THE EFFECTS OF IONS AND pH ON THE TRANSPORT OF SUGARS INTO RAT LIVER LYSOSOMES

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1. Introduction

A recent hypothesis linking proteolysis in the lysosome with the evolutionary origin of polypeptide hormones postulated that small molecular weight metabolites derived from digested macromolecules permeate the lysosomal membrane by transport processes, and that these processes could be subject to regulation [1]. We have presented evidence that sugar permeation of rat liver lysosomes is mediated by a transport process [2]. This paper reports our initial attempts to determine whether this process may be regulated.

One known mechanism by which regulation of transport occurs, is by linking the process to ionic and pH gradients across the membrane. In intestinal mucosa [3] and kidney tubules [4] sugar transport is coupled to the Na^+ gradient, while the accumulation of catecholamines in chromaffin granules is thought to be dependent on an H^+ electrochemical gradient [5,6]. Since pH and ionic gradients are known to exist across the lysosomal membrane [7,8], we have carried out experiments to ascertain whether these gradients may be involved in regulating sugar transport across lysosomal membranes.

2. Experimental

The osmotic protection method of studying sugar transport in lysosomes, and all experimental details have been described [2]. The method depends upon the fact that isotonic sugar solutions containing sugars which cannot penetrate the lysosomal membrane provide osmotic protection for lysosomes. Solutions of sugars which are able to penetrate the lysosomal membrane fail to protect the lysosomes and the accumulation of sugar plus water causes rupture of the lysosomes. Thus the rate of rupture of the lysosomes as measured specifically by the release of lysosomal enzymes is a function of the rate of net uptake (accumulation) of permeant sugars.

3. Results

3.1. *The effect of pH on the net transport of D-glucose*

The effect of pH on the net transport of D-glucose across the lysosomal membrane is shown in fig.1. At each value, data were obtained using two different buffers. A high degree of similarity was found at each pH except at pH 5 where acetate/Tris values were high. This was thought to be due to permeation of acetic acid through the membrane [9], and consequently only the dimethylglutarate/Tris data is shown for this pH in fig.1. In separate experiments intermediate pH values (5.5, 6.5 and 7.5) were studied to confirm the general shape of the curve. The rate of D-glucose accumulation is shown to increase in a sigmoidal manner with respect to pH, with the midpoint of the curve at pH ~6.5–6.9.

3.2. *The effect of monovalent cations on net sugar transport*

A concentration-dependent effect of KCl on the accumulation of D-glucose and D-ribose is shown in fig.2. The rate of accumulation of both sugars at 25°C, as measured by the release of aryl sulphatase was

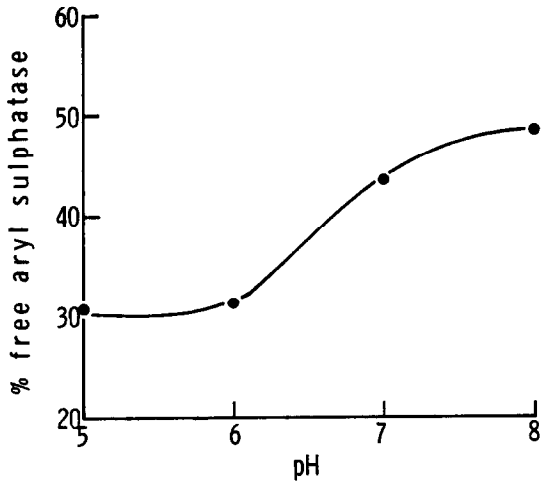

Fig.1. The effect of pH on the net transport of D-glucose into lysosomes at 25°C. The value plotted at each pH was the mean of that obtained using two different buffers (except at pH 5), and represents at least 3 separate experiments with each buffer. The lysosomes were incubated in 0.25 M D-glucose at the appropriate pH in the following buffers at 0.01 M: at pH 5, acetic acid/Tris and dimethylglutarate/Tris; at pH 6, MES [2(*N*-morpholino) ethanesulphonic acid]/Tris and dimethylglutarate/Tris; at pH 7, TES [*N*-(trishydroxymethyl) methyl)-2-aminoethanesulphonic acid]/Tris and Tricine [*N*-((trishydroxymethyl)-methyl) glycine]/Tris; at pH 8, Bicine [*N,N*-(bis-2-hydroxyethyl) glycine]/Tris and dimethylglutarate/Tris.

greatly decreased in the presence of 0.025 M KCl. Similar results were also obtained at 37°C. This effect was not due to lowering the sugar concentration since in a control experiment in 0.2 M D-ribose the release of aryl sulphatase actually increased with respect to that in iso-osmolar D-ribose (data not shown).

The effect of KCl is not specific (table 1). A variety of monovalent cation chlorides at 0.025 M caused the percentage free enzyme activity of lysosomes suspended in D-ribose to decrease from 48 to ~18 after 15 min incubation. This effect also was not mediated specifically by Cl^- since NaCl, acetate and sulphate all had similar effects at 0.025 M (data not shown).

