The Effect of Zinc and Other Metals on the Stability of Lysosomes[1] (36521)

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In our recent study on the effect of various chelating agents on the stability of lysosomes (1) we showed the stabilization of these organelles by agents such as diethyldithiocarbamate and penicillamine. We also demonstrated that zinc ions stabilize and cupric ions labilize the lysosomal membrane. We postulated that this effect may be related to changes in metal-catalyzed lipid peroxidation, to binding of the metal to components of the membrane with a resulting change in the conformation of the macromolecules involved, and/or to an effect on some enzyme(s) controlling the integrity of the membrane.

It was our aim to study the mechanism(s) of the beneficial effect of zinc on various tissue injuries, which is usually explained by the interaction of zinc with several zinc-dependent enzymes (2). This paper, however, shows that zinc stabilizes lysosomal membranes by a mechanism which is restricted to the surface of the membrane.

Materials and Methods. Lysosome-rich suspensions were prepared from the livers of female rats by the method previously described (1). The livers were perfused *in situ* with ice-cold saline, weighed and homogenized (1:10, w/v) in cold 0.25 *M* sucrose–0.01 *M* phosphate buffer (pH 7.4), which contained no EDTA. Special care was used to follow the same homogenization procedure and to use the same set of all-glass homogenizers each time in order to reduce variations in the degree of lysosome disruption. The material sedimenting between 800*g* and 15,000*g* was resuspended in 0.25 *M* sucrose containing 0.01 *M* PO_4 (pH 7.4), 1.5 ml/g tissue. Although this fraction contains mitochondria as well as lysosomes, the activity of an enzyme characteristic of lysosomes (β-glucuronidase) was used to evaluate membrane stability. For this reason we refer to the preparation as a lysosomal fraction. Samples of the suspension were adjusted to pH 5.0 with two volumes of 0.25 *M* sucrose–0.1 *M* acetate buffer containing the substances being tested. The final concentrations of these agents are given in the individual tables or figures. Samples were incubated with gentle shaking for 30 min at 37°. When lysosomes were incubated at a pH other than 5, they were adjusted to the appropriate pH with 2 vol of 0.25 *M* sucrose–0.01 *M* phosphate–0.01 *M* acetate buffer. After incubation the suspensions were centrifuged at 15,000*g* and the supernatants were assayed for released β-glucuronidase activity (3). The effect of these metals on the activity of purified β-glucuronidase was also determined. The enzyme (Type B-1, bovine liver) was purchased from Sigma Chemical Corp. and assayed in the presence of 1 m*M* of each agent. All metallic ions tested were chlorides of reagent grade; 8-hydroxyquinoline (8-HQ) was obtained from Fisher Sci. Company; 8-methoxyquinoline was purchased from K & K Laboratories.

In studies on the binding of zinc to the liver lysosomal fraction the 15,000*g* pellet was resuspended in 0.25 *M* sucrose and aliquots were treated for 15 min at 25° under the conditions given in Table II. Excess zinc was removed by thoroughly washing the individual samples seven times with sucrose–phosphate, pH 7.4. The washed lysosomes were resuspended in the same medium and a portion was removed and analyzed for total protein (4) and total content of zinc. The remainder was treated with 0.1%

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Triton X-100 to lyse the membranes, and was centrifuged at 20,000*g* for 20 min. The portion taken for total zinc and the pellet which contained the membrane fraction were hydrolyzed with redistilled nitric acid. Both the pellet (membrane) and the supernatant (soluble fraction) were assayed for zinc-using a Perkin-Elmer atomic absorption spectrophotometer, Model 303.

