a; Mills *et al.*, 1983). There is a discrepancy in the M_r observed by gel filtration, this being 140 000 for the chick-embryo tendon enzyme and 110 000 for PDI from other sources. This may reflect a genuine difference in quaternary structure, but could also indicate non-specific interaction between chick-embryo tendon PDI and some other component of the crude preparations on which this measurement was made. The evidence overall indicates a strong degree of homology of this enzyme from widely different sources.

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