EFFECT OF IONOPHORES AND TEMPERATURE ON INTRALYSOSOMAL pH

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1. Introduction

The digestion of macromolecules in the lysosomes is brought about by specific hydrolases which are characterized by having an acid pH optimum. This property implies that the interior of the lysosomes must have a pH low enough to allow the hydrolases to carry out their catalytic activity effectively [1]. Until recently, only semiquantitative data on intralysosomal pH, obtained by microscopic observation of the colour of phagocytosed dyes [2–5], have been available. In 1973, however, Reijngoud and Tager [6] and Goldman and Rottenberg [7] estimated the intralysosomal pH in isolated rat liver lysosomes by measuring the distribution of the weak base methylamine across the lysosomal membrane. The distribution of a lipid-soluble weak acid or base across the membrane of vesicular structures has been widely used to determine the internal pH [8–10].

Reijngoud and Tager [6] found that the intralysosomal pH was lower than the pH of the medium and that it increased as the external pH was increased. This ΔpH across the lysosomal membrane was found both in a sucrose and in a salt medium. They concluded that the lysosomal membrane is relatively permeable to protons [6]. Goldman and Rottenberg [7] obtained similar results to those of Reijngoud and Tager [6] in a sucrose medium, but found that the ΔpH was almost completely eliminated when the lysosomes were suspended in a salt medium. Goldman and Rottenberg [7] concluded that the lysosomal membrane is permeable not only to protons but to K^+ and Na^+ as well.

In this paper, we show that the apparent discrepancy is caused by the fact that Reijngoud and Tager [6] carried out their experiments at 25°C, while Goldman and Rottenberg [7] worked at 0°C. At the lower temperature, the lysosomal membrane is permeable to K^+ and Na^+, whereas at 25°C it is impermeable to these cations unless a specific ionophore is present. A temperature-dependent alteration in the permeability of lysosomal membranes to salts has recently been described by Davidson and Song [11].

2. Materials and methods

2.1. *Materials*

^3H_2O, [^{14}C]methylamine and [^{14}C]sucrose were obtained from Radiochemical Centre, Amersham, England, gramicidin from Calbiochem, San Diego, USA, and Triton WR 1339 from Haas and Rohm, Philadelphia, USA. Nigericin was a gift from Dr W. Pettinga, Eli Lilly and Co., Indianapolis, USA.

2.2. *Isolation of rat liver lysosomes*

Lysosomes were isolated by the method of Trouet [12] from the livers of Triton WR 1339-treated rats as described previously [6].

2.3. *Determination of intralysosomal pH*

The intralysosomal pH was determined by measurement of the distribution of [^{14}C]methylamine between the lysosomes and the medium as described [6]. The lysosomes (1–3 mg protein/ml) were incubated in a final vol of 1 ml (expts. of table 1 and fig.1) or 7 ml (expt. of fig.2) with 0.5 μCi ^3H_2O, and either about 1 nmol [^{14}C]methylamine (about 0.1 μCi) or about 1 nmol [^{14}C]sucrose (about 0.1 μCi) per ml. Other additions are indicated in the legends to table 1 and figs.1 and 2. After 1 min (table 1 and fig.1), or at the times indicated in fig.2, the lysosomes in 1 ml reaction mixture were separated from the medium by rapid

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Table 1
Effect of nigericin and gramicidin on intralysosomal pH in a KCl or NaCl medium at 25°C

Expt.	Addition	pH_{in} with		ΔpH with	
		KCl	NaCl	KCl	NaCl
1	None	6.49	6.40	1.01	1.10
	Nigericin	7.20	6.70	0.30	0.80
	Gramicidin	6.94	7.12	0.56	0.38
2	None	6.60	–	0.90	–
	Valinomycin	6.60	–	0.90	–

Rat liver lysosomes were incubated in a medium (final vol, 1 ml) containing 20 mM MES, 20 mM MOPS, sufficient Tris to bring the pH to 7.5, ^3H_2O, [^{14}C]methylamine, 1% ethanol, and either 130 mM KCl or 130 mM NaCl. Where indicated, 2 μg nigericin, 10 μg gramicidin or 10 μg valinomycin were also present. Temperature, 25°C. Incubation time, 1 min. In parallel incubations, [^{14}C]sucrose was substituted for [^{14}C]methylamine in order to determine the sucrose space (58% of total pellet vol in expt. 1 and 59% in expt. 2). For further experimental details, see Materials and methods).

centrifugation (2 min in an Eppendorf centrifuge, Model 3200, run at full speed). The further procedure was exactly as described in [6]. The [^{14}C]methylamine distribution between the lysosomal pellet and the supernatant was corrected for adherent water and non-osmotic space, obtained from the incubations with [^{14}C]sucrose. pH_{in} was calculated from the distribution of [^{14}C] methylamine as described in [6].

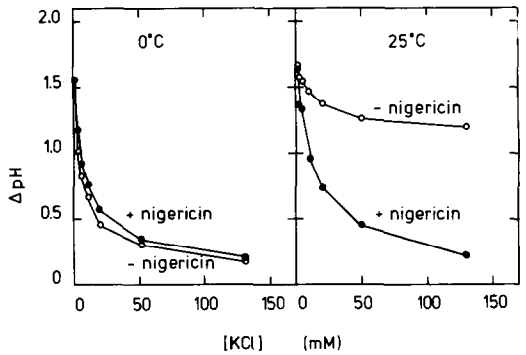

Fig.1. Effect of KCl at various concentrations and of nigericin on ΔpH across the lysosomal membrane at 25°C and at 0°C. Rat liver lysosomes were incubated in a medium containing 25 mM MES, 25 mM MOPS, sufficient Tris to bring the pH to 7.5, 2% ethanol, ^3H_2O, [^{14}C]methylamine, KCl at the concentration indicated and mannitol at a concentration such that the osmolarity of KCl + mannitol was 260 mOsM. Where indicated, 2 μg nigericin were also present. Temperature, 25°C. Incubation time, 5 min. The sucrose space, determined in parallel incubations with [^{14}C]sucrose instead of [^{14}C]methylamine, was 66% of the total pellet vol. For further experimental details, see Materials and methods.

