Permeability properties of lysosomal membranes

Review

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Lysosomes are involved in the cellular degradation of material either initially present in the cell or brought into the cell by phagocytosis or endocytosis. This degradative process, which occurs in an acidic environment, produces metabolites such as peptides and amino acids, nucleosides, and sugars which may subsequently accumulate within the lysosome. It is therefore important for osmotic reasons, and for optimal utilization of these metabolites, to have a system whereby the degradative products can pass through the lysosomal membrane. This can be achieved either by passive diffusion or via a specific transport mechanism. Furthermore, maintenance of the acidic milieu necessary for optimal activity of the lysosomal enzymes requires concentration of hydrogen ions within the lysosome. This hydrogen-ion gradient may be maintained either by a Gibbs-Donnan equilibrium or via an ATP-dependent proton pump in the lysosomal membrane. It is these permeability and hydrogen-ion-concentrating properties of the lysosomal membrane which will be discussed in this review.

Our interest in the mechanism by which the products of lysosomal digestion cross the lysosomal membrane to reach the cytosol arose from a hypothesis concerning the evolutionary origins of polypeptide hormones. Most polypeptide hormones are synthesized as propolypeptides which are cleaved at sites usually marked by pairs of basic amino acids (Docherty & Steiner, 1982). In considering the problem posed by the existence of prohormones, it was suggested that they could be explained if the evolutionary origin of polypeptide hormones was from a more primitive process of lysosomal proteolysis in unicellular organisms (Hales, 1978; Hales & Docherty, 1979). Polypeptide fragments produced by this means within the lumen of the secondary lysosome would potentially be capable of interaction with membrane components in the structure of the secondary lysosomal membrane. Such membrane proteins could include plasma-membrane transporters. One of the most important processes regulated by polypeptide hormones is membrane transport. It was therefore suggested that the membrane of the secondary lysosome would contain transport mechanisms.

Studies on the permeability properties of the lysosomal membrane to metabolites such as sugars and amino acids had up to that time been interpreted as indicative of simple diffusion (Reijngoud & Tager, 1977). We therefore undertook a reinvestigation of the problem as it related to sugar permeability. In this review we describe the methods available for the study of metabolite permeability of lysosomes and
the results obtained for sugars, peptides, and amino acids, and how these latter results pertain to the genetic disorder cystinosis. We will then briefly review experiments on the hydrogen-ion-concentrating properites of the lysosomal membrane.

**The Permeability of Lysosomes to Sugars**

One of the most readily applicable methods for studying the permeability of lysosomes to sugars and other metabolites is the osmotic-protection method, which is based on the experiments of Berthet et al. (1951). When placed in media of low osmotic pressure, rat liver lysosomes rupture, as shown by the release of their specific enzyme content. On the other hand, lysosomes placed in isotonic solutions of impermeable substances such as sucrose remain intact in solution for hours. Between these two extremes, a time-dependent rupture of lysosomes occurs in isotonic solutions of substances such as glucose. These substances are believed to enter the lysosome as a result of the concentration gradient established in isotonic solutions. Water is then drawn into the organelle with resultant swelling and eventual rupture. The extent to which different substances protect lysosomes against such osmotic rupture is a measure of the impermeability of the lysosomal membrane towards them.

The use of osmotic swelling to measure permeability has a precedent in the early investigation of red-cell permeability. The 'haemolysis-point' method (Jacobs, 1934) involved suspending red cells in isotonic solutions of various compounds. Permeant compounds caused osmotic lysis of the cells, and the time taken to reach a degree of haemolysis (measured by a decrease in turbidity) was an indirect measure of the rate of entry of the compound. Much of our early understanding of red-cell sugar transport was based on the results obtained using an osmotic-swelling method (LeFevre, 1961), and this method has also been used to investigate the sugar permeability of chromaffin granules (Perlman, 1976), and Golgi apparatus vesicles (White et al., 1980).

The most extensive early studies of lysosomal sugar permeability using osmotic protection were those of Lloyd (1969). He observed, in agreement with the vacuolation experiments (see below) of Cohn and Ehrenreich (1969), that various disaccharides did not cause lysosomal breakage. He also compared the behaviour of a wide range of monosaccharides and hexitols. Quite marked differences were observed between the relative permeabilities of these substances. The hexitols sorbitol, mannitol, and inositol provided good osmotic protection. At the extremes of the monosaccharides causing osmotic lysis were erythritol (very fast) and D-glucose (very slow). The large differences which were observed between the sugars and hexitols were attributed to differences in their rates of diffusion across the lysosomal membrane.

