Regulation of lysosomal α-mannosidase in macrophages

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Lysosomal storage diseases are usually the result of the deficiency of a single lysosomal enzyme, and they lead to the accumulation of large amounts of undegraded macromolecules in lysosomes, with consequent severe clinical effects. Deficiency of lysosomal α-mannosidase occurs genetically in animals and man (Ockerman, 1973). It can also be induced in animals by prolonged ingestion of the plant Swainsona canescens (Dorling et al., 1978), from which the indolizidine alkaloid swainsonine, a potent inhibitor of α-mannosidase (Dorling et al., 1980), has been purified. In an attempt to create a model storage disease in tissue culture, we have exposed macrophages to swainsonine.

Mouse peritoneal macrophages were routinely cultured in medium 199 containing 10% heat-inactivated pig serum, 100 μg of penicillin/ml and 100 μg of streptomycin/ml, as previously described (Dean et al., 1979). For experiments, the cultures received fresh media and at intervals the media were removed. The adherent cells lysed by 0.1% Triton X-100 in phosphate-buffered saline. Mannosidase activity in both fractions was measured at pH4.5 with 4-methylumbelliferyl α-D-mannopyranoside as substrate: 1 unit of activity releases 1 μmol of methylumbelliferyl/h. The lysosomal marker lactate dehydrogenase was assayed with NADH and pyruvate as substrates, and 1 unit of its activity oxidizes 1 μmol of NADH/min.

Preliminary experiments demonstrated that cellular acid α-mannosidase activity in macrophages decreased during cultivation (Fig. 1a), unlike other lysosomal enzymes (Jessup & Dean, 1980). This loss of activity was not due to specific secretion, since no corresponding increase in extracellular activity was observed. The loss was most pronounced in cultures incubated in serum-free medium (80% loss in 24 h), but was limited to 20–40% in the presence of various sera at 10% (v/v). Expansion of the lysosomal system by phagocytosing latex spheres did not sustain the mannosidase activity (Fig. 1a). Macrophage α-mannosidase was susceptible to zymosan-induced secretion (Fig. 1b), in common with other lysosomal hydrolases (Dean et al., 1979). This release was not due to cell lysis, since an equivalent release of cytoplasmic lactate dehydrogenase was not observed (results not shown); nor did incubation with zymosan alter the total mannosidase activity of the cultures (113 units and 105 units per culture for zymosan-treated and controls respectively).

Swainsonine could completely inhibit the acid α-mannosidase of macrophage lysates (at micromolar concentrations). This effect could be reversed by dilution (as reported by Dorling et al., 1980). When macrophages were exposed in culture to low concentrations (<0.1 μM) of swainsonine for either 15 min or 24 h, enzyme activity in lysed cells was no longer detectable. However, if the cells were washed four times with phosphate-buffered saline before harvesting, essentially all the activity could be recovered. This and other results (not shown) suggest that the inhibitor can rapidly penetrate through cell membranes.

In the hope of inducing lysosomal storage, we exposed macrophages to swainsonine (0.1 μM) for prolonged periods, changing the media every 2–3 days, and maintaining the swainsonine concentration. Media were removed at chosen times and residual extracellular swainsonine was removed by washing as described above. For this experiment we used alkali-inactivated pig serum, which has negligible α-mannosidase activity (cf. heat-inactivated pig serum). Mannosidase activity in control cultures declined from 93.4 ± 6.5 (mean ± s.d.) units per culture at day 0, to 11.1 (SD. 1.1) at day 14. In contrast, in swainsonine-treated cells, mannosidase activity increased 3.1-fold (82.3 ± 3.7 and 257.8 ± 12.4 units per culture on days 0 and 14 respectively). Similar increases in total protein (1.9-fold) and lactate dehydrogenase (3.1-fold) were also observed. The activity of lysosomal hexosaminidase increased more markedly (from 717.7 ± 67.5 to 2035 ± 246 units per culture, a 27.6-fold increase) in the same cultures.

Clearly swainsonine has effects beyond the direct inhibition of lysosomal mannosidase. The increase in lysosomal enzymes that we have shown could be due to more general effects on cellular glycoprotein metabolism, since the inhibition in vivo of neutral α-mannosidase affects glycoprotein processing (Elbein et al., 1981). Swainsonine will thus probably be more useful in studies...
on glycoprotein processing than in creating a model lysosomal storage disease.

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A comparison of the mechanism of the oxygenation of quercetin with that of haem

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The plant flavonol, quercetin, undergoes oxidative decarbonylation to the corresponding depside (Fig. 1a) with the loss of the C 3 atom as CO (Simpson et al., 1960) and the incorporation of two atoms of oxygen at C 2 and C 4 (Westlake et al., 1959). The oxygenation can be catalysed by the enzyme quercetinase (Simpson et al., 1963) or non-enzymically using strong base in non-aqueous solvent (Nishinaga & Matsuura, 1973). The isolation and purification of quercetinase has been described by Oka et al. (1971).

The physiological degradation of haem to biliverdin is initiated by the hydroxylation of a methene bridge carbon atom to yield hydroxyhaem (Fig. 1b). The subsequent macrocyclic ring cleavage step to produce biliverdin is similar to that of quercetin oxygenation in that a carbon atom is eliminated as CO and two oxygen atoms are inserted at the adjacent carbon atoms (Fig. 1b). 18O labelling studies (Brown & King, 1978) have shown that these atoms are derived from two different oxygen molecules (Two-Molecule Mechanism) and not from one oxygen molecule (One-Molecule Mechanism). The way in which a Two-Molecule Mechanism is attained has not been elucidated, due in part to the difficulty in isolating the hydroxyhaem intermediate.

A study of the mechanism of quercetin oxygenation might thus provide an insight into the mechanism of the macrocyclic ring cleavage of haem. Although previous 18O studies on quercetin by Krishnamurty & Simpson (1970) had shown that both oxygen atoms were derived from molecular oxygen, it was not possible to distinguish between a One-Molecule and a Two-Molecule Mechanism. We now present the determination of the mechanism of the oxygenation of quercetin by cinnyric acid and non-enzymically using strong base in non-aqueous solvent (Brown, 1963) or non-enzymically using strong base in non-aqueous solvent.

Fig. 1. (a) Macrophages in medium 199 containing: heat-inactivated (56°C, 30 min) pig serum.
- alkali-treated (pH 9, 56°C, 60 min) pig serum; 
- acid-treated (pH 3, 56°C, 30 min) foetal-calf serum, 
- 1 μl of latex (0.8 μm diameter; 5% (v/v) suspension)/ml, 
- no additions.
(b) Cellular (△) and secreted (○) α-mannosidase in cultures (medium 199 with 10% heat-inactivated pig serum) alone (△, ○) or exposed to 50 μg of zymosan/μl (△, ○).