Results. Effect of certain metals on the stability of the lysosomal membrane. Results from the direct incubation of isolated lysosomes with various metals, 8-HQ, and Zn:8-HQ complexes are presented in Table I. As we showed earlier (1) zinc ions significantly stabilize lysosomes yet do not interfere with the determination of β-glucuronidase activity. Cadmium and lead ions also increase lysosomal stability with relatively little or no effect on purified β-glucuronidase. Although Au^{3+} almost completely decreases the activity of β-glucuronidase released in this fragility test, this parallels the inhibition of enzyme activity. Thus it is not possible from these data to determine whether or not gold alters membrane stability. The known labilizing effects of Cu^{2+} (1) and Hg^{2+} (5) are evident even though both metals partially inhibit the marker enzyme. Of the metals tested only Mn^{2+} and Ni^{2+} do not alter lysosomal fragility under the conditions used in this study.

The three stoichiometric complexes of Zn:8-HQ differed markedly in their effects on isolated lysosomes. The unsaturated complexes (1:1 and 1:2) are even more effective stabilizers than an equivalent amount of zinc alone (1 m*M*). In contrast, the saturated (1:3) complex, as well as 8-HQ itself, are significantly labilizing. This labilizing effect of 8-HQ is related to its chelating properties since 8-methoxyquinoline (which has no chelating capacity) does not affect the stability of lysosomes.

Effect of various concentrations of zinc, Zn:8-HQ (1:1) and 8-HQ. The magnitude of the stabilization of lysosomes by zinc and Zn:8-HQ (1:1) is dependent on the concentration of the substance tested. The results of one typical experiment are shown in Fig. 1; they verify and extend the results presented earlier (1). Furthermore, the labilizing effect of 8-HQ alone is shown to be strongly concentration dependent.

Zinc binding to biomembrane. Potentiation of the stabilizing effect of zinc by partial

TABLE I. Effect of Zinc, Zinc:8-Hydroxyquinoline Complexes and Some Other Metals on the Fragility of Liver Lysosomes and the Activity of Purified β-Glucuronidase.

Sample[a]	Lysosome fragility[b] (% of control)	Purified β-glucuronidase (% of control)
Control, no treatment	100 ± 3.5	100 ± 1.2
Zn^{2+}, 1 m*M*	62 ± 2.2[d]	100 ± 1.4
Zn:8-HQ, 1:1	52 ± 4.8[d]	99 ± 4.7
1:2	55 ± 3.7[d]	95 ± 4.5
1:3	143 ± 12[c]	105 ± 2.7
8-HQ	278 ± 44[c]	109 ± 4.2
8-Methoxyquinoline	95 ± 3.9	—
Cu^{2+}	195 ± 19[d]	88 ± 2.2[c]
Hg^{2+}	190 ± 21[d]	64 ± 0.12[d]
Cd^{2+}	78 ± 3.7[c]	99 ± 0.18
Mn^{2+}	93 ± 3.7	101 ± 1.4
Pb^{2+}	73 ± 3.3[c]	93 ± 1.5[c]
Ni^{2+}	99 ± 1.4	99 ± 0.7
Au^{3+}	1.5 ± 0.3[d]	2.3 ± 0.12[d]

[a] All substances tested at 1 m*M* final concentrations. Various stoichiometric complexes of zinc refer to 1 m*M* Zn^{2+} and 1, 2, or 3 m*M* 8-HQ. Results are the average of 3 to 6 determinations.

[b] Refers to β-glucuronidase released after 30 min incubation (pH 5.0, 37°).

[c] Results significantly different from controls: $p < .01$; [d] $p < .001$.