Docherty et al. (1979) decided to reinvestigate this problem with the aim of determining to what extent, within the limitations of the technique, known criteria of a sugar-transport process could be applied to the system. Where comparable studies were carried out their results agreed closely with those of Lloyd (1969). They concluded that their more extensive investigations were most readily explained by
the presence of one or more transport processes as evidenced by the following criteria: sugar specificity; sterospecificity; inhibition by cytochalasin B and phlorrhizin; competition; and a Q10 for uptake of approximately 2.8. It was proposed that sugar uptake took place by facilitated diffusion. Docherty et al. (1979) also discussed the fact that the ranking of the relative rates of net uptake would not necessarily reflect the order of permeability at physiological (as opposed to 0.25 M) concentrations. The high concentration of sugars dictated by the osmotic protection method makes it likely that the uptake of sugars is occurring at a concentration considerably in excess of their affinity constant for the transport process. Under these conditions the rate of net uptake, which is the difference between influx and efflux, can become quite slow. The reason for this is that influx proceeds at an almost maximal velocity whilst, due to rapid increase of the lysosomal sugar concentration, the rate of efflux also becomes very rapid with the difference between the two being quite small. This has been demonstrated mathematically by Stein (1967). For a series of compounds of decreasing affinity, at high concentrations the rate of net uptake may be in the inverse order of their relative affinities for the uptake process. Clearly the order will reverse at some point since substances with an extremely low affinity for the uptake process will not enter the lysosome at a measurable rate. Somewhat surprisingly, in view of the different cell types involved, the order of net uptake established for rat liver lysosomes by the osmotic protection method is the reverse of the affinities of these sugars for the transport mechanism in the human erythrocyte (Docherty et al., 1979). A prediction of this explanation of the reversed order is that some sugars with a slow rate of net uptake should have a higher affinity for the carrier than those with a high rate of uptake. They should therefore also inhibit the uptake of sugars with a high rate of net accumulation. This prediction was indeed verified experimentally (Docherty et al., 1979).

The affinity of glucose for the plasma-membrane transport system varies from tissue to tissue. The affinity constants for rat muscle, adipose tissue, and liver are 8 mM (Post et al., 1971), 7 mM (Whitesell & Gliemann, 1979), and 66 mM (Elliott & Craik, 1982) respectively. If the sugar-transport system in lysosomes is similar or identical to that of the plasma membrane then the above kinetic considerations indicate that the rate of net glucose uptake into lysosomes of muscle and adipose tissue should be considerably slower than that into liver lysosomes. This has been found to be true experimentally using the osmotic protection method (Docherty & Hales, 1980; K Docherty, GA Maguire & CN Hales, manuscript submitted).

The permeability of lysosomes to sugars can also be measured directly using radioactively labelled sugars. In order to study the uptake of radioactively labelled sugars it is necessary to use a highly purified preparation of lysosomes. One approach used to obtain such a preparation is to alter the buoyant density of the lysosomes by prior accumulation of low-density material. If rats are injected intra-peritoneally with Triton WR-1339 the detergent is accumulated in the lysosomes (Leighton et al., 1968). The modified lysosomes ('tritosomes') may be highly purified from a liver homogenate prepared 4 days after the injection (Trouet, 1974). Maguire et al.
(1983) investigated the entry of D-[\(^3\)H]glucose into tritosomes by a
method which involved separating them from the incubation medium by
centrifugation through silicone oil. The internal water space (2.2 ±
0.2 µl/mg of protein, mean ± SEM, n = 12) of the pelleted tritosomes
was 63% of the total water space of the pellet. The rate of entry of
D-[\(^3\)H]glucose at high concentrations was, however, too great for
accurate estimation of linear rates of influx. Using concentrations at
which it was possible to measure linear rates of influx (up to 20
mM), the rate of entry of D-[\(^3\)H]glucose exhibited concentration
dependence indicative of saturation with a \(K_m\) of 48 ± 18 mM (mean ±
SEM, n = 4). As discussed above for a system that operated by
facilitated diffusion, the relative rates at which sugars enter lysosomes
at high concentrations may be in the reverse order of their affinities,
whereas at low concentrations the relative rates will be in the order
of their affinities. At high concentration (125 mM) D-[\(^14\)C]ribose
entered purified lysosomes more quickly than D-[\(^14\)C]glucose. At low
concentrations (1 mM), this order was reversed, thus confirming the
presence of a transport system (Maguire et al., 1983). If a single
transport system was responsible for the entry of the sugars investi-
gated then mutual competition between the sugars would be expected.
The uptake of D-[\(^14\)C]glucose and D-[\(^14\)C] ribose was measured in the
presence of several competing sugars. The order of competition in
both cases (2 deoxy-D-glucose < D-glucose < D-mannose < D-ribose)
was similar to that deduced from the direct uptake study and the
osmotic protection study. Thus, it is likely that the sugars share a
transport system.

