PROTEOLYSIS AT NEUTRAL pH IN A LYSOSOMAL–CYTOSOL SYSTEM CAN NOT BE ATTRIBUTED TO THE UPTAKE OF PROTEINS INTO LYSOSOMES

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Received 18 May 1974

1. Introduction

Although intracellular protein degradation is a major factor in determining the cellular concentration of a protein, there is little known about the mechanism of this process [1,2]. It has not been possible to imitate this process in vitro; the energy requirement, noticed in liver slices and isolated cells is lost after cell disruption [3]. Auricchio et al. [4], however, suggested from experiments on the inactivation of tyrosine aminotransferase (TAT) in a liver homogenate at neutral pH, that this enzyme is taken up into and degraded within intact lysosomes. The formerly noticed [5] absence of TAT inactivation and protein degradation in general at neutral pH should, according to Auricchio [4], be attributed to a homogenization procedure, which ruptured the lysosomes. We have tested the validity of this hypothesis for a labelled protein fraction from rat liver cytosol. We did not find any evidence for the uptake of short- or long-lived cytosol proteins into intact lysosomes.

2. Materials and methods

Two rats of 200 g were intravenously injected with 1.0 mCi ^{3}H-Leu and after 4 days with 0.125 mCi ^{14}C-Leu. One hour after the last injection the rats were anaesthetized, the liver was perfused in situ with 0.25 M sucrose to remove as much as possible of the plasma proteins, and a cell sap fraction was prepared according to Bouma and Gruber [6]. This cytosol fraction was purified on Sephadex G75 according to Ansorge et al. [7] and a fraction consisting of proteins with a molecular weight higher than that of hemoglobin, was concentrated and dialyzed against 0.25 M sucrose + 0.06 M Tris–HCl buffer pH 7.4. This cytosol fraction showed negligible endogenous proteolytic activity under our incubation conditions: less than 0.2% of the radioactivity became trichloroacetic acid-soluble after 2 hr.

3. Results and discussion

With the double labelled fraction in which the ^{3}H and ^{14}C indicate proteins with a long and short half-life in vivo respectively, it is possible to compare the fate of both types of proteins in one experiment [8, 9]. The cytoplasmic protein fraction was added to an intact and to a disrupted mitochondrial-lysosomal fraction in the absence and in the presence of dithiothreitol, an activator of several lysosomal proteases, which can penetrate the lysosomal membrane (unpublished results). Fig. 1 shows that the proteolysis by a disrupted mitochondrial-lysosomal fraction is considerably more extensive than by intact particles. If proteins were taken up by intact lysosomes, one would at least have expected the degradation of short-lived ^{14}C-labelled proteins to proceed more quickly in the intact system, in which the low intralysosomal pH [10] favours digestion by the lysosomal cathepsins. The proteolysis by the intact particle fraction can probably be explained by the presence of disrupted lysosomes. The amount of free acid phophatase activity, which can be used as a marker for lysosomal integrity [11], was 7.5% at the beginning and 30% at the end of the incubation.

In vivo, protein degradation is energy dependent [12,13]. The addition of 10 mM ATP + $MgSO_4$ to the

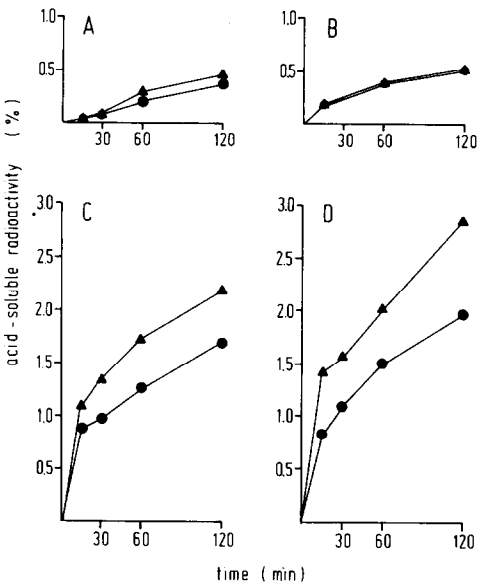

Fig. 1. Degradation of double labelled cytosol proteins by intact and disrupted lysosomes in vitro. A mitochondrial-lysosomal fraction obtained according to Bouma and Gruber [6], either intact or disrupted by 3 cycles of freezing and thawing, was incubated at 38°C with double labelled cytosol proteins prepared according to Ansorge et al. [7]. The incubation mixture contained in a volume of 3 ml: 8.4 mg cytosol protein, with 12 350 dpm ^{14}C-Leu and 80 730 dpm ^{3}H-Leu per mg protein respectively; 12.5 mg lysosomal-mitochondrial protein; 0.25 M sucrose; 0.05 M Tris–HCl buffer, pH 7.4 (38°C); dithiothreitol, when used, was present at a concentration of 2 mM. At the times indicated, 0.5 ml samples were added to 0.5 ml 20% trichloroacetic acid. The acid-soluble radioactivity was counted in Bray's solution in a Philips PW-4510 scintillation counter. A) intact lysosomes, no dithiothreitol; B) intact lysosomes, dithiothreitol present; C) disrupted lysosomes, no dithiothreitol; D) disrupted lysosomes, dithiothreitol present. ▲—▲ ^{14}C-Leu; ●—●^{3}H-Leu.

incubation mixture had, however, no effect. This is in agreement with the results of Brostrom and Jeffay [3].

As indicated in fig. 1, lysosomal proteases degrade short-lived ^{14}C-labelled proteins faster to acid-soluble fragments than the long-lived ^{3}H-labelled ones. This higher susceptibility of the short-lived proteins to proteolysis is a well-known phenomenon [9]. It has been found both with rather specific proteases like trypsin and chymotrypsin and with a mixture of proteases like pronase.

We have also investigated whether intact lysosomes take up proteins, without degradation to acid-soluble fragments. For this purpose, we incubated a mitochondrial-lysosomal fraction with double labelled cytosol proteins as described under fig. 1. Samples taken at several times up to 1 hr were centrifuged at 25 000 *g* for 10 min and the mitochondrial-lysosomal pellet was washed twice. A small and identical rise of total radioactivity was found both in the pellet of intact and of disrupted particles. Thus, there was no evidence for uptake of labelled proteins in intact lysosomes. Moreover, the $^{14}C/^{3}H$ ratio remained constant throughout, and was identical to that in the total mixture. The phenomenon is therefore probably due to an aspecific adsorption.

Summarizing, our results give no indication that lysosomes can take up proteins in vitro. Preliminary experiments in our laboratory on ornithine decarboxylase, a cytosol enzyme with an extremely short half-life (12–14 min) in vivo [14], have failed to show any inactivation of the enzyme by intact lysosomes in vitro. The inactivation of TAT in vitro observed by Auricchio [4] might be quite exceptional. Alternatively, these results might be explained if TAT had been taken up in lysosomes in vivo.

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