THE IDENTITY OF THE INSULIN DEGRADING THIOL-PROTEIN DISULFIDE OXIDOREDUCTASE (GLUTATHIONE–INSULIN TRANSHYDROGENASE) WITH THE SULFHYDRYL-DISULFIDE INTERCHANGE ENZYME

S. ANSORGE, P. BOHLEY, H. KIRSCHKE, J. LANGNER, I. MARQUARDT, B. WIEDERANDERS and H. HANSON
With the technical assistance of B. KAISER
Physiologisch-chemisches Institut der Universität, 402-Halle (Saale), DDR

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1. Introduction

In previous studies [1–3] we reported on a glutathione–insulin transhydrogenase from microsomes of rat liver. The substrate specificity of this enzyme is not restricted to insulin and glutathione. We were able to show that this enzyme catalyzes also the reduction of a number of other disulfide-containing proteins, e.g. trypsin, chymotrypsin, chymotrypsinogen, pepsin, bovine serum albumin, γ-globulin, oxytocin, lysozyme, ribonuclease, 'randomly-oxidized' ribonuclease, and the intermediate of proinsulin [4,5]. Because of the broad specificity we prefer the term thiol-protein disulfide oxidoreductase (TPO) instead of glutathione–insulin transhydrogenase (GIT) for this enzyme system (Katzen et al. [6]).

The specificity of action of the TPO on protein-bound disulfide groups as well as its intracellular localization suggested that this enzyme might be identical with or closely related to the sulfhydryl–disulfide interchange enzyme (ribonuclease-reactivating enzyme) discovered by Anfinsen et al. [7] and also by Venetianer and Straub [8]. Indications supporting this assumption were given by Katzen and Tietze [6], Givol et al. [9] and Narahara [10], but convincing evidence as to our knowledge was lacking until now.

The sulfhydryl–disulfide interchange enzyme has been shown to be present in several organs and was purified from bovine liver microsomes [7, 8, 11–13].

The present report gives conclusive evidence on the identity of the microsomal TPO and the sulfhydryl–disulfide interchange enzyme.

Preliminary results concerning this problem have been published [3–5].

2. Materials and methods

The purification of the microsomal TPO was performed as described previously [3]. 'Randomly-oxidized' ribonuclease was prepared according to Anfinsen et al. [14,15] by reduction of native ribonuclease (Serva, Heidelberg) with 2-mercaptoethanol followed by air-oxidation in the presence of 8 M urea. The conditions for the reactivation of 'randomly-oxidized' ribonuclease were similar to those described by Givol et al. [9] (for details see legend of fig. 1.). Amino acid composition of TPO-preparations was determined after hydrolysis with 6 M HCl at 110°C for 24 hr with an Automatic Amino Acid Analyzer Unichrom (Beckman, Munchen). Activity of ribonuclease was determined as described by Fiers [16].

3. Results and discussion

The following two experimental findings give evidence on the identity of the sulfhydryl–disulfide interchange enzyme [12, 13] and the microsomal TPO [3].

3.1. As shown in fig. 1, the reactivation of 'randomly-oxidized' inactive ribonuclease is strongly accelerated by the microsomal TPO from rat liver. The reaction

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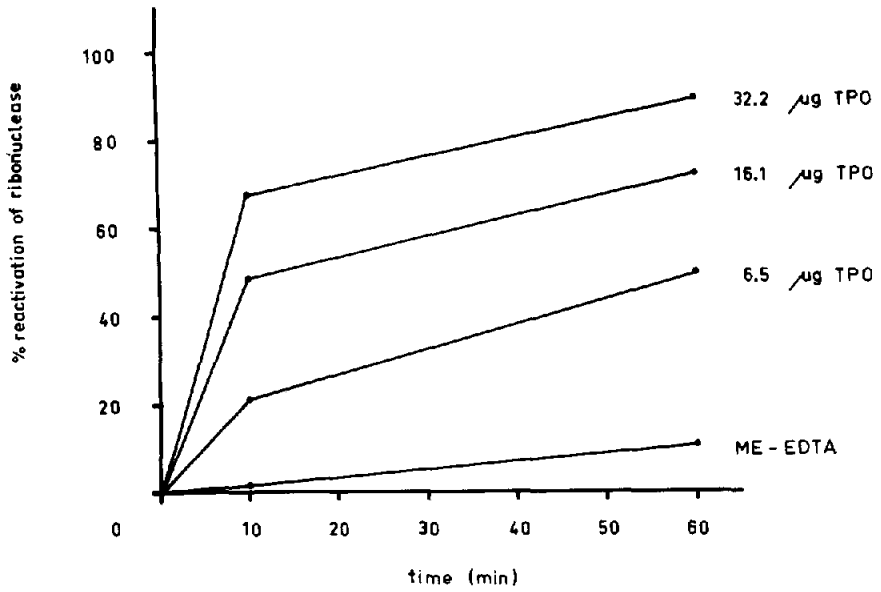