The Permeability of Lysosomes to Peptides and Amino Acids

The permeability of lysosomes to peptides and amino acids has
been studied by measuring vacuolation in intact cells, by the osmotic-
protection method, and by the efflux of radioactively labelled amino
acids from preloaded lysosomes. The basis of the vacuolation method
is that during endocytosis, soluble molecules are taken up passively and
non-specifically into endocytotic vesicles which fuse with primary
lysosomes to form secondary lysosomes. Substances which cannot
permeate the lysosomal membrane accumulate in the secondary
lysosome, and the increased osmolarity of the interior results in water
influx and vacuolation. The process of vacuolation can be observed in
intact cells by phase-contrast microscopy. Electron-microscopic studies
in which acid phosphatase has been localized in the vacuoles have
confirmed that they are indeed secondary lysosomes. Vacuolation was
used by Cohn and Ehrenreich (1969) to demonstrate that the
membrane of the secondary lysosome of rabbit polymorphonuclear
leucocytes was permeable to monosaccharides, and that
oligosaccharides had to be degraded before their constituent sugars
could escape from the secondary lysosome. The effect on the
lysosome of incubating mouse peritoneal macrophages with peptides
was also studied by Ehrenreich and Cohn (1969). L-amino-acid-
containing peptides did not cause vacuolation, presumably because of
hydrolysis to permeable amino acids. However, from experiments using
a series of di- and tri-peptides containing D-amino acids, they
concluded that the lysosomal membrane was impermeable to peptides of mol.wt. greater than 230.

The osmotic-protection method was used by Lloyd (1971) to study the permeability of lysosomes to peptides and amino acids. Because this method necessitated the use of isotonic (0.25 M) solutions, and few amino acids and peptides are sufficiently soluble, the study was somewhat limited. The results, however, were consistent with the observations of Ehrenreich and Cohn (1969), but, as with the vacuolation method, the limitations of the system meant that experiments which could differentiate between transport and non-transport mechanisms could not be attempted. Overall, these two approaches led to the conclusion that the lysosomal membrane was permeable to amino acids and peptides with a rough mol.wt.-cut-off limit of about 230.

A related technique was used to study the permeability of lysosomes to amino acid and peptide methyl esters at low concentrations (1-2 mM) (Goldman & Kaplan, 1973). L-amino acid methyl esters caused a gradual loss of lysosomal latency while the D-isomers did not. The explanation for this was that the methyl esters were more permeable than the free amino acids, and the L-amino acid methyl esters were more readily hydrolysed by lysosomal esterases to yield the free L-amino acids, which would accumulate (and cause lysis) faster than the permeable methyl esters. Reeves (1979) used this method to introduce radioactively labelled amino acids into rat liver lysosomes. When lysosomes were incubated in L-[3H]amino acid methyl esters at low concentrations (0.01 mM), they accumulated the respective L-[3H]amino acid but did not rupture. The efflux of the L-[3H]amino acid was then studied with a view to determining whether it exhibited any of the characteristics of a transport-mediated process, i.e. saturation and counter-transport. Lysosomes were incubated in 30 mM L-leucine, for 10 min at 37°C in order to preload them with L-leucine, and then the preparation was exposed to L-[3H]leucine methyl ester for 10 min at 22°C to allow accumulation of L-[3H]leucine. The lysosomes were then diluted 70-fold and the efflux of L-[3H]leucine measured. The efflux of L-[3H]leucine from the preloaded lysosomes was the same as that from control lysosomes, suggesting that if there was a transport system, then it did not saturate at 30 mM. Since hepatocyte amino-acid-transport systems have $K_m$ values of about 5 mM (Joseph et al., 1978) the results favoured a passive-diffusion model. However, the interpretation of the data as being indicative of the absence of a transport system may be criticized. It was assumed that a 10-min incubation with 30 mM L-leucine would result in equilibration, since preliminary experiments on the efflux of L-[3H]leucine showed that 90% of the radioactive amino acid had left the lysosome in 10 min. If, however, there is a transport system in lysosomes, the rate of equilibration of 30 mM L-leucine would be much slower (Stein, 1967; see above). Hence, the internal concentration of L-leucine after a 10-min incubation in 30 mM L-leucine may not have been close to 30 mM, and the conclusions regarding nonsaturability were not justified. A similar criticism can be applied to Reeve's counter-transport experiment, which also failed to favour a transport system. Indeed as described in the next section, evidence has been presented that amino acids may permeate the membrane of lysosomes by a transport process.
Cystinosis

