A Possible Role of Cytoplasmic Thioltransferase in the Intracellular Degradation of Disulfide-containing Proteins *

KENT AXELSSON and
BENGT MANNERVIK

Department of Biochemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm, Sweden

Little is known about the chemical reactions which *in vivo* lead to formation and scission of disulfide bonds in proteins. It is probable that thiol-disulfide interchange mediates these reactions. Glutathione (GSH **), which is the most abundant low-molecular-weight thiol,[1] is a likely reductant for the scission of protein disulfide groups in the cell. Thiol-disulfide interchange reactions take place spontaneously, but they are probably enzyme-catalyzed to be amenable to control *in vivo*. In rat liver cytosol the enzyme thioltransferase catalyzes reduction of sulfur – sulfur bonds of various substrates by use of glutathione as a reductant.[2,3] The present report shows that homogeneous thioltransferase[2] catalyzes the reduction of protein disulfide bonds provided that they are sterically accessible, and indicates the possible involvement of thioltransferase in the intracellular degradation of disulfide-containing proteins.

Bovine serum albumin was selected as a model substrate for the reactions considered. The molecule contains 17 intracatenary disulfide bridges and one cysteinyl residue.[4] The reactivity of the disulfide bonds of albumin was assayed before and after treatment with proteolytic enzymes (Table 1). In untreated albumin only 0.68 disulfide bond per albumin molecule was accessible to reduction by GSH. This amount probably represents the mixed disulfides of albumin and GSH or cysteine.[5] Thioltransferase increased the rate of reduction of these disulfide bonds significantly, but did not change the number of accessible bonds. Pretreatment of albumin with proteases increased 5- to 10-fold both the number of disulfide bonds reduced and the velocity of the thioltransferase-catalyzed reduction. The rate of the spontaneous reduction was only slightly enhanced after proteolysis. A corresponding experiment was made with urea-treated albumin. Qualitatively similar results were obtained with egg-white lysozyme, ovalbumin, and ribonuclease, which all contain disulfide bonds. The effect of increasing the GSH concentration was investigated by a change from 0.5 mM in the standard assay system to the range (5 – 10 mM) prevailing in rat liver cytosol.[6] Table 1 shows that for both proteolytically modified and urea-denatured albumin the physiological concentrations of GSH gave the highest rates of the reactions catalyzed by thioltransferase.

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** Abbreviation: GSH, glutathione.

Table 1. Effect of cytoplasmic thioltransferase and GSH on the velocity of disulfide reduction in bovine serum albumin denatured by treatment with proteases or urea.

Treatment	Reaction	Velocity/μM min^{-1} [a] GSH concentration			Accessible disulfide bonds per molecule of albumin [b]
		0.5 mM	5 mM	10 mM	
Control	Spontaneous	1.0	9.2	n.d.	
	Thioltransferase-catalyzed	27	112	n.d.	0.68
Protease	Spontaneous	1.3	22	34	
	Thioltransferase-catalyzed	140	900	810	10.6
Urea	Spontaneous	3.5	43	55	
	Thioltransferase-catalyzed	162	1170	1580	2.7

[a] The amount of thioltransferase used in the actual measurements was 0.07 unit, but the values given for the enzymatic velocity have been recalculated to correspond to the apparent mean concentration of thioltransferase in liver cytosol. For this calculation a factor of 1.4 has been used to convert unit per g wet weight to unit per ml cytosol (see Tables II and III in Ref. 6). Velocities refer to initial rates; zero order kinetics were applicable for 10 to 15 % of the course of the reaction. The enzymatic velocity was obtained by subtraction of the rate of the spontaneous reaction. n.d. = not determined. [b] The number of accessible bonds was not determined for the spontaneous reaction in this experiment, but was in a separate investigation found not to differ significantly from the values obtained in the presence of enzyme (see also Ref. 9).

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The mechanisms of intracellular degradation of proteins are generally assumed to involve conformational changes, which make the covalent bonds available to cleavage.[7,8] Partial denaturation may be brought about by interaction with the cellular membranes, removal of cofactors, or limited proteolysis. Native proteins appear to have structures which protect internal disulfide linkages as well as peptide bonds from cleavage. Thus, the non-enzymatic scission of disulfide bonds in proteins by reduction with GSH has been studied by Davidson and Hird, and it was found that most disulfide bonds were inaccessible unless some degradation or denaturation made them available.[9] These findings were confirmed by the present investigation. However, the broad substrate specificity of the cytoplasmic thioltransferase in rat liver,[2,3] led us to assume that the thioltransferase could participate in the scission of disulfide bonds in the degradation of proteins. Table 1 shows that the enhancement of disulfide reduction in a denatured protein in the cytosol of a liver cell may be >100-fold at 0.5 mM GSH and >40-fold at 5 mM GSH, if the data are recalculated to the apparent mean concentration of cytoplasmic thioltransferase in liver (as determined in the post-microsomal fraction of a liver homogenate). It is important to note that in none of the proteins studied did the number of susceptible bonds increase in the presence of thioltransferase as compared with the number found in the non-enzymatic reaction. Thus, the enzyme cannot by itself elicit changes of the tertiary structure of the substrate protein molecule which render the disulfide bonds more susceptible to reaction. The effect of the thioltransferase appears to be limited to enhancing the rate of reaction of the groups already available for the spontaneous reaction with GSH.

It is generally believed that most of the degradation of proteins takes place in lysosomes, and thioltransferase activity has been demonstrated in these organelles.[10] However, the role of lysosomes in protein degradation, although most important, is not unique, and some of the degradation may be located to the cytoplasm.[11] We, therefore, suggest that also the cytoplasmic thioltransferase may be involved in the reduction of disulfide bonds in intracellular protein degradation.

Experimental. Protein solutions at concentrations in the range 0.1 – 0.6 mM were preincubated for 20 h at 30 °C with a mixture of proteases (α-chymotrypsin, trypsin, and Pronase P, each at 0.4 μM concentration) in 40 mM Tris-HCl buffer (pH 8.1) containing 10 mM $CaCl_2$. When urea was used as a denaturant the proteins were preincubated for 20 min in 0.1 M sodium phosphate buffer (pH 7.5). The rate of disulfide reduction was measured at 30 °C essentially as described in Ref. 2. The reaction system contained: 0.13 M Tris-HCl buffer (pH 8.1), 0.1 mM NADPH, 0.5 mM GSH, 2 units of glutathione reductase, 0.07 unit of purified thioltransferase[2] and substrate. The various substrates were intact or pretreated disulfide-containing proteins, which were added to give a final concentration corresponding to 0.03 – 0.2 mM intact protein in the assay system. The spontaneous reduction of protein disulfide groups by GSH was measured in the absence of thioltransferase.

Determination of accessible disulfide bonds was made in the same assay system. The decrease of A_{340nm} was used as a measure of accessible disulfide bonds; controls showed that the concentration of NADPH was always in excess of the reducible disulfide bonds.

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