- Batke, J. (1989a) FEBS Lett. **251**, 13–16
- Batke, J. (1989b) Trends Biochem. Sci. **14**, 481–482
- Batke, J. (1991) J. Theor. Biol. **152**, 41–46
- Batke, J., Asbóth, G., Lakatos, S., Schmitt, B. & Cohen, R. (1980) Eur. J. Biochem. **107**, 389–394
- Brooks, S. P. J. & Storey, K. B. (1991) Biochem. J. **278**, 875–881
- Chock, P. B. & Gutfreund, H. (1988) Proc. Natl. Acad. Sci. U.S.A. **85**, 8870–8874
- Christen, P., Cogoli-Greuter, M., Healy, M. J. & Lubini, D. (1976) Eur. J. Biochem. **63**, 223–231
- Creighton, D. J., Migliorini, M., Pourmotabbed, T. & Guha, M. K. (1988) Biochemistry **27**, 7376–7384
- Ehrlich, R. S. (1987) J. Biol. Chem. **262**, 269–271
- Endre, Z. H. & Kuchel, P. W. (1986) Biophys. Chem. **24**, 337–356
- Datta, A., Merz, J. M. & Spivey, H. O. (1985) J. Biol. Chem. **260**, 15008–15012
- Friedrich, P. (1984) Supramolecular Enzyme Organization: Quaternary Structure and Beyond, Oxford, Pergamon Press & Budapest, Akadémiai Kiadó
- Friedrich, P. (1985) in Organized Multienzyme Systems: Catalytic Properties (Welch, G. R., ed.), pp. 141–176, Academic Press, Orlando, London
- Friedrich, P., Apró-Kovács, A. V. & Solti, M. (1977) FEBS Lett. **84**, 183–186
- Harris, J. I. & Waters, M. (1976) Enzymes 3rd. Ed. **13**, 1–49
- Jacobson, K. & Wojcieszyn, J. (1984) Proc. Natl. Acad. Sci. U.S.A. **81**, 6747–6751
- Jencks, W. P. (1964) Prog. Phys. Org. Chem. **2**, 63–128
- Keleti, T., Ovádi, J. & Batke, J. (1989) Prog. Biophys. Mol. Biol. **53**, 105–152
- Kvassman, J. & Pettersson, G. (1989) Eur. J. Biochem. **186**, 265–272
- Kvassman, J., Petterson, G. & Ryde-Petterson, U. (1988) Eur. J. Biochem. **172**, 427–431
- Leslie, A. G. W. & Wonacott, A. J. (1984) J. Mol. Biol. **178**, 743–772
- Lienhard, G. E. & Jencks, W. P. (1966) J. Am. Chem. Soc. **88**, 3982–3995
- Masters, C. J. & Winzor, D. J. (1981) Arch. Biochem. Biophys. **209**, 185–190
- Mastro, A. M., Babich, M. A., Taylor, W. D. & Keith, A. C. (1984) Proc. Natl. Acad. Sci. U.S.A. **81**, 3414–3418
- Orosz, F. & Ovádi, J. (1987) Biochim. Biophys. Acta **915**, 53–59
- Ovádi, J. (1991) J. Theor. Biol. **152**, 1–22
- Ovádi, J. & Keleti, T. (1978) Eur. J. Biochem. **85**, 157–161
- Ovádi, J., Salerno, C., Keleti, T. & Fasella, P. (1978) Eur. J. Biochem. **90**, 499–503
- Ovádi, J., Mohamed-Osman, I. R. & Batke, J. (1983) Eur. J. Biochem. **133**, 433–437
- Ovádi, J., Mátrai, Gy., Bartha, F. & Batke, J. (1985) Biochem. J. **229**, 57–62
- Patthy, L. (1978) Eur. J. Biochem. **88**, 191–196
- Patthy, L. & Vas, M. (1978) Nature (London) **276**, 274–276
- Patthy, L., Váradi, A., Thész, J. & Kovács, K. (1979) Eur. J. Biochem. **99**, 309–313
- Rae, C., Berners-Price, S. J., Bulliman, B. T. & Kuchel, P. W. (1990) Eur. J. Biochem. **193**, 83–90
- Rashevsky, N. (1960) Math. Biophys. **1**, 13
- Rognstad, R. (1991) Arch. Biochem. Biophys. **286**, 555–561
- Solti, M. & Friedrich, P. (1979) Eur. J. Biochem. **95**, 551–559
- Srere, P. A. (1967) Science **158**, 936–937
- Vander Jagt, D. L., Daub, E., Krohn, J. A. & Han, L. P. B. (1975) Biochemistry **14**, 3669–3675
- Vas, M. & Batke, J. (1990) Eur. J. Biochem. **191**, 679–683
- Vértessy, B. G. & Ovádi, J. (1987) Eur. J. Biochem. **164**, 655–659
- Vértessy, B. G., Orosz, F. & Ovádi, J. (1991) Biochim. Biophys. Acta **1078**, 236–242
- Wu, X., Gutfreund, H., Lakatos, S. & Chock, P. B. (1991) Proc. Natl. Acad. Sci. U.S.A. **88**, 497–501

reduced in the course of proteolytic degradation in lysosomes. The available evidence appeared contradictory. On the one hand, several investigators had shown that the presence of thiol is necessary for the efficient degradation of disulphide-containing proteins by lysosomal enzymes and that this requirement could not be attributed solely to the thiol-requirement of some of the proteinases. On the other hand, cystine accumulates in the lysosomes of cells of cystinosis patients, in which the lysosome membrane cystine porter is absent, and this accumulation correlates with the degradation of proteins that contain cystine residues.