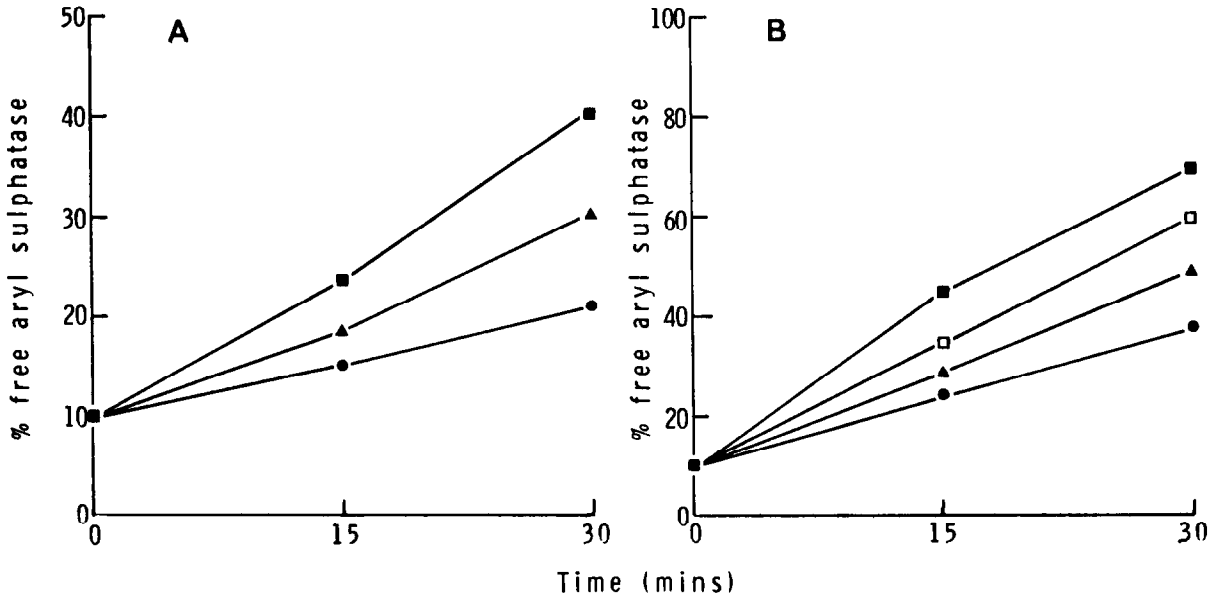

Fig.2. The effect of KCl on the net transport of (A) D-glucose and (B) D-ribose at 25°C. The lysosomes were incubated in the following solutions in 0.01 M TES/Tris at pH 7: (—▪—) 0.25 M sugar; (—▫—) 0.24 M sugar + 0.005 M KCl; (—▲—) 0.23 M sugar + 0.01 M KCl; (—●—) 0.2 M sugar + 0.025 M KCl. The values are the mean of the free activity (% of total) of 3 separate experiments.

Table 1
The effect of monovalent cation chlorides on the accumulation of D-ribose at pH 7 (0.01 M TES/Tris) and 25°C

D-ribose	Salt	% Free aryl sulphatase time (min)	
		0	15
0.25 M	0	11.2	48.9
0.2 M	0.025 KCl	10.6	20.6
0.2 M	0.025 NaCl	9.7	16.6
0.2 M	0.025 LiCl	9.4	18.0
0.2 M	0.025 RbCl	9.7	16.9
0.2 M	0.025 CSCl	10.0	17.3

The lysosomes were incubated in 0.25 M D-ribose, and 0.2 M D-ribose + various cation chlorides at 0.025 M. All values are the mean of the free activity (% of total) of 2 separate experiments

3.3. *The effect of divalent cations on the net transport of D-glucose*

The effect of Mg^{2+} was studied at a physiological concentration (1 mM). $MgCl_2$ decreased substantially the release of enzyme over a 30 min period. $CaCl_2$ (1 mM) had a similar effect (fig.3).

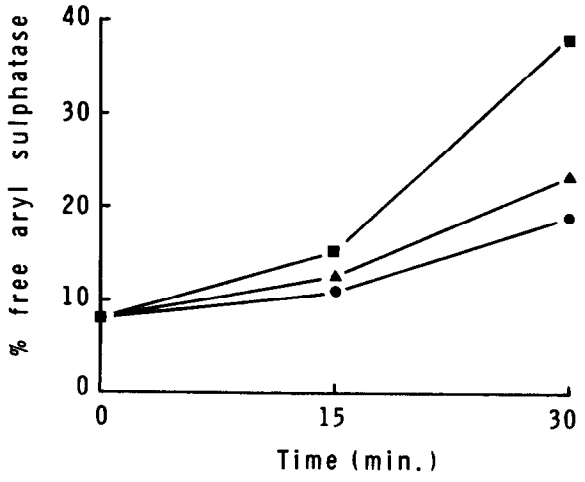

Fig.3. The effect of divalent cations on the net transport of D-glucose. The lysosomes were incubated in the following solutions in 0.01 M TES/Tris at pH 7: (–■–) 0.25 M D-glucose; (–▲–) 0.25 M D-glucose + 1 mM $CaCl_2$; (–●–) 0.25 M D-glucose + 1 mM $MgCl_2$.