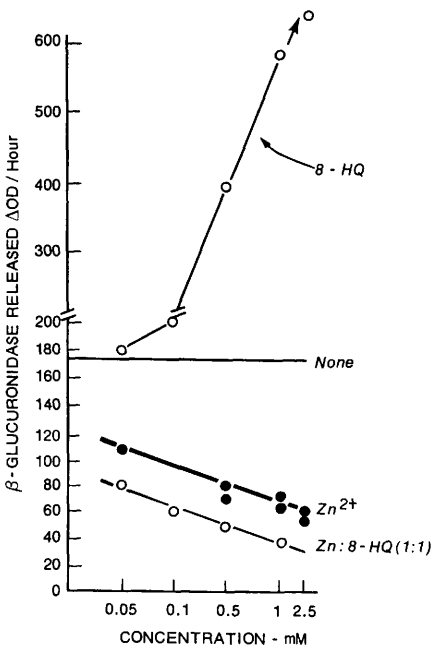

FIG. 1. Effect of different concentrations of zinc, 8-hydroxyquinoline and of zinc + 8-hydroxyquinoline on the stability of isolated liver lysosomes. Aliquots of the lysosomal suspension containing 10 to 15 mg protein were incubated at 37° in 0.25 *M* sucrose, 0.1 *M* acetate buffer (pH 5.0), and the substances being tested. The total volume was 3.0 ml. After 30 min the samples were centrifuged at 15,000*g* and the supernatants assayed for released β-glucuronidase. Results are presented as activity of released enzyme (increased absorption at 550 nm × 1000).

complexing with 8-HQ (Table I) suggests that zinc is acting in some way on the surface of the membrane. The unsaturated Zn:8-HQ complexes do not permeate the cell membrane (6). These are just the agents which are the most effective stabilizers.

We measured the amount of zinc bound to the membrane and present in the interior of lysosomal particles prepared from the livers of adult rats (Table II). The ratio of bound (membrane) and soluble (interior) zinc is approximately 2:1. When the particles are treated with 1 m*M* zinc at either pH 6.0 or 7.4 and then washed free of unbound metal, the total content of zinc increases 10-fold while the distribution between the membrane and interior is unchanged. In contrast, incubation with the unsaturated Zn:8-HQ complexes increases the zinc content of the membrane fraction only.

Effect of pH in the presence of Ca^{2+} *or* Zn^{2+}. The above data indicate that zinc does in fact stabilize the lysosomal membrane by some as yet unknown mechanism(s) related to the surface of the membrane. We thought that zinc might alter the activity of certain enzymes located at the membrane which control the integrity of the lysosomal membrane. The existence of phospholipases in lysosomes has been established (7, 8). Of those that have been characterized, one is located within the lysosome, has greatest activity at pH 4 and is inhibited by calcium; the second is in the membrane, functions optimally at pH 7–8 and is activated by calcium.

We compared the effect of zinc with calcium on lysosome stability between pH 4 and 8.5. The results of one representative experi-

TABLE II. Distribution of Zn^{2+} Between Membrane and Interior of Control, Zn^{2+} and Zn:8-HQ-Treated Liver Lysosomal Fraction.[a]

Sample	Total zinc (μg/mg protein)	Membrane bound (% of total)	Ratio (bound/free)
Control	0.27 ± 0.071	61.0	2:1
Zn^{2+}, 1 m*M*, pH 6.0	3.32 ± 0.42	65.0	2:1
7.4	3.31 ± 0.07	65.0	2:1
Zn:8-HQ, 1:1	3.66 ± 0.24	95.0	10:1
1:2	3.11 ± 0.18	97.0	10:1

[a] Aliquots of a lysosome-rich fraction, prepared from the rat liver as given under "Methods," were incubated for 15 min at 25° in the presence of 2 vol of saline and the above substances at 1 m*M* final concentration. For details see "Methods." Results are the average of three separate incubations and are presented as mean ± SE.

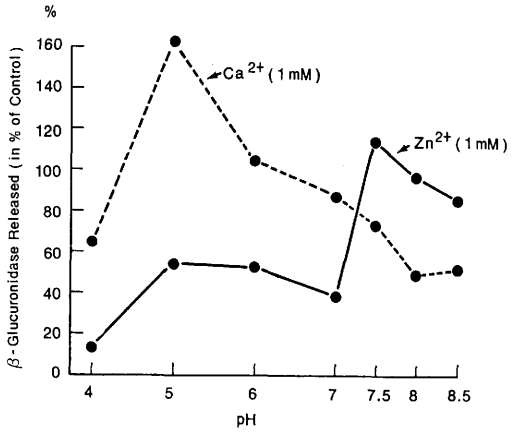

FIG. 2. Effect of zinc and of calcium on the stability of isolated liver lysosomes at different pH values. The conditions of incubation are the same as for Fig. 1 except that the buffer contained 0.1 *M* acetate and 0.05 *M* PO_4 adjusted to the appropriate pH. The results are presented as the activity of released β-glucuronidase calculated as the percentage of enzyme released in the control sample at each pH.