I proposed that the apparent conflict resolves if disulphide-containing proteins are indeed reduced during lysosomal proteolysis and if cysteine is the reducing agent. If this is the case, the cysteine that is generated in the process, and which accumulates in cystinosis, arises from the reducing agent.

Subsequently a report (Pisoni *et al.*, 1990) appeared describing the properties of a human fibroblast lysosome membrane porter specific for cysteine and its close analogue cysteamine. The pH-profile of its activity is unusual: it transports cysteine poorly below pH 6, and shows maximal activity at around pH 7.4. This observation was taken by Pisoni *et al.* (1990) as indicating that the porter's role is to transport cysteine into the lysosome. By contrast, most of the other lysosome membrane porters are assumed to mediate the efflux of metabolites from the lysosome. Pisoni *et al.* (1990) presented their observations as supporting evidence for the role I had earlier proposed for cysteine in the lysosomal breakdown of disulphide-containing proteins.

Recently two reports have appeared in which it is proposed that the reduction of disulphide bridges in lysosomes is a crucial step in protein processing by antigen-processing cells. Collins *et al.* (1991) have synthesized a conjugate in which [¹²⁵I]iodotyrosine is linked to proteins through a disulphide moiety. [¹²⁵I]iodotyrosine is liberated when these conjugates are endocytosed and taken to the lysosomes, indicating disulphide reduction in this subcellular compartment. Jensen (1991) has shown that lysosomal reduction of disulphide bridges in the insulin molecule is a key component in A-chain recognition by class II-restricted T lymphocytes.

I am concerned that in these subsequent publications the stoichiometry of the mechanism I proposed for intralysosomal disulphide reduction has not been fully appreciated. Consider the digestion of a protein that contains cystine but no cysteine moieties. If, as I proposed (Lloyd, 1986), the reducing agent is free cysteine, the cysteine consumed will be exactly balanced by the cysteine liberated when the disulphide moieties in the protein are reduced and its peptide linkages hydrolysed by the cathepsins. There will also be an equivalence between the cystine content of the proteins entering the lysosomes by endocytosis or autophagy and the cystine released from the lysosomes via the cystine porter. It follows that there is no requirement for a net influx of cysteine from the cytoplasm across the lysosome membrane. Pisoni *et al.* (1990) postulate that the lysosome membrane cystine porter constitutes a major route for the delivery of cysteine to the lysosomal compartment. While this may be potentially so, I question the existence of a significant flow of cysteine into lysosomes under physiological conditions. If there is an inward flow, it is likely to constitute an anaplerotic pathway, serving only to maintain the cysteine concentration within the lysosomes by replacing any cysteine that undergoes autoxidation to cystine.

Many cystine-containing proteins also have cysteine residues. When such proteins are degraded in the lysosomes, the consequence will be a net production of both cysteine and cystine. Even allowing for some loss of cysteine by autoxidation, a net efflux of both amino acids across the membrane is likely. Whether cysteine is transported out on the cysteine-specific porter, despite

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Lysosomal handling of cystine residues: stoichiometry of cysteine involvement

In an earlier contribution to this journal (Lloyd, 1986) I addressed the question of whether cystine residues in proteins are

the latter's poor performance at the pH of the lysosomal interior, or on one of the neutral amino acid porters (Pisoni *et al.*, 1990) is a matter for speculation.

The mechanism of antigen processing envisaged by Jensen (1991) and Collins *et al.* (1991) involves the reduction of disulphide linkages in the antigen and the recycling to the cell surface of antigen fragments displaying free cysteine moieties. This mechanism, in which proteolysis does not proceed to completion and free cysteine is not a product, does imply an influx of cysteine into and an efflux of cystine from the lysosome. However, this only applies to antigen-processing cells and even in these cells the process is unlikely to be a quantitatively significant component of total lysosomal protein processing.

The message of this Letter does not weaken the original

argument (Lloyd, 1986) that cysteine is the physiological reducing agent for disulphide reduction in lysosomes. I suggest however that direct experimental evidence for this hypothesis is still lacking.

John B. LLOYD

Cellular Pharmacology Research Group, Department of Biological Sciences, Keele University, Staffordshire ST5 5BG, U.K.

Collins, D. S., Unanue, E. R., & Harding, C. V. (1991) *J. Immunol.* **147**, 4054–4059

Jensen, P. E. (1991) *J. Exp. Med.* **174**, 1121–1130

Lloyd, J. B. (1986) *Biochem. J.* **237**, 271–272

Pisoni, R. L., Acker, T. L., Lisowski, K. M., Lemons, R. M. & Thoene, J. G. (1990) *J. Cell Biol.* **110**, 327–335

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