3.4. *The effect of pH and ions on the osmotic stability of lysosomes*

The effect of the ionic environment or pH on the stability of lysosomes in isotonic sugar solutions could be due to an effect on the osmotic stability of the lysosomes themselves, the movement of ions or water, or to a change in the rate of sugar accumulation by lysosomes. In order to investigate the first two possibilities, the degree and time course of osmotic breakage at different osmotic pressures and under different pH and ionic conditions was investigated. The stability profile in sucrose (fig.4) shows the existence of populations of lysosomes of varying osmotic fragility, each apparently disrupted immediately on encountering a lower sucrose concentration. Similar sucrose stability profiles were obtained at pH 5, 6 and 8. The lysosomes were found to be less stable in solutions containing sucrose and KCl (0.025 M) than in solutions containing only sucrose. This decrease in stability probably represented the ability of KCl to permeate slowly the lysosomal membrane causing osmotic breakage [10]. On the other hand $MgCl_2$ and $CaCl_2$ at 1 mM were found

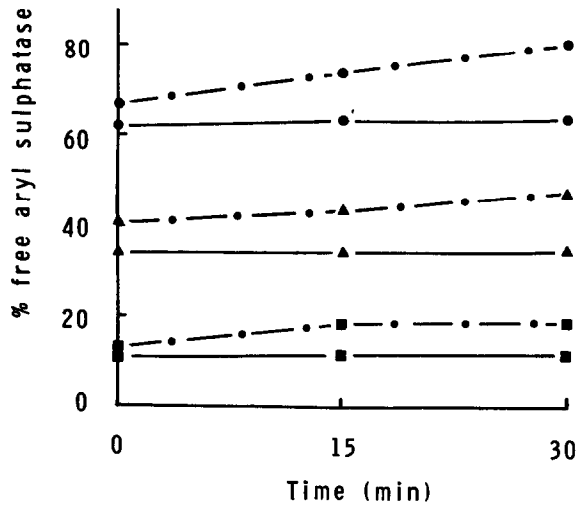

Fig.4. The stability profile of rat liver lysosomes in sucrose. The lysosomes were incubated at 25°C in the following sucrose solutions buffered at pH 7 in 0.01 M TES/Tris: (–■–) 0.25 M sucrose; (–▲–) 0.1 M sucrose; (–●–) 0.05 M sucrose. The effect of 0.025 M KCl on the osmotic stability of the lysosomes is also shown (–·–), where the lysosomes were suspended in: (–■–) 0.2 M sucrose + 0.025 M KCl; (–▲–) 0.05 M sucrose + 0.025 M KCl and (–●–) 0.025 M KCl.

to stabilise the lysosomes against osmotic breakage. Both salts decreased the percentage free lysosomal enzyme activity in 0.1 M and 0.05 M sucrose by ~8%, while 1 mM KCl did not affect lysosomal stability in sucrose.

4. Discussion

Since the pH of the suspending solution appears to have no effect on the osmotic stability of the lysosomes, then the data shown in fig.1 can be interpreted as an effect of pH on the accumulation of sugars in the lysosomes. There are two ways in which this could be affected by the medium pH. If the net transport is coupled to a pH gradient across the membrane, then a continuous relationship between rate of accumulation and pH would be expected. If, on the other hand transport was dependent on ionisation of a specific active site, then a sigmoidal curve could be expected. The effect shown in fig.1, favours the latter alternative. Further evidence for this is the observation that the proton gradient uncouplers, dinitrophenol (0.1 mM) and carbonyl cyanide *p*-trichloromethoxyphenylhydrazone (5 μM) had no effect on the accumulation of D-glucose at pH 7 (data not shown).

As monovalent salts tend to increase the breakage of lysosomes (fig.4), the decreased osmotic breakage of lysosomes in sugar solutions containing monovalent salts (fig.2, table 1) demonstrates that the salts are inhibiting the accumulation of sugars. This inhibition would appear to be non-specific for the salts tested, although allowing for the lack of sensitivity of the method it is not possible to eliminate an order of specificity in the effect. The effect may represent the binding of cations to a negative charge on the carrier. Difficulty however arises in interpreting the divalent cation effect (fig.3), since this would appear to at least partially represent a stabilising effect on the lysosome. It is not possible to rule out a direct inhibitory effect of the divalent cations on the net transport of the sugar.

The inhibition of sugar accumulation by pH and ions reported here provides further support for the concept of sugar permeation of the lysosomal membrane by facilitated [2] rather than passive diffusion [11]. Using the present methodology it is impossible to predict exactly in what way intralysosomal changes in pH or ion content might operate. The osmotic protection method does not measure the affinity of sugar for the transport carrier, but rather the net movement of sugar in the opposite direction to that expected to operate in vivo. Experiments are conducted at very high sugar concentrations, and we have no information about what concentration of metabolites might be produced inside the lysosome in vivo. Nevertheless, coupled to the known differences in the ionic content and pH of the cytosol and lysosome [12], these data do indicate that sugar transport across the lysosome membrane may be regulated by ionic and pH gradients and changes therein [13,14].

Acknowledgements

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