Nephropathic cystinosis is a genetically determined disorder, inherited in an autosomal recessive fashion and characterized by the Fanconi syndrome and progressive renal failure. The leucocytes and fibroblasts of such patients contain 50-100 times the normal amount of non-protein cystine (Schneider et al., 1967), which is contained within the lysosomes (Schulman et al., 1969). The disorder can best be explained as either a defect in the metabolic interconversions between cystine and (the smaller and therefore more permeable) cysteine, or as a defect in the permeability of the lysosomal membrane to cystine. In order to test the latter theory, lysosomes from leucocytes (Steinhertz et al., 1982; Gahl et al., 1982a), transformed lymphoblasts (Jonas et al., 1982a), and fibroblasts (Jonas et al., 1982b) of cystinotic patients were loaded with $^{35S}$-cystine and the rate of efflux compared with that in lysosomes from normal patients. The lysosomes were loaded with cystine using the mixed disulphide of cysteine and glutathione or using cystine methyl ester. Despite an initial report to the contrary (Steinhertz et al., 1982), the efflux of cystine was considerably slower in the lysosomes from cystinotic cells than in those from normal cells (Gahl et al., 1982a; Jonas et al., 1982a). The efflux of cystine was enhanced by ATP, which suggested to Jonas et al. (1982b) that it may be dependent upon the functioning of a lysosomal proton pump. Gahl et al (1982b) demonstrated that the efflux of cystine from lysosomes from leucocytes of normal patients was not only stimulated by ATP, but also demonstrated saturability, thus indicating the presence of a cystine-transport system in the lysosomal membrane. Lysosomes from leucocytes of cystinotic patients exhibited no transport of cystine, while the lysosomes from leucocytes of individuals heterozygous for cystinosis exhibited half the normal rate of cystine transport. These observations are consistent with the hypothesis that the disorder cystinosis is caused by a defect in the transport of cystine across the lysosomal membrane.

The Lysosomal-Membrane Proton Pump

Two different mechanisms have been proposed for the maintenance of the acidic interior of lysosomes. The proton gradient is thought to be maintained either by a Gibbs-Donnan-type equilibrium, whereby fixed or impermeable acidic molecules within the lysosome buffer the pH within a certain range, or by an energy-dependent proton pump located in the lysosomal membrane. Evidence for the maintenance of the acidic environment within the lysosome by a Gibbs-Donnan equilibrium is based on studies on the permeability of lysosomal membranes to ions, which demonstrated that the membrane was permeable to protons and monovalent cations (Goldman & Rottenberg, 1973; Henning, 1975). However, these experiments were performed at about 4°C, but at more physiological temperatures (25°C and 37°C) the lysosomal membrane was found to be impermeable to monovalent cations. It was then suggested that the Gibbs-Donnan equilibrium involved movement of protons with a charge compensatory movement of anions (Reijngoud & Tager, 1975).

Evidence for a lysosomal-membrane proton pump was originally based on the observation that added ATP stimulated proteolysis in the
lysosome (Mego et al., 1972). Since then lysosomal membrane ATPase activity has been reported (Schneider, 1974; 1977; Titani & Wells, 1974), and ATP-dependent enhancement of methylamine (Schneider, 1979) and acridine orange (Dell'Antone, 1979) uptake into lysosomes has also been reported. These latter uptake experiments have come in for criticism however (Holemans et al., 1980), on the grounds that the ATP effect was non-specific or that the ATP-dependent process was due to impurities in the lysosomal preparation. Recent convincing evidence for a lysosomal proton pump comes from experiments using fluorescein isothiocyanate-dextran, which is phagocytosed by cells and accumulates in the lysosome. The fluorescence of this compound is sensitive to changes in pH. Thus, intact cells (Ohkuma and Poole, 1978), or isolated lysosomes (Ohkuma et al., 1982) loaded with the reagent were placed in a spectrofluorimeter, and the effect of various additions on the intralysosomal pH recorded. In the presence of Mg\(^{2+}\), the addition of ATP caused acidification of the isolated lysosomes. This was reversed by the protonophore carbonyl cyanide p-trifluoromethoxyphenylhydrazone, suggesting the presence of an electrogenic ATP-driven proton pump (Ohkuma et al., 1982). The electrogenic nature of this proton pump is in disagreement with the observations of Schneider (1981) from methylamine-uptake experiments that suggested that the proton pump was electroneutral.