ment (Fig. 2) show that zinc stabilizes lysosomes from pH 4.0 to 7.0. Evaluation of the results above pH 7 is difficult because of the possible formation of insoluble zinc hydroxide; this may explain why lysosomal stability was not affected at higher pH. Calcium ions showed an opposite effect: at acid pH there was a marked labilization of the lysosomal membrane. This pattern does not correspond to the response of the known phospholipases to calcium (7, 8).

Discussion. We have presented more information on the stabilization of lysosomal membranes by zinc supporting the concept that this effect is a membrane phenomenon. In particular, we showed that an increase in the amount of zinc bound to the membrane coincides with a decrease in the release of β-glucuronidase from isolated lysosomes incubated in the presence of zinc or Zn:8-HQ. This complex does not permeate the membrane, binds exclusively to the surface, still stabilizing the membrane integrity.

We do not yet know the precise mechanism by which zinc stabilizes biomembranes. In principle, a metal might interact with reactive groups of membrane components, or it might alter the activity, conformation or binding capacity of enzymes responsible for controlling the membrane integrity. The results presented in this paper do not reflect the metal and pH dependences of the known phospholipases (7, 8). On the basis of these results, it is certainly not possible to conclude that zinc is not acting by such a mechanism. However, we favor the nonenzymatic concept of a direct interaction of zinc with membrane components for the reasons discussed below.

Functional groups of macromolecular components of biomembranes might react with metals under either an increase or a decrease in membrane stability. For example, metals might interact with thiol groups; this has been postulated as the mechanism of the labilizing effects of mercury on lysosomes (5). The presence of phospholipids in membranes offers the possibility of a metal–phosphate interaction. Binding of Zn to the phosphate moiety and the base of the ATP molecule was shown by Rimai and Heyde (9) who suggested that metal–phosphate interaction may play the fundamental role in ATPase mechanisms.

Alternatively, a close correlation between lipid peroxidation and distortion of biomembranes has been found (10). Such peroxidation is catalyzed by metals forming redox systems, for example, Fe^{2+}/Fe^{3+} and Cu^{+}/Cu^{2+}. The fact that the stabilizing properties are not restricted to zinc but also are shown by cadmium and lead, all of which are metals having only one oxidation state, suggests that they may act by interfering with oxidation of membrane components. This hypothesis is under investigation.

Summary. The effects of zinc, certain other metals (Cu, Hg, Pb, Cd, Ni and Au), 8-hydroxyquinoline (8-HQ), and various stoichiometric complexes of Zn:8-HQ on the release of β-glucuronidase from lysosomes isolated from rat liver were tested. Zinc and Zn:8-HQ (1:1 and 1:2 complexes) markedly stabilized lysosomes in a concentration-related fashion. The saturated 1:3 complex and 8-HQ alone showed a labilizing effect, while the nonchelating substitute of 8-HQ (8-methoxyquinoline) did not affect lysosomal stability. Cd and Pb also stabilized lysosomes, although

they were both less effective than zinc.

The distribution of zinc between the membrane and the interior of isolated liver lysosomes is 2:1. After treatment with zinc, the distribution is unchanged but the total content of zinc increased 10-fold. In samples treated with unsaturated complexes of Zn:8-HQ (1:1 or 1:2) the total amount of zinc present is the same as in zinc-treated particles, but the metal is bound exclusively to the membrane fraction.

The effect of pH on lysosomal fragility in the presence of zinc was almost opposite to that observed when calcium was tested. The results of this study favor the concept that the stabilizing effect of zinc occurs at the surface of the membrane. The effect does not seem to be related to the function of known phospholipases.

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