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Short Communication

THE ACCUMULATION IN LYSOSOMES OF THE ANIONIC DETERGENT LINEAR ALKYLBENZENE SULFONATE

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Abstract—This in vitro study provides evidence that the anionic detergent linear alkylbenzene sulfonate (LAS) enters energized lysosomes from rat liver. Because LAS is lipophilic and negatively charged, the uptake is due to the presence of a positive inside potential induced by energization of lysosomes. This accumulation could explain some damage caused by sublethal doses of detergent in some aquatic species, as it has been found that LAS inhibits lysosomal enzymes such as acid phosphatase and alkaline phosphatase.

Keywords—Anionic detergents Lysosomes Toxicity

INTRODUCTION

The increasing use of anionic detergents in households and in industry gives rise to problems of pollution in natural water systems and presents a risk to aquatic organisms [1–7]. A previous paper [8] indicated that the anionic detergent linear alkylbenzene sulfonate (LAS) influences lysosomal function because it inhibits some lysosomal enzymes such as acid phosphatase (ACP) and alkaline phosphatase (ALP), which are found in the liver and gills of the teleost *Channa punctatus*. Acid phosphatase and ALP are hydrolytic enzymes of lysosomes. The authors [8] suggested that even at low concentration of surfactant, cellular damage occurs. This damage could be responsible for the sublethal long-term toxicity described in the literature [1–7].

This paper supports this hypothesis; we provide evidence that LAS, because it is negatively charged and lipophilic, is accumulated in lysosomes from rat liver. The accumulation is a consequence of the presence in lysosomes of an ATP-driven electrogenic proton pump that induces an acidic internal pH and a positive inside potential, which drives the accumulation of negative ions within lysosomes. High levels of accumulation can cause the membrane to leak. However, even at low levels, the accumulation enhances the inhibitory effect of LAS on ACP and ALC and the consequent toxic effect.

MATERIALS AND METHODS

Rat liver lysosomes were prepared according to Sawant et al. [9] and resuspended before use at 0°C in a medium containing 0.125 M KCl, 10 mM Mops (3-[N-Mozphalino] propanesulfonic acid) pH 7.4, and 5 mM $MgCl_2$. Acridine Orange (AO) (Merck) was purified according to Pal and Schubert [10]. Uptake of AO by lysosomes [10] was followed spectrophotometrically at 20°C, with an Aminco DW-2a UV-VIS spectrophotometer, by measuring the change in dye absorbance at 492 nm, with 540 as the reference wavelength. The anionic detergent LAS was a gift of A. Orio. All the other reagents employed were of the highest analytical grade commercially available.

Linear alkylbenzene sulfonate was added to energized lysosomes or mitochondria. After 8 min lysosomes (or mitochondria) were centrifuged for 5 min at 15,000 rpm in a Sorvall RC 2B (Rotor SS34) and the supernatant collected for the monitoring of LAS. Concentrations of LAS in the medium were monitored by HPLC [11]. In some cases, reversed-phase HPLC separation was carried out using a C_8 column (Lichrosper RP-18, 250 × 4-mm i.d., 5-μm particle sitze) with gradient elution as follows: eluent A: H_2O with 0.15 M $NaClO_4$; eluent B: acetonitrile/H_2O (70:30 v/v) with 0.15 M $NaClO_4$. Fluorescence wavelengths were 232 nm for excitation and 290 mm for emission; the flow rate was 1 ml/min. Protein concentration was determined by the Lowry method [12]. The data reported in the histograms are the mean values obtained from five measurements with different lysosomal preparations. The measurements showed a maximum discrepancy of about 4%.

