FURTHER EVIDENCE FOR A PROTON PUMP IN MOUSE KIDNEY PHAGOLYSOSOMES: EFFECT OF NIGERICIN AND 2,4-DINITROPHENOL ON THE STIMULATION OF INTRALYSOSOMAL PROTEOLYSIS BY ATP

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SUMMARY. The lipid soluble acid 2,4-dinitrophenol completely abolished the stimulatory effect of ATP on intralysosomal proteolysis in mouse kidney phagolysosomes at pH 8. The protonophore had no effect in the absence of ATP at pH 8 but inhibited intralysosomal proteolysis in unbuffered media. The ionophorous antibiotic nigericin also prevented the action of ATP at pH 8 but had no effect in the absence of ATP in unbuffered media. Nigericin also inhibited intralysosomal proteolysis at pH 8 in the absence of ATP. These observations support the hypothesis that the phagolysosome membrane contains an ATP-driven proton pump which functions to maintain intralysosomal acidity.

We have proposed the existence of an ATP-driven proton pump in the phagolysosome membrane (1) which functions to maintain the intralysosomal acidity required for hydrolytic activities of cathepsins and other acid hydrolases. This hypothesis is based on the stimulation of intralysosomal proteolysis by ATP-Mg during incubations of mouse liver and kidney phagolysosomes filled with intravenously injected, formaldehyde-treated [^{125}I]-labelled albumin. At pH 8, intralysosomal proteolysis is inhibited but additions of ATP restore proteolysis to normal rates. The fact that some proteolysis occurs at pH 8 suggests that acidity is maintained in the absence of energy source. Reijngoud and Tager (2) have shown that intralysosomal pH remains about 1 pH unit lower than extralysosomal pH. These authors suggest that a Donnan equilibrium may suffice for maintenance of intralysosomal acidity but they do not exclude the existence of a proton pump as a possible auxiliary mechanism. A Donnan equilibrium would be established by the large quantities of acidic lipoprotein (3) and glycolipid (4) known to be present in lysosomes. De Duve et al (5) believe that a proton pump is mandatory to explain the intralysosomal accumulations of certain basic substances such as chloroquine and neutral red.

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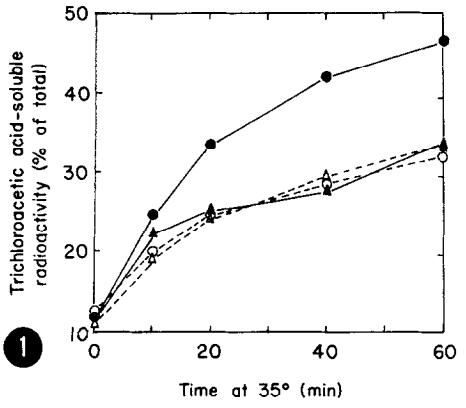
If ATP drives a proton pump, then the resulting pH gradient should be dissipated by certain ionophorous substances particularly those known to transport protons across lipid membranes, and intralysosomal proteolysis will be inhibited. Some studies with the antibiotic nigericin which exchanges K^+ for H^+ (6,7), and 2,4-dinitrophenol, a lipid-soluble acid which acts as a proton carrier across membranes (8,9) are described in this communication.

MATERIALS AND METHODS. Preparation of mouse kidney phagolysosome suspensions containing intravenously injected formaldehyde-treated radioiodinated bovine serum albumin and the assay of intralysosomal proteolysis in these particles have been described (10,11). The suspensions were incubated in 12 ml 0.25 M sucrose containing 5 mM $MgCl_2$, 50 mM β-mercaptoethanol and other additions as described in legends to figures or tables. The buffer used was potassium borate, pH 8. ATP (neutralized to pH 8 with NaOH) was added to preincubated media (35°) just prior to phagolysosomes to a final concentration of 2.5 mM. Nigericin stock solution was prepared by dissolving in 95% ethanol and dilution in distilled water to a concentration of 2.2 μM nigericin and 3.3% (v/v) ethanol. A stock solution of 0.12 M 2,4-DNP was prepared in ethanol. Ethanol was added to all phagolysosome suspensions to the same concentration during incubations whether they contained nigericin or DNP or not.

Nigericin was a gift from Dr. David Wong, Eli Lilly Research Laboratories. ATP (disodium salt) and 2,4-DNP were purchased from Sigma Chemical Co., St. Louis, Mo.

RESULTS. Fig. 1 shows a typical experiment demonstrating that 1 mM 2,4-DNP completely abolished the stimulatory effect of ATP on intralysosomal proteolysis in mouse kidney phagolysosomes incubated at pH 8 but had no effect on the rate of proteolysis at pH 8 in the absence of ATP. Fig. 2 shows that 0.2 mM 2,4-DNP inhibited the stimulatory effect of ATP about 44% with respect to a control containing ATP but no DNP. Low concentrations of 2,4-DNP enhanced the stimulatory effects of ATP. I can offer no explanation for this stimulation at the present time. 2,4-DNP inhibited intralysosomal proteolysis in unbuffered media without ATP (table 1). Nigericin also inhibited the stimulation of intralysosomal proteolysis at pH 8 by ATP (figure 3). Unlike 2,4-DNP however, the antibiotic inhibited proteolysis somewhat at pH 8 in the absence of ATP and had no effect in unbuffered media even in the presence of K^+ and a concentration of 750 nm (table 1).