Fig. 1. Reactivation of 'randomly-oxidized' inactive ribonuclease by the microsomal TPO. Reactivation mixtures contained in a final volume of: 0.5 ml 0.1 M Tris buffer, pH 7.5; 1 mM 2-mercaptoethanol (ME); 1 mM EDTA, 10 µg 'randomly-oxidized' ribonuclease and 0, 6.5, 16.1, and 32.2 µg microsomal TPO. Incubations were carried out at 37°C. After the given times 0.1 ml of incubation mixture was added to 0.9 ml 0.1 M acetate buffer, pH 5.0. Activity of ribonuclease was determined at pH 5.0 as described by Fiers [16].

depends on time and enzyme concentration. The values measured were similar to those reported by Givol et al. [9] who used disulfide interchange enzyme from bovine liver.

3.2. Further evidence concerning the identity is provided by the comparison of the amino acid composition of these two enzymes (table 1). Although these enzymes were isolated from different animals by different methods they show a high degree of identity in the content of each amino acid.

Only recently Varandani [17] has isolated and characterized a GIT from rat liver. Since he used acetone powders of liver homogenates as starting material, it is impossible to decide whether this GIT is of microsomal origin and whether it is identical with the TPO (or GIT) isolated in our laboratory. However, apart from the same specificity there are some indications, such as similar K_M-values for insulin and glutathione and a similar molecular weight, proving that these two enzymes are identical proteins. Accordingly, we believe that all GIT's purified from different tissues and species as yet (summarized in ref. [18]) are identical with the ribonuclease-reactivating enzyme, too.

In addition to the microsomal TPO, we found rat liver, as well as other tissues, to contain two further TPO's, both localized in the cytosol fraction [2, 4, 5]. These enzymes are distinguished from one another by their molecular weights of 70 000–90 000 and about 10 000 respectively [2, 4, 5]. Like the microsomal TPO, the low molecular weight TPO of the cytosol was shown also to be capable of enhancing the reactivation of scrambled ribonuclease [4]. There is some evidence that these two enzymes are structurally related to the microsomal TPO, too [5].

The possible physiological function of this enzyme system deserves comment. On the one hand the microsomal enzyme is thought to be involved in the formation of 'correct' disulfide bonds during the biosynthesis of disulfide-containing proteins [19]. On the other hand, the TPO system catalyzes the reduction of protein-bound disulfide groups under physiological conditions [5]. These apparently opposite reactions seem to be based primarily on the same enzymatic mechanism, namely on the reduction of protein-bound disulfide groups. Whether the final result of the TPO-catalyzed reaction is the partially or

Table 1
Comparison of the amino acid composition of the microsomal TPO from rat liver [3] and the sulfhydryl–disulfide interchange enzyme from bovine liver [12].

Residue	Moles amino acid per 100 Moles of total imino acids	
	TPO from microsomes of rat liver	Sulfhydryl–disulfide interchange enzyme from bovine liver [12]
Lysine	10.70	10.37
Histidine	2.68	2.16
Arginine	2.10	1.98
Aspartic acid	12.38	11.46
Threonine	4.81	4.23
Serine	4.38	4.47
Glutamic acid	14.50	15.84
Proline	3.97	3.85
Glycine	6.18	6.46
Alanine	9.36	9.40
Valine	5.44	5.16
Methionine	0.93	0.83
Isoleucine	4.37	4.47
Leucine	9.22	9.05
Tyrosine	2.30	2.26
Phenylalanine	6.51	7.00
S-Carboxymethyl-cysteine		0.93
1/2 Cystine	Trace	
Cysteic acid	0.00	
Sum	99.83	99.92
Cysteic acid (after oxidation)	1.39	

The values reported are the average of analyses of two different preparations of the TPO from rat liver (with exception of the value for cysteic acid after oxidation).

totally reduced inactive protein or the 'correct'-folded native structure seems to depend particularly on the thermodynamic and kinetic stability of the protein structure itself [12], and on the thiol concentration in the environment of the enzyme. For example, as we have found by the spectrophotometric assay [6], chymotrypsinogen and native ribonuclease are reduced 2–3 times more slowly than chymotrypsin and 'randomly-oxidized' ribonuclease, respectively by the microsomal TPO [5].

The present findings on the different actions of TPO's studied in vitro suggest that this enzyme system might have at least a 2-fold function in vivo, i.e. in the formation of the native protein structure during the biosynthesis as well as in the breakdown of disulfide-containing proteins.

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