Translocation of sugars into rat liver lysosomes

Evidence against a common carrier for D-glucose and D-ribose

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A conflict exists in the literature concerning the mode of translocation of D-glucose and D-ribose across the lysosome membrane. The more rapid net uptake of ribose, when measured by the osmotic-protection technique, has been attributed either to its smaller size and lower hydrogen-bonding capacity, or to a lower affinity for a transport system shared by both sugars. The latency of acid β -hexosaminidase in isolated rat liver lysosomes was measured after preincubation for periods up to 1 h in various solutions containing glucose and/or ribose, and in some cases sucrose. After confirmation of the superior osmotic protection afforded by glucose (than by ribose), it was shown that a solution 0.125 M in both glucose and ribose provided protection intermediate between that given by 0.25 M-glucose and that given by 0.25 M-ribose. This result is inconsistent with the common-carrier hypothesis.

INTRODUCTION

It is widely agreed that neutral monosaccharides such as glucose can cross the lysosome membrane (for reviews see Lloyd, 1973; Goldman, 1976; Reijngoud & Tager, 1977; Docherty *et al.*, 1983; Hales *et al.*, 1984). This ability is of considerable physiological importance, since monosaccharides are a major product of intralysosomal catabolism of polysaccharides.

There is, however, a divergence of opinion over whether translocation across the membrane is by passive diffusion or by a carrier-mediated mechanism. An early study (Lloyd, 1969) of the apparent rates of entry of various physiological and non-physiological sugars into isolated rat liver lysosomes provided no evidence for a carrier-mediated mechanism: the differential rates observed seemed wholly explicable on the basis of physico-chemical parameters such as molecular size, charge and hydrogen-bonding capacity. Thus the pentoses entered more rapidly than the hexoses, and the pentitols than the hexitols, which last were almost non-penetrant.

Subsequently Hales and his colleagues (Docherty *et al.*, 1979; Docherty & Hales, 1979; Hales *et al.*, 1982; Maguire *et al.*, 1983) have published evidence that sugars enter isolated rat liver lysosomes by a facilitated-diffusion system. Uptake of hexoses and pentoses was found to exhibit some degree of stereospecificity and mutual competition, to be inhibitable by cytochalasin B or phlorizin, and to have a high Q_{10} . Docherty *et al.* (1979) also offer an alternative interpretation of the data of Lloyd (1969). Accepting that, for example, pentoses enter lysosomes more rapidly than hexoses, they argue that this may be a consequence of the former having a lower affinity for the membrane transporter. The osmotic-protection method used both by Lloyd and by Hales and colleagues involves incubating isolated intact lysosomes in 0.25 M solutions of the test sugar, and measuring the rate at which the latency of lysosomal enzymes is lost. The method provides an indication of the net rate of a sugar's accumulation which, as Docherty *et al.* (1979) point out, is the difference between the influx and efflux

rates. Basing their argument on the theory of Wilbrandt & Rosenberg (1961), they reason that, for a carrier-mediated process and at concentrations of sugar considerably in excess of the K_m for the carrier, the rate of efflux will rapidly approach the rate of influx. Thus the net flux will diminish, with the consequence that for a series of compounds the rates of net uptake may be in the reverse order to the relative affinities of the compounds for the carrier.

The contrasting paradigms of Lloyd and Hales can be exemplified by their data on D-glucose and D-ribose. Using the osmotic-protection method, with differences only in detail, both Lloyd (1969) and Docherty *et al.* (1979) report that D-ribose enters lysosomes at a higher net rate than D-glucose. Lloyd (1969) explains this result in terms of the smaller molecular size of D-ribose and its lower number of hydroxy groups. Docherty *et al.* (1979) interpret the same data to indicate transport of both sugars on a single carrier, but with D-glucose having a significantly greater affinity. Maguire *et al.* (1983) subsequently determined a K_m (48 ± 18 mM for D-glucose) for the putative sugar-transport system.

