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Disulphide reduction in lysosomes

The role of cysteine

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Published evidence indicates that cystine-containing proteins have their disulphide bonds reduced during proteolysis in lysosomes. However, the intralysosomal accumulation of cystine in the cells of patients with cystinosis has been seen as evidence that protein cystine residues are not reduced. The data are reconcilable and fully in harmony if it is postulated that cysteine from the cytoplasm is the physiological reducing agent.

When proteins that contain disulphide bonds are degraded within lysosomes, are those disulphides reduced? The available evidence appears to be conflicting.

Arguments for the reduction of cystine residues arise from studies on the stimulatory effects of thiols on lysosomal proteolysis. Mego (1973) observed that thiols stimulate protein degradation in intact lysosomes, but this could have been due simply to activation of the thiol-dependent cathepsins. Griffiths & Lloyd (1979), however, demonstrated that degradation of ^{125}I-labelled bovine insulin by rat liver lysosomal enzymes proceeded even when the thiol proteinases were inhibited by leupeptin, and that this residual proteolytic activity was stimulated by cysteine. Subsequently Kooistra *et al.* (1982) showed that the degradation of bovine insulin or albumin by purified cathepsin D was thiol-stimulated. Since cathepsin D is not affected by thiol, and since no other enzyme was present, it was concluded that the role of thiol was the non-enzymic reduction of disulphide linkages, facilitating access by the proteinase to susceptible linkages in the substrate. This view is reinforced by subsequent data from Mego (1984), who showed that thiols activate proteolysis by cathepsin D only if the substrate protein contains disulphide bridges. A further insight into the role of thiol is provided by Kooistra *et al.* (1982): proteolysis proceeds optimally only if thiol is present together with proteinase in the incubation mixture; a pre-treatment with thiol will not suffice, nor is thiol able to reduce all the disulphide linkages in a protein in the absence of proteinase. There would therefore appear to be a synergism between disulphide reduction and enzyme-catalysed peptide bond hydrolysis, each component process opening out the substrate molecule and so permitting further attack.

Evidence that unreduced cystine is a product of intralysosomal proteolysis derives chiefly from the study of cystinosis, a human metabolic disease that is inherited as a Mendelian recessive (Schneider & Schulman, 1982). Cystinosis is characterized biochemically by a high cystine concentration in the lysosomes, with normal concentrations in the cytoplasm and extracellular fluids. The cystine of cystinotic fibroblasts may be depleted by incubation *in vitro* with the thiol cysteamine, and it is found that the rate of reaccumulation correlates with the presence of cystine-containing proteins in the culture medium (Thoene & Lemons, 1982). This and earlier (Thoene *et al.*, 1977; Thoene & Lemons, 1980) evidence indicates that cystine accumulates in lysosomes of cystinotic cells as a consequence of lysosomal digestion of exogenous and/or endogenous cystine-containing proteins.

These data on cystinotic cells are consistent with the metabolic defect being one of cystine reduction, perhaps through an under-production of the physiological reducing agent (Kooistra *et al.*, 1982). More recent work, however, has shown that the defect in cystinosis is the absence of a cystine-transport system that is present in the lysosome membrane of normal cells (for a review see Kooistra *et al.*, 1984). Cystine leaves normal lysosomes as cystine, without prior reduction to cysteine.

Mego (1984) explicitly draws attention to the paradox presented by these results. If cystine residues in proteins are reduced during proteolysis, why do cells need a cystine-transport system in the lysosome membrane and why does its absence (in cystinosis) cause profoundly elevated intralysosomal concentrations of cystine?

I suggest that the resolution of this paradox lies in the identity of the reducing agent responsible for intralysosomal reduction of protein disulphide bonds. Two candidates have been canvassed. The first is cysteamine (Thoene *et al.*, 1976; Kooistra *et al.*, 1982). This substance is a normal component of the cytoplasm, albeit in low concentration, is able to cross the lysosome membrane in both its reduced and oxidized (cystamine) form (Kooistra *et al.*, 1982), and reduces cystine in cystine-loaded isolated lysosomes (Gahl *et al.*, 1985). However, this hypothesis fails to explain why cystine accumulates in cystinotic cells: cysteamine is present in normal concentrations in these cells (Orloff *et al.*, 1981). The other candidate for physiological reductant is reduced glutathione (GSH). However, it appears that GSH cannot cross the lysosome membrane (Mego, 1984, 1985; Gahl *et al.*, 1985).

