The Relative Protein Disulphide Isomerase (PDI) Activities of Gonadotrophins, Thioredoxin and PDI

Chuah Chong Chew, Thierry Magallon*, Nadine Martinat*, François Lecompte, Yves Combarnous* and James P. Gosling

Department of Biochemistry, University College Galway, Ireland. *Station de Physiologie de la Reproduction, INRA Centre de Recherches de Tours, 37380 Nouzilly, France.

The three-dimensional configuration assumed by a polypeptide chain occurs spontaneously, with or without the participation of molecular chaperones, and is due to the combined interactions of the amino acids in the chain. In the case of disulphide-bond containing proteins, one or more enzymes catalyse the random cleavage and correct reformation of nascent protein disulphide bonds, e.g. protein disulfide-isomerase (PDI).

PDI was first discovered in the nineteen sixties and was purified from bovine liver by Anfinsen and coworkers. The PDI active site contains reducible disulphide groups that participate in the reactivation of denatured protein. Generally, its activity is measured with respect to the catalysis of correct disulphide formation, and therefore reactivation, of reduced RNAase. Alternatively oxidized denatured ('scrambled') RNAase can be used as substrate and disulphide isomerization leading to reactivation measured.

That thioredoxin (TRX), a ubiquitous, monomeric, multifunctional protein may have a role analogous to that of PDI was first suggested because its active site includes an amino acid sequence (Trp-Cys-Gly-Pro-Cys) similar to that in the active site of PDI (Trp-Cys-Gly-His-Cys). These vicinyl Cys residues of TRX and PDI can undergo reversible oxidation to form disulphide bonds.

Recently Boniface and Reichert [1] pointed out that the ß-subunits of gonadotropic hormones also contain similar vicinyl Cys residues. Bovine, ovine or porcine LH-ß have the sequence His-Cys-Gly-Pro-Cys at positions 89-93 and bovine, ovine, and porcine FSH-ß have a similar sequence (His-Cys-Gly-Lys-Cys) in the same position. They found experimentally that purified ovine FSH and bovine LH preparations were about 60 and 300 times as active as thioredoxin, respectively.

We used the assay of Pigiet et al. [2] with reduced, denatured RNAase as substrate to compare the activities of gonadotropins (pLH and pFSH), PDI and thioredoxin.

Ribonuclease Assay. The enzyme RNAase acting on RNA catalyses two successive reactions culminating in the cleavage of pyrimidine nucleotide 3' esters. The principle of the assay used is based on the second reaction in that RNAase is allowed to hydrolyse cytidine cyclic 2';3'-phosphate (CcMP) to cytidine 3'-phosphate. The following conditions were used: 0.44 mmol/L CcMP, 0.1 mol/L MOPS-NaOH, pH 7.0 and 5-50 µg/mL RNAase at 25°C. The procedure involved addition of the MOPs buffer and CcMP to a quartz cuvette. Then the enzyme was added, rapidly mixed and the increase in absorption at 284 nm was continuously monitored. The substrate solution was always freshly prepared and the blank used was the reaction mixture without the enzyme. With due care reliable results were obtained and the assay was linear up to a rate of change of 0.1 absorbance units per minute (Figure 1).

Reduction and Denaturation of RNAase. Reduced, denatured RNAase was prepared by incubating the native enzyme (30 mg) overnight in 1.5 mL of 0.1 mol/L Tris-HCl containing 0.15 mol/L dithiothreitol and 6 mol/L guanidine hydrochloride at pH 8.6. The reduced RNAase was then removed from the denaturation reagents by means of Sephadex G-25 equilibrated with 0.01 mol/L HCl. The fractions containing denatured RNAase were pooled, sparged with nitrogen gas, sealed, and stored at -20°C. This was to ensure that no oxygen was present and that thus no renaturation would occur until purposely initiated.

Reactivation of RNAase. Reoxidation and renaturation of RNAase was initiated by diluting the reduced enzyme in 0.1 mol/L Tris, 1mM EDTA and different kind of renaturing gonadotropins etc. at 25°C and pH 7.4. At various times aliquots were assayed and the results obtained were graphed as % total activity against time. The total activity was arbitrarily taken to be the maximum activity observed in the same renaturation experiment.

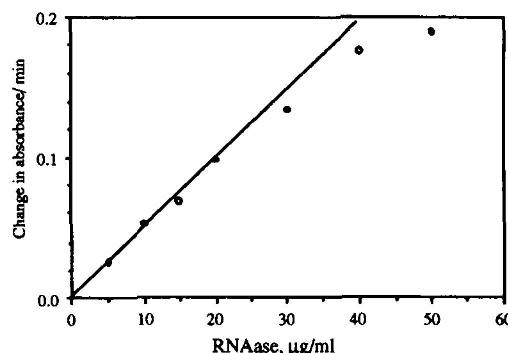

Figure 1. Plot of RNAase initial velocity (change in absorbance at 284 nm) against RNAase concentration.

Table 1. Relative specific activities of TRX, pLH, pFSH and PDI

Enzyme	Source	Mol wt	Slope*	Relative slope
PDI	Bov. liver	107 000	0.0253	95
TRX	E. coli	11 000	0.000265	1
pLH	Porcine	30 000	0.00125	6
pFSH	Porcine	32 000	0.00125	6

*Slope of reciprocal of time in hours for 50% activation of 1 µmol/L RNAase plotted against PDI, TRX etc. concentration in µmol/L.

The half life for reactivation was defind as the time (h) taken for recovery of 50% of total activity. The concentration of denatured RNAase was calculated from the extinction coefficient, 9200 $cm^{-1}.M^{-1}$ at 275 nm, and was about 1 µmol/L.

The above results show that pLH, pFSH, and thioredoxin reactivate inactive denatured RNAase, indicating that pLH, pFSH and TRX may all act in the same way as PDI. However a comparison of the activities of the four protein shows considerable differences (Table 1). PDI is the most efficient, catalysing maximum reactivation with the lowest molarity and at the highest rate; thioredoxin is the least efficient and the hormones are intermediate. The differences may be caused by differences in the amino acid sequences in or near the vicinyl cysteine residues refered to in the introduction, by differences in other parts of the proteins or, of course, by the purities or conditions of the preparations used.

A survey of 11 different preparations of porcine and ovine LH and FSH identified none with greater activity than those above. Purified alpha and beta subunits were inactive.

The biological role of the PDI-like activity of gonadotropins is unclear. Boniface and Reichert suggested the possibility that the bound hormone may catlyse disulphide interchange in its receptor. Or, complete hormone molecules may promote correct disulphide pairing in nascent α and β-hormone chains.

Koedam and van den Brande (3) have recently reported that IGF-I and IGF binding protein-3 also have PDI-like activity.

We thank R. B. Freedman, University of Kent for the gift of PDI.

1. Boniface, J.J. and Reichert, L.E., Jr (1990). Science, 247, 61-63

2. Pigiet, V.P and Schuster, B. J.(1986) Proc.Natl.Acad.Sci. USA. 83, 7643-7647

3. Koedam, J.A. and van den Brande J.L. (1994) Biochem. Biophys. Res. Commun. 198, 1225-1231.