The present paper seeks to resolve this conflict of interpretation. We have studied the osmotic protection afforded to isolated rat liver lysosomes when suspended in a solution containing both D-glucose and D-ribose (each 0.125 M). On the Hales hypothesis, these two sugars would both compete for the sugar transporter. If D-glucose has the greater affinity, the translocation of D-ribose would be powerfully inhibited and the rate of accumulation of D-glucose itself perhaps a little decreased, owing to the lower concentration in the incubation medium. The mixed solution of D-glucose and D-ribose should therefore provide even better osmotic protection than 0.25 M-D-glucose. If, however, these sugars enter lysosomes by simple diffusion, their rates of entry will be directly proportional to concentration, and the osmotic protection afforded by the mixed solution should lie between that provided by 0.25 M-D-glucose and that by 0.25 M-D-ribose.

MATERIALS AND METHODS

Materials

Sucrose and D-glucose were from BDH Chemicals, Poole, Dorset, U.K.; D-ribose was from Sigma Chemical Co., Poole, Dorset, U.K.; 4-methylumbelliferyl 2-acetamido-2-deoxy- β -D-glucopyranoside and 4-methylumbelliferone were from Koch-Light, Haverhill, Suffolk, U.K.

Preparation and incubation of lysosomes

Rat liver lysosomes were prepared as previously described (Lloyd, 1969). The lysosome-rich fraction, sedimenting between 1100 g (10 min) and 22500 g (10 min), was gently resuspended in the appropriate test sugar solution (4 ml/0.1 g original pulp wt.) which had been adjusted to pH 7.0; the lysosome suspension was kept on ice before incubation. The lysosome suspension was then placed in an incubation bath at 25 °C, and duplicate samples were withdrawn for enzyme assay at 0, 30 and 60 min.

Assay of free and total β -hexosaminidase activity

A 50 μ l portion of the lysosome suspension was incubated for 1 min at 25 °C with 250 μ l of 0.16 M-citrate buffer, pH 5.0, containing 0.25 M-sucrose, in the absence or presence of 0.36% Triton X-100. The enzyme assay was initiated by the addition of 100 μ l of 10 mM-4-methylumbelliferyl 2-acetamido-2-deoxy- β -D-glucopyranoside in 0.25 M-sucrose; this yielded a final assay volume of 400 μ l containing 0.1 M-citrate buffer and 2.5 mM-4-methylumbelliferyl 2-acetamido-2-deoxy- β -D-glucopyranoside in the absence or presence of 0.2% Triton X-100. The assay was terminated after 1 min by the addition of 2 ml of 1 M- Na_2CO_3 . The liberated 4-methylumbelliferone was determined by measuring the fluorescence ($\lambda_{\text{excitation}} = 354 \text{ nm}$, $\lambda_{\text{emission}} = 444 \text{ nm}$) on a Perkin-Elmer 3000 fluorescence spectrometer.

The activities of the enzyme measured in the absence and presence of Triton X-100 are designated the free activity and the total activity respectively.

RESULTS

Fig. 1 shows the behaviour of a lysosome-rich fraction of rat liver when suspended and incubated in 0.25 M-sucrose, -D-glucose or -D-ribose. The mean initial free activity of β -hexosaminidase, shown as a percentage of the total activity, was 11% in sucrose, 12% in glucose and 20% in ribose. During the 60 min incubation period there was virtually no increase in free activity when lysosomes were in sucrose, whereas lysosomes in glucose or ribose displayed a steady increase. In agreement with earlier work (Lloyd, 1969; Docherty *et al.*, 1979), glucose offered rather better osmotic protection than ribose. Fig. 1 also shows that a solution of 0.125 M in both glucose and ribose offered osmotic protection intermediate between that afforded by 0.25 M-glucose or 0.25 M-ribose.