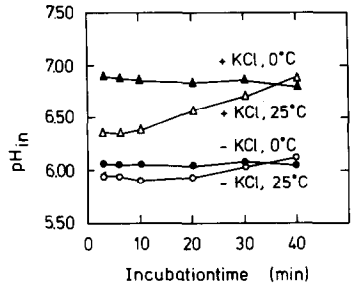

Fig.2. Effect of KCl, incubation temperature, and incubation time on ΔpH across the lysosomal membrane. Rat liver lysosomes were incubated in a medium (final vol, 7 ml) containing 25 mM MES, 25 mM MOPS, sufficient Tris to bring the pH to 7.5, 250 mM sucrose, ^3H_2O, [^{14}C]methylamine, and (where indicated) 50 mM KCl. Temperature, 25° or 0°C as indicated. At the time intervals indicated, 1 ml samples of the incubation mixture were removed and the lysosomes centrifuged down. For further experimental details, see Materials and methods. The sucrose space was determined in parallel incubations in which [^{14}C]sucrose was substituted for [^{14}C]methylamine.

3. Results

Fig.1 shows the effect of increasing concentrations of KCl on the △pH across the lysosomal membrane, calculated on the basis of the distribution of [^{14}C] methylamine. At 25°C, the △pH in the absence of KCl was 1.66; this corresponds to a methylamine $_{in}$/methylamine $_{out}$ distribution ratio of 45.7. As the KCl concentration was increased to 50 mM, the △pH gradually decreased to 1.26. At concentrations of KCl higher than 50 mM, there was little further change in △pH. Even when the KCl concentration was 125 mM, a △pH of 1.20 was observed at 25°C (methylamine distribution ratio 15.8). Dramatically different results were obtained at 0°C. Increasing concentrations of KCl led to a decrease in △pH until at 125 mM KCl the △pH was 0.18 (methylamine distribution ratio 1.5).

Nigericin, an ionophore that induces a K^+–H^+ excange across membranes [see 13], had no effect on the △pH at 0°C (fig.1). At 25°C, however, nigericin brought about a decrease in △pH in the presence of KCl. This effect of nigericin increased progressively as the concentration of KCl was increased.

The results of another experiment with ionophores are summarized in table 1. The effect of the ionophores is dependent on the type of cation in the medium. Nigericin decreased the △pH from 1.01 to 0.30 in a KCl medium, but had only a small effect in a NaCl medium. Gramicidin, on the other hand, decreased the △pH markedly both in a KCl and in a NaCl medium. These results are in accordance with the known specificity of nigericin for K^+, and the wide specificity for monovalent cations of gramicidin [13]. Valinomycin had no effect on the △pH (table 1, expt. 2; see Discussion).

In the experiment of fig.2, lysosomes were incubated at 0°C and at 25°C for varying times. In a sucrose medium, the intralysosomal pH was lower than that of the medium (△pH 1.4) and remained relatively constant both at 0°C and at 25°C throughout the incubation period of 40 min. In a KCl medium, however, the incubation temperature had a marked effect on the internal pH. At 0°C, the △pH was only 0.59 even after 3 min, when the first measurement was made, and remained constant. At the higher temperature, the △pH was 1.13 during the first 10 min of incubation, and then decreased slowly to a value of 0.58 after 40 min.

4. Discussion

The results presented in this paper show clearly that the apparent discrepancy between our findings [6] and those of Goldman and Rottenberg [7] with regard to the permeability of the lysosomal membrane to monovalent cations is due to the difference in incubation conditions. At 0°C, the incubation temperature used by Goldman and Rottenberg [7], the lysosomal membrane is completely permeable to monovalent cations (see also [11]) and to protons. At 25°C, however, the lysosomal membrane is impermeable to monovalent cations like Na^+ and K^+ (see also [11]), unless specific ionophores are present. The increase in permeability at the lower temperature may be caused by a thermal transition in membrane lipids [14,15], as suggested by Davidson and Song [11].

The fact that the intralysosomal pH, as measured by the distribution of methylamine, is a function of the pH of the medium not only at 0°C [7] but also at 25°C [6], shows that the lysosomal membrane is permeable to protons at both temperatures. At the lower temperature, this movement of protons can be accompanied by transport of monovalent cations in the opposite direction, so that electroneutrality is maintained; furthermore, the intralysosomal pH will be determined by a Gibbs-Donnan equilibrium.

The question arises of the nature of proton transport at 25°C. Valinomycin, which promotes a charge-uncompensated transport of K^+ (see [13]) has no effect on the △pH at 25°C. Thus the transport of protons at this temperature can not be electrogenic. Indeed, when nigericin, which brings about an electroneutral K^+–H^+ exchange, is added at 25°C, there is an influence on the △pH, the magnitude of which depends on the concentration of K^+ in the medium. Thus in the presence of the latter ionophore, a Gibbs-Donnan equilibrium is set up, exactly as at 0°C.

We propose that at 25°C (in the absence of ionophores) the dependence of the intralysosomal pH on that of the medium is brought about by a movement of protons accompanied by a charge-compensating movement of anions. Using the terminology of Mitchell [16] we propose that the lysosomal membrane contains a proton–anion symport or its formal equivalent, an hydroxyl ion–anoin antiport. The nature of the anoin taking part in this symport or antiport remains to be determined; it could perhaps be phosphate (see [17]).

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