PROTEIN DISULPHIDE ISOMERASE ACTIVITY IN COLLAGEN-SYNTHESISING TISSUES OF THE CHICK EMBRYO

Richard HARWOOD
Department of Biochemistry, University College, PO Box 78, Cardiff CF1 1XL, Wales

and

Robert B. FREEDMAN
Biological Laboratory, University of Kent, Canterbury CT2 7NJ, England

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1. Introduction

Remarkably little is known about the process of formation of native disulphide bonds during the biosynthesis of proteins; neither the mechanism, the catalyst(s) nor the ultimate source of oxidising equivalents is definitely established [1,2]. This process is of particular interest in the biosynthesis of the genetically-distinct collagen types I and II in which the characteristic triple-helical collagen conformation is assembled in the precursor form, procollagen, where the three polypeptides are joined together by inter-chain disulphide bonds between the carboxyl-terminal non-helical extension regions of the precursor chains [3–6]. Although these carboxyl-terminal regions are subsequently excised extracellularly, there is considerable evidence that the formation of these disulphide bonds is an essential step in the post-translational chemical and conformational changes which lead to the formation of native collagen [7,8].

Protein disulphide isomerase (EC 5.3.4.1), an enzyme first described [9,10] catalyses thiol/protein disulphide interchange and hence converts disulphide pairings in proteins to the most stable accessible arrangement. The enzyme is quite widely distributed and it was proposed [11] that it functions to ensure the rapid attainment of the native disulphide pairing during protein synthesis. Evidence relating to this role for the enzyme has recently been summarised [1].

Since the mechanism of formation of the inter-chain disulphide bonds in procollagen is unknown, it was interesting to determine if protein disulphide isomerase is present in tissues specialised for the synthesis and secretion of this protein. We report here the detection of protein disulphide isomerase activity in microsomal fractions from embryonic chick tendon and cartilage cells which synthesise type I and type II procollagen, respectively.

2. Materials and methods

2.1. *Preparation of matrix-free cells and isolation of microsomal fractions*

Isolated tendon and cartilage cells were obtained by digestion of leg tendons and sternal cartilages from day 17 chick embryos with bacterial collagenase and trypsin using a modification [12] of the procedures in [13,14]. The removal of the extracellular connective tissue matrix makes possible the subcellular fractionation of these cells and microsomal preparations were isolated from tendon (3.9×10^9 cells) and cartilage (2.4×10^9 cells) cells by methods in [12]. The characterisation of such fractions by electron microscopy and marker enzyme analysis is in [12].

Liver tissue was dissected from day 17 chick embryos and microsomal fractions isolated by differential centrifugation as above [12].

2.2. *Assay of protein disulphide isomerase activity*

Protein disulphide isomerase was assayed by the reactivation of 'randomly' reoxidised ribonuclease as in [15]. Samples of microsomal membranes were incubated with the substrate and dithiothreitol at 30°C in 50 mM Tris–HCl (pH 7.5) buffer containing 0.25 M sucrose, 25 mM KCl, and 5 mM $MgCl_2$. Samples were removed for ribonuclease assay at various time intervals (see fig.1) and the regenerated activity was determined against a high molecular weight RNA substrate by following the change in E_{260} relative to E_{280} using the dual-wavelength mode of a Perkin-Elmer 356 spectrophotometer. Ribonuclease activities were estimated from the linear change over the first 2–3 min; a plot of ribonuclease activity against time of withdrawal gave a linear time course for 30 min, from which protein disulphide isomerase activity was obtained. One unit of ribonuclease activity is defined as that producing a relative absorbance change of 1 unit/min; one unit of isomerase activity is then defined as that catalysing re-activation of ribonuclease at the rate of 1 unit/min [15]. Correction was made for background re-activation by dithiothreitol alone.

Protein concentrations were determined as in [16] with bovine serum albumin as standard.