Fig. 2(a) shows the results of incubations in a solution containing 0.125 M-sucrose and either 0.125 M-glucose or 0.125 M-ribose. The initial free activity of β -hexosaminidase was approx. 10%, and a slow increase was seen during the incubation period. The glucose-containing mixture provided better osmotic protection than the ribose-containing mixture.

Fig. 1. Free β -hexosaminidase activity of a lysosome-rich fraction of rat liver after incubation at 25 °C in various solutions for periods up to 60 min

Each value shown (mean $\pm$ S.E.M. for three experiments) is the free activity (% of total activity) after incubation in 0.25 M-sucrose (●), 0.25 M-glucose (■), 0.25 M-ribose (▲), or 0.125 M-glucose/0.125 M-ribose (○).

Fig. 2. Free β -hexosaminidase activity of a lysosome-rich fraction of rat liver after incubation in various solutions at 25 °C for periods up to 60 min

Each value shown (mean $\pm$ S.E.M. for three or four experiments) is the free activity (% of total activity) after incubation in 0.25 M-sucrose (●); 0.125 M-sucrose/0.125 M-glucose (■), or 0.125 M-sucrose/0.125 M-ribose (▲) (a); or 0.125 M-sucrose (○), 0.125 M-glucose (□), or 0.125 M-ribose (△) (b).

Fig. 2(b) shows the results of incubations in 0.125 M-sucrose, -glucose or -ribose. The mean initial free activity of β -hexosaminidase was 33% in sucrose, 43% in glucose and 50% in ribose. A further major increase in free activity was seen during incubations in glucose or ribose, but not in sucrose.

DISCUSSION

As explained in the Introduction, the hypothesis advanced by Hales would lead to the prediction that a

solution 0.125 M in both glucose and ribose would afford better osmotic protection than a solution of 0.25 M-glucose, since the ribose component would behave as an impermeant sugar. Fig. 1 shows that this prediction is not borne out. Fig. 2(a) reinforces the validity of this test of the hypothesis, showing that a solution 0.125 M in both glucose and an impermeant sugar (sucrose) is indeed a better osmotic protector than 0.25 M-glucose. Furthermore, the osmotic protection provided by this equimolar solution of sucrose and glucose is a consequence of the presence of both sugars: Fig. 2(b) shows that suspension in 0.125 M-glucose or 0.125 M-sucrose leads to initial free activities much higher than that seen after suspension in the mixture of sugars. We therefore conclude that the results reported in the present paper are inconsistent with the hypothesis that glucose and ribose enter lysosomes on a common carrier whose K_m for ribose is greater than that for glucose. Our data do not disprove the existence of a carrier for glucose, nor the possibility that ribose enters on another carrier. The simplest explanation, however, is that glucose and ribose both enter lysosomes by simple diffusion, ribose entering more rapidly because of its smaller size and lower hydrogen-bonding capacity.

Reijngoud & Tager (1977) and Docherty & Hales (1979) have previously reported data that indicate a marked heterogeneity of lysosomal susceptibility to osmotic rupture, but the phenomenon has not received much attention. In the present experiments it was found that lysosomes suspended in 0.125 M-sucrose have an initial free activity of some 30%, and that this value did not increase on further incubation for up to 60 min. This must indicate a heterogeneity in the lysosome population with respect to intrinsic internal osmolarity. The lysosomes that fail to break on suspension in 0.125 M-sucrose must have an internal osmotic pressure not greatly above that value, whereas the remainder of the population, whose internal osmolarity is higher, break immediately. The data also indicate that ribose enters lysosomes with

a higher net rate than glucose, irrespective of the concentration or the total osmolarity of the test-sugar solutions investigated. We therefore conclude that subpopulations of lysosomes having different internal osmolarities do not display any variation in the relative permeability properties of their membranes to these two sugars.

We thank the National Kidney Research Fund and the National Fund for Research into Crippling Diseases for grants in support of this work.

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Received 5 May 1987; accepted 3 June 1987