DISCUSSION. In order to transport protons across membranes, 2,4-DNP must



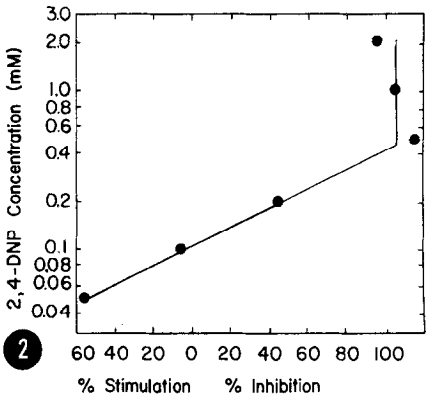


Fig. 1. Intralysosomal Proteolysis of ^{125}I-labelled, Formaldehyde-Treated Bovine Albumin in the Presence and Absence of 2.5 mM ATP and the Effects of 2,4-Dinitrophenol. Albumin-filled mouse kidney phagolysosomes were incubated at 35° in 0.25 M sucrose, 5 mM $MgCl_2$, 50 mM mercaptoethanol and 25 mM potassium borate buffer, pH 8. 2,4-DNP was dissolved in ethanol and all samples contained 0.4% ethanol (v/v). ATP-Mg ●—●; ATP-Mg and 1 mM 2,4-DNP ▲—▲; 5 mM Mg O- - O; 5 mM Mg and 1 mM 2,4-DNP Δ- -Δ.

Fig. 2. Effect of 2,4-DNP concentrations on Intralysosomal Proteolysis in Mouse Kidney Phagolysosomes at pH 8 in the Presence of 2.5 mM ATP. The experiment was the same as described in fig. 1 except that all samples contained ATP-Mg and the concentrations of 2,4-DNP were varied. % stimulation or inhibition refers to controls without DNP. For example, 1 mM 2,4-DNP completely abolished the stimulatory effect of ATP (as in fig. 1), and 0.05 mM DNP further enhanced the effect of ATP by about 56% (stimulated proteolysis 134% instead of 86%).

cross the membrane into the acid compartment in the non-protonated form. The pH in the acid compartment also should be low enough to permit protonation. In unbuffered media, these criteria apply to the mouse kidney phagolysosome suspensions shown in table 1. At pH 8 in the absence of ATP, the intralysosomal pH may be too high to allow protonation of 2,4-DNP and no effects were observed. Inhibition of the stimulatory effect of ATP at pH 8 therefore may be explained by the action of a proton pump which lowered the intralysosomal pH sufficiently to allow protonation of the 2,4-DNP.

The effects of nigericin are also consistent with a proton pump. Nigericin forms lipid-soluble complexes with K^+ or Rb^+ and carries these ions across lipid membranes only at high pH values (6). Inside the acid compart-

TABLE 1

Effect of 2,4-Dinitrophenol and Nigericin on Intralysosomal Proteolysis in Mouse Kidney Phagolysosomes Incubated in Unbuffered Media

Trichloroacetic Acid-Soluble Radioactivity Produced in 40 Min Incubation, % of Total				
No. additions	0.2mM 2,4-DNP	1 mM 2,4-DNP	2 mM 2,4-DNP	750 nM Nigericin
29.8	20.3	12.0	5.4	--
23.3	--	--	--	24.3

The data shown represent two separate experiments. 12 ml suspensions of phagolysosomes were incubated at 35° in 0.25 M sucrose, 50 mM mercaptoethanol, 5 mM $MgCl_2$ and the above additions, for 60 min. Nigericin and 2,4-DNP were added in 95% ethanol and ethanol was added to all samples, including the controls, to the same concentration. The control and the experimental suspension in the nigericin experiment also contained 10 mM KCl.

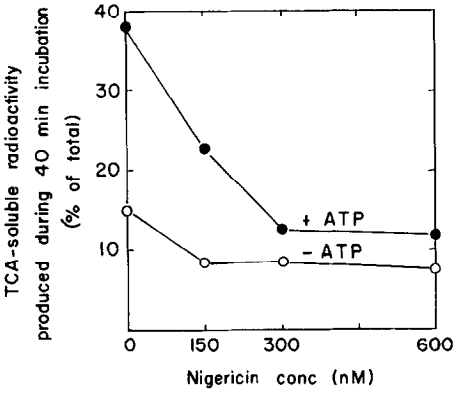


Fig. 3. Effect of Nigericin on Intralysosomal Proteolysis in Mouse Kidney Phagolysosomes in the Presence and Absence of ATP. Experimental conditions are described in the legend to fig. 1 and in Materials and Methods. Nigericin was added in 3.3% ethanol and all samples contained 1% ethanol (v/v).

ment, the antibiotic acts as a proton carrier and exchanges K^+ for H^+ thus dissipating a pH gradient. Nigericin therefore inhibited intralysosomal proteolysis at pH 8 in the presence or absence of ATP. Although the inhibi-

tory effect of nigericin at pH 8 without ATP is shown by only one point in fig. 3 (the control), the effect was reproducible. The inhibition was considerably greater in the presence of ATP due to the greater pH gradient. In unbuffered media, nigericin had no effect because the pH was not sufficiently high to permit complexing with external K^+. These observations can best be explained in terms of an ATP-driven proton pump.

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