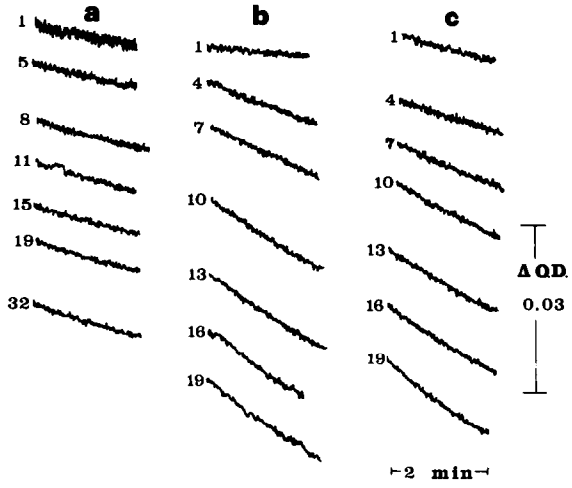

Fig.1. Protein disulphide isomerase activity of chick embryo tissue fractions. Each spectrophotometer trace is a ribonuclease assay performed on an aliquot withdrawn at the time indicated between 1 min and 32 min after setting up an isomerase incubation. Assays from a single incubation are represented as a vertical series. The rate of increase of ribonuclease activity is the measure of isomerase activity. (a) Control incubation of 50 μg randomly reoxidised ribonuclease with 10 μM DTT; (b) as (a) but in the presence of tendon microsomes (0.82 mg protein); (c) as (a) but in the presence of liver microsomes (0.98 mg protein).

3. Results and discussion

The results obtained in this study indicate that it is possible to detect protein disulphide isomerase activity reproducibly in microsomal fractions derived from embryonic chick tendon and cartilage cells and liver tissue. Figure 1 shows the re-activation of 'randomly' re-oxidised ribonuclease in the presence of dithiothreitol alone and on the addition of tendon and liver microsomes. The observed specific activities for the fractions studied were 0.34 units/g protein, 0.22 units/g protein, and 0.07 units/g protein for tendon, liver and cartilage microsomes, respectively.

It is interesting that the protein disulphide isomerase activities in collagen-synthesising tissues are comparable to that observed in chick embryo liver. In studies on the tissue distribution of protein disulphide isomerase in bovine [17] and sheep tissues (D. Hillson and R.B.F., in preparation) the liver was found to have the highest specific activity of those tested. The distributions of protein disulphide isomerase found in these two studies indicated that activity was highest in tissues which secrete protein, so the high level of isomerase in chick embryo tendon microsomes may reflect their very active synthesis of procollagen [13].

The constituent chains of collagen types I and II are synthesised in precursor forms, pro-α chains, having both amino- and carboxyl-terminal extension peptides [3–6]. These precursor polypeptides are assembled into the respective triple-helical procollagen molecules within the lumen of the endoplasmic reticulum of the cells concerned [6]. Comparative studies of the molecular size of these procollagens under reducing and non-reducing conditions indicate that the pro-α chains are linked through disulphide bonds and analyses of the location of these bonds have demonstrated that they are restricted to the carboxyl-terminal, non-helical extensions [3–6]. Intra-chain disulphide bonds have also been shown to exist within both the amino- and carboxyl-terminal domains of the type I procollagen molecule [18,19]. The possibility of a relationship between the process of inter-chain

disulphide bond formation between pro-α chains and that of triple-helix formation is strongly suggested by the close correlation observed between the timing of the two processes [20,21]. Since the enzymes responsible for some of the post-translational modifications of the pro-α chains, e.g., lysine hydroxylase, do not function on the triple-helical procollagen molecule, the timing of the attainment of this conformation may well be critical in determining the extent of these modifications in a particular procollagen type. The rate of inter-chain bonding and therefore triple-helix formation, does seem to vary according to cell type and is markedly slower in cartilage cells than tendon cells [20,21]. The cartilage (type II) procollagen exhibits a higher degree of secondary modification than tendon (type I) procollagen and these considerations indicate that a clear understanding of the mechanisms involved in the disulphide bonding process is of considerable importance. The detection of protein disulphide isomerase in embryonic chick tendon and cartilage cells, in which procollagen represents the predominant secretory protein synthesised, suggests that the enzyme may well be involved in the assembly of procollagen; catalysing the formation of the correct intra- and inter-chain disulphide bonds found in the peptide extensions. It is particularly noteworthy that the level of the enzyme is consistently lower in cartilage cells, where the rate of inter-chain disulphide bond formation between pro-α chains is slower than in tendon cells.

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