RESULTS AND DISCUSSION

In lysosomes, the ATP-driven proton pump induces a proton influx into the internal matrix. In the absence of permeant anions, the consequent acidification is not very marked, because the positive inside potential induced by the proton influx opposes the proton pumping. In the presence of chloride ions, the positive inside potential induces the accumulation of chloride ions through a selective channel [13] and the accumulation of the negative charges allows a more marked proton pumping. In this condition (chloride ions in the medium as in physiologic conditions) the internal pH in lysosomes is between 4 and 4.5. The acidification in lysosomes may be monitored following the change in absorbance of the dye AO (Fig. 1). The dye AO is a weak base and, following the classical accumulation mechanism of weak bases [14], it is accumulated in acidic compartments of the cell. The accumulation is accompanied by a change in absorbance of AO in the suspension of lysosomes, allowing monitoring of the acidification. In the presence of the negative detergent LAS, a release of the dye is induced. Because the dye measures the acidification of the internal matrix, LAS appears to reduce internal acidification (Fig. 1a). Similarly, if LAS is added before ATP (Fig. 1b), acidification is less or, by

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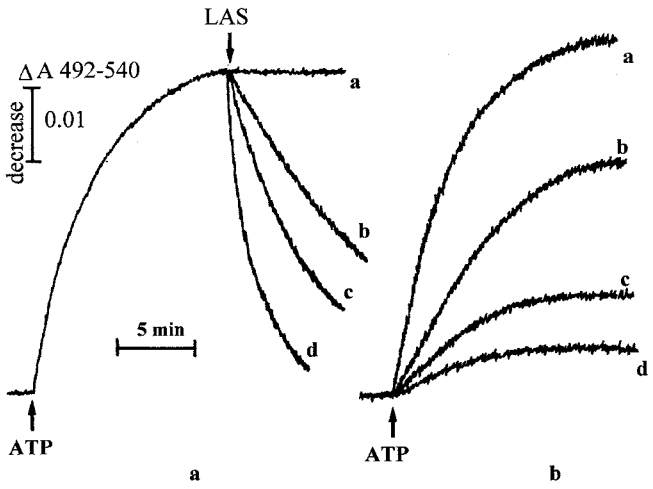

Fig. 1. The figure shows a typical experiment of uptake of Acridine Orange (2 μM) when lysosomes (0.4 mg/ml) are energized by ATP (2 mM). Because the dye spectrophotometric change measures ΔpH, internal acidification can be monitored. LAS induces reduction of acidification if added either before (**a**) or after energization (**b**). Concentrations of LAS: a—0 μM, b—100 μM, c—200 μM, d—300 μM.

increasing the dose, does not occur. On the basis of the experimental data, we suggest that LAS, because it is negative and lipophilic, behaves as the chloride ion and therefore accumulates inside lysosomes. The accumulation gives rise to a membrane leak (as with a detergent) and consequent ΔpH collapse.

As shown in Fig. 2, acidification inside lysosomes is induced without energization. Lysosomes are resuspended in a K^+-free medium and, because lysosomes contain in the internal matrix about 0.1 M K^+ [13] (in the presence of nigericin, an electroneutral H^+/K^+ exchanger), a K^+ efflux and an equivalent H^+ influx is observed. Because internal acidification is not induced by the proton pump, but by an electroneutral exchange, a positive potential is never produced. If LAS is added to the suspension of lysosomes after acidification of the internal matrix by nigericin (Fig. 2), no reduction of ΔpH is observed.

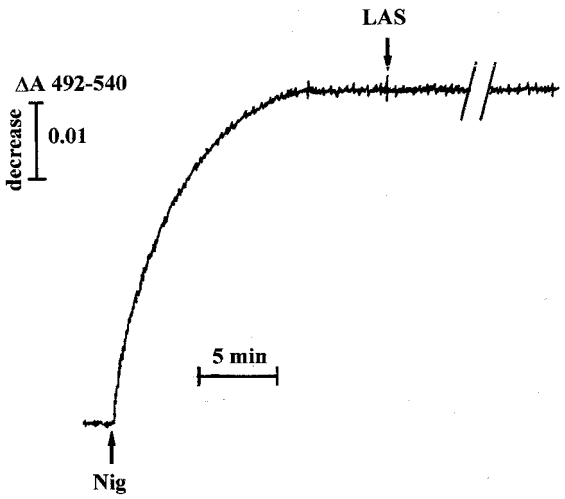

Fig. 2. This figure illustrates a typical experiment of internal acidification of lysosomes being induced by 4 μg nigericin. Lysosomes (1 mg/ml) are resuspended in a potassium-free medium (medium composition: 0.25 M sucrose, 10 mM Tris-Cl, 2 mM $MgCl_2$). Because nigericin is an electroneurtal H^+/K^+ exchanger, the potassium efflux is accompained by an equivalent proton influx. The consequent acidification is measured by absorbtion change of the dye Acridine Orange (5 μM). Addition LAS (0.5 mM) does not induce any proton leak.

