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THE RELATIVE PERMEABILITY OF LYSOSOMES FROM *TETRAHYMENA PYRIFORMIS* TO CARBOHYDRATES, LACTATE AND THE CRYOPROTECTIVE NONELECTROLYTES GLYCEROL AND DIMETHYLSULPHOXIDE

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SUMMARY

Lysosomes in homogenates of *Tetrahymena pyriformis* have been shown to be permeable to the cryoprotective nonelectrolytes glycerol and dimethylsulphoxide, to the hexoses glucose, galactose and α-methylglucose and to the hexitols mannitol, sorbitol and inositol. They are impermeable to the disaccharides sucrose, maltose and melibiose and to the trisaccharide raffinose. The presence of a charged group has a great influence on permeability, the lysosomes being impermeable to the gluconate and glucuronate anions and only slightly permeable to the lactate anion and the glucosaminium cation.

INTRODUCTION

Lysosomes have now been described in many types of cells[1] and, although some of the earlier studies on rat liver lysosomes concerned their permeability[2–4], it was only recently[5] that a systematic study was made of their permeability to carbohydrates. As nothing is known about the permeability characteristics of lysosomes from the ciliate protozoan *Tetrahymena pyriformis*, it was of interest to study their response to carbohydrates and, in addition, to the cryoprotective agents glycerol and dimethylsulphoxide, as preliminary results[6] had shown dimethylsulphoxide to protect these lysosomes against damage during freezing.

MATERIALS AND METHODS

Dimethylsulphoxide (Laboratory Reagent Grade), glycerol (A.R.), glucose (A.R.) and lithium lactate were from Hopkin and Williams, Chadwell Heath, Essex. Mannitol was the product of British Drug Houses, Poole, Dorset. β-Glycerophosphate (Grade 1), Triton X-100 and all other carbohydrates were supplied by Sigma (London), London S.W.6.

Growth and harvesting of cells

T. pyriformis, strain S, was grown in the dark at room temperature[7]. Cells were harvested between 5 and 7 days after inoculation, washed with 0.25 M sucrose in the manner described previously[8] (except that centrifugation was at 1500 × *g* for 2.5 min) and resuspended in 0.25 M sucrose at a concentration between 10^7 and $5 \cdot 10^7$ cells per ml before disruption at 4° by exposure to pH 11 for between 45 sec and 1 min. Acid phosphatase (orthophosphoric monoester phosphorohydrolase, EC 3.1.3.2) was assayed in triplicate in the manner described previously[8]; all assays contained 0.25 M sucrose in addition to the sucrose which was present in the homogenates.

Assessment of permeability of the lysosomal membrane

After preparation, homogenates were placed in ice and an aliquot diluted with 4 vol. of the solution under examination. At timed intervals thereafter, samples of the diluted homogenates were assayed for acid phosphatase activity. For the determination of total activity, assay mixtures contained 0.05 % Triton X-100 and latency is defined as the percentage of the total activity unmasked by incorporation of Triton X-100 into the assay mixture. As there is no indication in the literature that any of the compounds under examination has a direct lytic action on biological membranes at the concentrations studied, it was assumed that a decrease in latency of acid phosphatase was the result of permeation of the substance through the lysosomal membrane.

RESULTS

In Fig. 1 it can be seen that the latency of acid phosphatase in an homogenate diluted with 0.25 M sucrose decreased only slightly during the subsequent 2-h period, while the latency in homogenates diluted with 0.25 M glucose or 0.25 M α-methylglucose decreased dramatically (although in approximately the same fashion). In another experiment the latency was well maintained on dilution with the trisaccharide raffinose, although it decreased on dilution with the hexitols sorbitol and inositol.

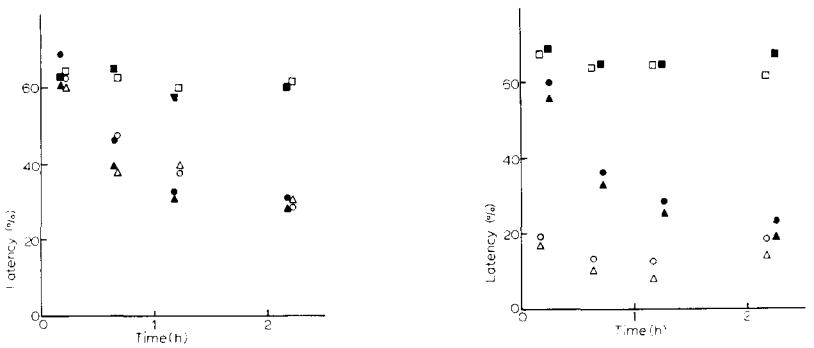

Fig. 1. Latency of acid phosphatase in two homogenates prepared with 0.25 M sucrose. (1) After dilution with sucrose (□), with glucose (△), with α-methylglucose (○). (2) After dilution with raffinose (■), sorbitol (▲), with inositol (●). The concentration of the diluting solutions was 0.25 M.

Fig. 2. Latency of acid phosphatase in two homogenates prepared with 0.25 M sucrose. (1) After dilution with melibiose (□), with glycerol (△), with dimethylsulphoxide (○). (2) After dilution with maltose (■), with galactose (▲), with mannitol (●). The concentration of the diluting solutions was 0.25 M.

Fig. 2 shows the latency of acid phosphatase to remain high after dilution with maltose but to decrease on dilution with galactose and mannitol. Similarly, latency was well maintained with the disaccharide melibiose, although dilution with glycerol or dimethylsulphoxide resulted in a much more rapid decrease in latency than was seen with hexoses or hexitols.

