Degradation of proteins by lysosomes

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SUMMARY

Chapter I is a review of intracellular protein turnover and, in particular, of the role of cellular proteases in this process. Our analysis of the literature leads to the hypothesis that lysosomes are involved.

We began by studying the degradation of proteins added to intact lysosomes in vitro. The experiments, and the results of a reinvestigation of reports claiming the existence of this process are given in chapter II. After a brief introduction, treating the requirement of cellular integrity in protein turnover (II-A), we show, in sections B-D, that the presumed uptake of proteins into lysosomes in vitro and their subsequent degradation inside these organelles, either is caused by artefacts (B and D) or at any rate is not a general phenomenon (C). Uptake of proteins into lysosomes in vitro, if possible at all, thus has not (yet) been demonstrated.

Chapter III is concerned with protein degradation by disrupted lysosomes. Section A is a review of the problems involved and the relationship between in vitro experiments, and the situation in vivo. Section B shows that lysosomal extracts are able to digest a number of native proteins essentially completely. The resistance of some other proteins to degradation cannot, therefore, be ascribed to their resistance to acid denaturation but must be caused by the absence of susceptible peptide bonds at the surface of the native molecules. This section also shows the involvement of thiol cathepsins in protein degradation, and from this observation the existence of a reducing environment within lysosomes in vivo is inferred.

In chapter III-C we describe the role of individual cathepsins in proteolysis by disrupted lysosomes in more detail. Cathepsin D is not essential for degradation of proteins by liver lysosomal enzymes. In the digestion of some denatured proteins, cathepsins B1 and D appeared to be interchangeable, but in the degradation of native proteins, serum albumin at any rate, the activity of a thiol enzyme, probably cathepsin B1, is rate-limiting. The degradation of native proteins like serum albumin and ribonuclease proceeds mainly as an all-or-none reaction. Besides the better known lysosomal peptidases, viz. cathepsins B1, C and D, some other thiol-dependent (endo)protease(s) are involved. Finally, we have shown that hepatocyte lysosomes contain a set of proteases able to degrade serum albumin rapidly and extensively.

In our study on the role of individual cathepsins in lysosomal protein digestion, we have used three antibiotics which strongly inhibit cathepsin B1 (antipain and leupeptin) and cathepsin D (pepstatin). If these inhibitors could be introduced into lysosomes in vivo, e.g. by including them into liposomes, they might be used to prove or disprove the possible involvement of lysosomes in intracellular protein turnover.