A third candidate, although the most obvious, has not been considered. Cysteine is abundant in the cytoplasm and can penetrate the lysosome membrane in normal and cystinotic cells (Kooistra *et al.*, 1982; Gahl *et al.*, 1982). Cysteine may also arise in the lysosomes by degradation of cysteine-containing proteins. Like any other thiol, it could by two sequential reactions fully reduce a protein cystine residue (Fig. 1), but unlike other reductants would

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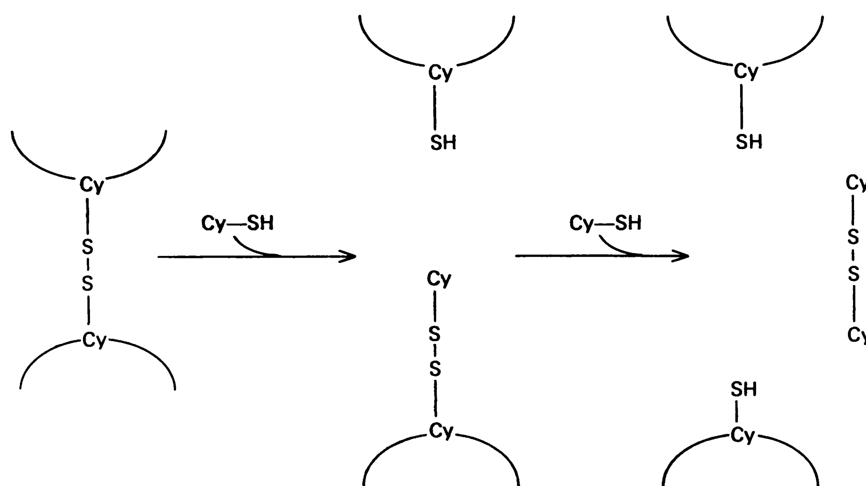

Fig. 1. Diagram illustrating the reduction of a protein disulphide bridge by two consecutive reactions with cysteine (Cy-SH)

Note that cystine is a product of this reaction sequence and that it arises from the reductant and not from the cystine residue reduced.

in the process generate a molecule of cystine. This cystine, arising not from the protein itself but from the reductant, would still require a cystine transporter for efflux from the lysosome. Reduction by cysteine is plausible on thermodynamic grounds. Cysteine and protein-bound cystine must have very similar redox potentials, and a high cysteine/cystine concentration ratio in the lysosomes will be maintained by the reductive effect of cytoplasmic GSH. Whether intralysosomal disulphide interchange is enzyme-catalysed must remain an open question. There is currently no evidence of a lysosomal disulphide reductase, and cysteamine has been shown to reduce cystine in a simple buffer solution, albeit at pH 7.4 (Thoene *et al.*, 1976).

When Mego (1984) argues that 'the defect in cystinosis appears to be impairment of cystine transport from lysosomes, which suggests that protein disulphide bonds...are not reduced in these lysosomes', the reasoning is invalid if cysteine is the physiological reducing agent. Finally, Shen *et al.* (1985) report the cytotoxicity of a disulphide-linked methotrexate–poly(D-lysine) conjugate. Their results indicate that, if the disulphide reduction takes place in lysosomes, the reductant is not cysteamine or GSH, and conjecture that reduction takes place enzymically 'in a prelysosomal compartment'. They state, however, that their 'results cannot rule out the possibility that the disulphide spacer in the conjugate is cleaved in lysosomes by a nonenzymatic and pH-independent reduction with a hydrogen donor other than glutathione and cysteamine.' Non-enzymic reduction by cysteine would seem to offer the simplest explanation of their interesting results.

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