Conclusions

The properties of sugar entry into lysosomes satisfy all the criteria which have been tested of a facilitated diffusion transport system (Stein, 1967). This lysosomal transport system, which appears to be similar to that in the plasma membrane, can arrive at its destination in the lysosome directly via the rough-endoplasmic-reticulum/Golgi-apparatus route, or it may be derived from the plasma membrane during endocytosis. Recent work has indicated that elements of the plasma membrane are indeed in dynamic equilibrium with intracellular counterparts (Schneider et al., 1979; Stanley et al., 1980; Widnell et al., 1982). It is not at present clear whether the sugar-transport system is present in the primary lysosome. If the transporter was present only in secondary lysosomes, it could be concluded that the system was derived from the plasma membrane, but until a preparation consisting exclusively of primary lysosomes can be purified, this question cannot be answered definitively.

Whether the lysosomal sugar transporter is regulated is not known. Regulation of fat-cell glucose transport appears to be effected not by an activation of pre-existing transporters, but by movement of an intracellular pool of transporters to the cell surface (Suzuki & Kono, 1980; Cushman & Wardzala, 1980). It is possible that the lysosomal glucose-transport system is part of this intracellular pool. An effect of ions on the uptake of sugars into rat liver lysosomes, as measured by the osmotic protection method, has been observed (Docherty & Hales, 1979). Whether this has any role in regulating the lysosomal sugar-transport system is not clear.

A review of the literature on the permeability of lysosomes to amino acids and peptides does not at present allow one to conclude whether transport systems exist. Only in the case of cystine (Gahl et
al., 1982b) has a transport system clearly been demonstrated. This lysosomal cystine-transport system is similar to that of the plasma membrane, at least in so far as it is energy dependent (Rosenberg & Downing, 1965). Schulman et al. (1971) demonstrated that the rate of uptake of \[^{35}S\]cystine into leucocytes from cystinotic patients was the same as into those from normal patients. These authors, however, did not provide any kinetic evidence that they were studying a transport process. If in cystinosis the cystine transport system is absent from the lysosome (Gahl et al., 1982b) while present in the plasma membrane, this may be taken as evidence that the transport system of lysosomes is not derived from or the same as that of the plasma membrane. Alternatively, the abnormality in cystinosis could be a defect in the mechanism whereby the plasma-membrane transport system is translocated from the plasma membrane to the lysosome (J. P. Luzio, personal communication). Some instances of familial hypercholesterolaemia provide a precedent for such a change in which the particular defect is in the mechanism whereby the plasma-membrane LDL receptor is internalized (Brown & Goldstein, 1979).

Evidence appears to favour the existence of a lysosomal proton pump, and it is interesting that the electrogenic proton pump described by Ohkuma et al. (1982) is more similar to the proton pump described in the chromaffin granule (Njus et al., 1981) and the insulin-secretory granule (Hutton & Peshavaria, 1982) than to the mitochondrial proton pump. This is in keeping with the predicted similarities between the lysosome and the secretory granule as described by Hales (1978).

It is becoming clear that the membranes of the cellular organelles involved in the processes of lysosomal degradation and of secretion are not merely delimiting lipid bilayers, but contain functional integral protein components. Examples of such components include the signal-peptide-translocating apparatus of the rough endoplasmic reticulum (Kriebich et al., 1978), the intracellular mannose-6-phosphate receptors involved in the transport of lysosomal enzymes to their site of function (Gonzalez-Noriega et al., 1980), and the proton pumps of the chromaffin (Njus et al., 1981) and the insulin-secretory granule (Hutton & Peshavaria, 1982). In this review we have described the presence of such components in the lysosomal membrane. It will be interesting to see what new lysosomal-membrane components will be discovered. One candidate is a system for the specific uptake of cytosolic proteins for degradation in the lysosome.

References

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