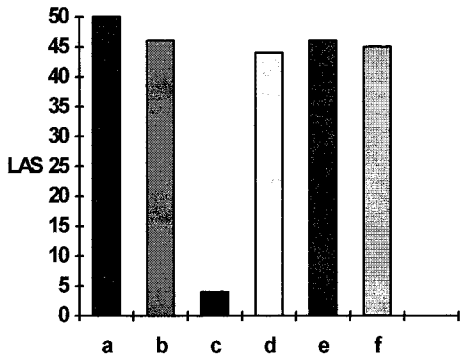

Fig. 3. The histograms provide the LAS concentration (μM). Histograms a, b, c, d, e: medium composition—50 μM LAS, 0.125 M KCl, 10 mM Mops-chloride, 5 mM $MgCl_2$. Histogram f: medium composition—0.25 M sucrose, 10 mM Mops-chloride, 5 mM $MgCl_2$, 50 μM LAS.

The data reported in the histograms further support our hypothesis that a positive potential causes LAS accumulation. In histogram a (Fig. 3), the concentration of 50 μM LAS in the medium without lysosomes (or mitochondria) is reported. In histogram b, the conditions are the same as in histogram a, but the LAS concentration is monitored after centrifugation of the medium containing 1 mg/ml of lysosomes in the absence of ATP. In this condition (histogram b) lysosomes are deenergized: the external LAS concentration is 45 μM and about 5 nmoles/mg of protein of LAS are passively bound to the lysosomes. In lysosomes energized by ATP (histogram c), the LAS concentration in the medium after centrifugation is very low (below 5 μM). A comparison of the data in histograms b and c of Fig. 3 allows us to conclude that LAS is accumulated only in energized lysosomes. Because energization gives rise to a positive inside potential, the potential is responsible for the accumulation of LAS. This conclusion is supported by the results reported in histograms d and e of Fig. 3. In histogram d the LAS concentration was monitored after centrifugation of the medium containing 1 mg/ml of mitochondria energized by ATP. In histogram e, the same experiment as in histogram d was performed with nonenergized mitochondria. No accumulation occurs after energization, because energization in mitochondria gives rise to a negative inside potential. The same results in mitochondria and lysosomes are obtained with higher and lower concentrations of LAS, indicating that, in any case, LAS is not accumulated by mitochondria and is accumulated by lysosomes, whereby a positive potential occurs.

In the experiments shown in histogram f, 1 mg/ml of lysosomes were resuspended in a medium in which 0.25 M sucrose was substituted for 0.125 M KCL. Four micrograms of nigericin was added and after centrifugation the concentration of LAS was measured (histogram f). In this condition a positive potential is not present because nigericin is an electroneutral exchanger. No significative accumulation of LAS was observed, thus confirming the results of Fig. 2 and also confirming that in the absence of an applied positive potential, even in the presence of an acidic internal pH, no LAS accumulation occurs.

We conclude that the data presented in the histograms demonstrate that the proton leak shown in Fig. 1 is the result of a positive potential accumulation that causes a membrane leak. We also conclude that the experiment of Fig. 2 suggests that

no accumulation of LAS occurs if acidification is not the result of a correlated positive potential.

CONCLUSIONS

The behavior of LAS in lysosomes may support and complete the data previously reported by Gupta et al. [8]. The authors [8] found that LAS inhibits ACP and ALC in solution. The present study suggests that the inhibitory effect and consequent toxic effect are enhanced by the fact that LAS is accumulated in lysosomes. Therefore, even if cytoplasmic concentration is low, the accumulation induces high internal concentrations and the inhibition of ACP and ALC, which are lysosomal enzymes, is more effective. The accumulation does not exclude other toxic effects; because LAS is lipophilic, it can induce a membrane leak when it accumulates inside lysosomes, with a consequent loss of ions from the internal matrix not only of lysosomes, but also of other acidic vesicles.

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