All of the compounds tested above are unchanged, and it was decided to study the effect of charged groups on permeability. Fig. 3 shows that dilution of an homogenate with 0.25 M potassium gluconate or 0.25 M potassium glucuronate resulted in only a slight decrease in latency of acid phosphatase over the subsequent 2-h period. This decrease probably does not differ significantly from that found in the control homogenate diluted with sucrose and, in any case, it is clearly seen that the presence of an anionic grouping had a profound effect on the permeability of these molecules as compared to glucose.

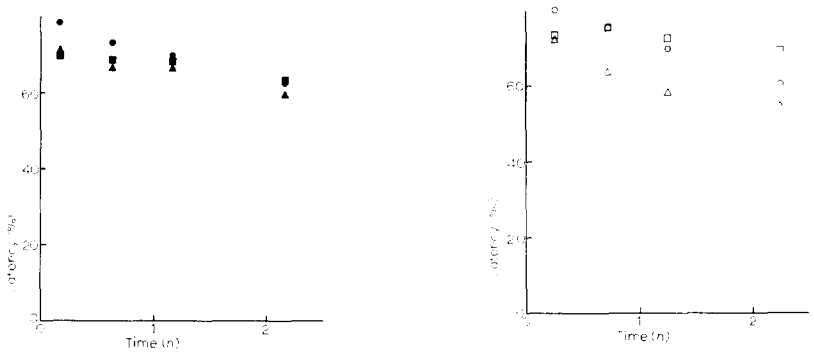

Fig. 3. Latency of acid phosphatase in an homogenate prepared with 0.25 M sucrose after dilution with sucrose (■), with potassium glucuronate (▲), with potassium gluconate (●). The concentration of the diluting solutions was 0.25 M.

Fig. 4. Latency of acid phosphatase in an homogenate prepared with 0.25 M sucrose after dilution with sucrose (□), with lithium lactate (△), with glucosamine·HCl which had been adjusted to pH 7.0 (○). The concentration of the diluting solutions was 0.25 M.

Dilution with lithium lactate and glucosamine·HCl resulted in decreases in latency (Fig. 4) which were greater than those found with the sucrose control although not as great as those found with hexoses and hexitols.

DISCUSSION

In Figs. 1 and 2 it is shown that the latency of acid phosphatase decreased only slightly when homogenates prepared in 0.25 M sucrose were diluted with identical concentrations of the disaccharides maltose or melibiose or with the trisaccharide raffinose. This is in agreement with the findings for rat liver lysosomes[5] where the latency of nitrocatechol sulphatase was always maintained when the lysosomes were resuspended in solutions of disaccharides. The results with monosaccharides are also in agreement with those for rat liver lysosomes as glucose, galactose and α-methylglucose all appear to permeate rapidly into both types of lysosome. There is, however, con-

siderable difference between the two types in their permeability to hexitols. As shown in Figs. 1 and 2 mannitol, sorbitol and the cyclic hexitol inositol all permeate the lysosomes of *T.pyriformis*, while they permeate very slowly, if at all, into rat liver lysosomes, even at 25°. This permeability to mannitol and sorbitol was also found with homogenates prepared by passing a suspension of cells through a nylon Millipore filter (mean pore diameter 14 μ) so it would appear that the permeability to hexitols is, in fact, a property of the lysosomes of *T.pyriformis* and not an artifact of the isolation procedure which exposes the lysosomes to high pH. It may be that the intracellular digestive system of *T.pyriformis* is adapted to the utilisation of hexitols found predominantly in the vegetable matter of their natural habitat[9,10].

The permeability to glucose of the lysosomes from *T.pyriformis* is not affected by the incorporation of 0.2 mM CN^- into the diluted homogenate (D. LEE, unpublished results), and it seems that their permeability to hexoses is not mediated by an active transport system. It appears, therefore, that they will be permeable to all uncharged molecules with molecular dimensions similar to (and less than) that of glucose.

Rat liver lysosomes have been shown[2] to be permeable to simple salts such as NaCl, KCl, Na_2SO_4 and $CaCl_2$ and, as it seems probable that the lysosomes from *T.pyriformis* will also be permeable to such simple salts, the influence of the organic ion only will be considered in the following discussion. The lysosomes from *T.pyriformis* are impermeable or only very slightly permeable to gluconate and glucuronate in spite of the fact that their molecular weight is closer to that of glucose than to a disaccharide and the presence of an anionic grouping must be having considerable influence on the permeability of the molecule. This is also found with lactate which appears to penetrate the lysosomes much more slowly than glucose in spite of its lower molecular weight. Another example of the influence of a charged group in a small molecule is shown by the fact that lysosomes from *T.pyriformis* and from rat liver[2,3] are permeable to glycerol although impermeable, or only slightly permeable, to β-glycerophosphate. Nevertheless, if the cell has the ability to maintain a sufficiently low intralysosomal pH[11–13] it seems possible that organic acids could pass from the lysosomes into the cell sap in the uncharged form.

The presence of a cationic grouping in the molecule also has a considerable effect on the permeability as can be seen by comparing the decrease in latency found with glucose (Fig. 1) with that found with glucosamine (Fig. 4). However, the fact that the lysosomal membrane shows some permeability towards glucosamine does not preclude the possibility that an initially low intralysosomal pH which allows the passage of uncharged organic acids could not be followed by a progressively higher pH which subsequently enhances the passage of organic bases[11,12].

The precipitous decrease in latency of acid phosphatase found when homogenates were diluted with glycerol and dimethylsulphoxide, shows that these compounds traverse the lysosomal membrane with great ease and suggests that the protective effect of dimethylsulphoxide during the freezing of lysosomes[6] might be produced by the same mechanism as operates with whole cells[14,15].

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